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TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

WALTER WYMAN, Surgeon-General

HYGIENIC LABORATORY.—BULLETIN No. 36

M. J. ROSENAU, Director

APRIL, 1907

STUDIES UPON HYPERSUSCEPTIBILITY AND IMMUNITY

By

M. J. ROSENAU

and

JOHN F. ANDERSON

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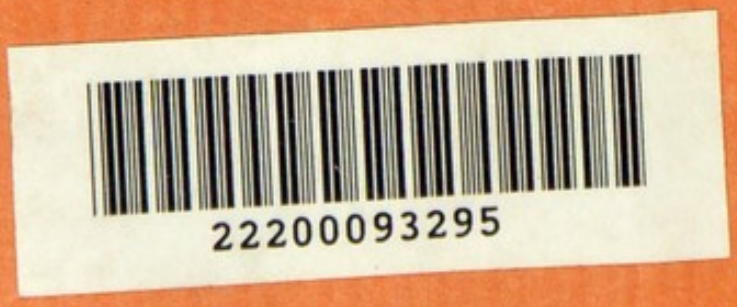
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CONTENTS.

STUDIES UPON HYPERSUSCEPTIBILITY AND IMMUNITY.[a]

By MILTON J. ROSENAU,
Passed Assistant 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.

We have shown[b] that horse serum is apparently a bland and harmless substance when injected into a normal guinea pig, but this injection renders the guinea pig susceptible to a subsequent injection of horse serum. At least ten days must elapse between the first and the second injection for this hypersusceptibility to manifest itself.

The present bulletin gives the results of our further work upon this interesting phenomenon. We have endeavored to obtain a deeper insight into the cause and nature of hypersusceptibility and have attempted to localize the phenomenon in certain fluids, cells, or organs of the body.

We foresaw last year that the problem of hypersusceptibility has an important bearing upon the question of immunity and expressed the opinion[c] that "resistance to disease may be largely gained through a process of hypersusceptibility. Whether this increased susceptibility is an essential element or only one stage in the process of resistance to disease, must now engage our attention." We can not escape the

[a] Manuscript submitted for publication April 27, 1907.

[b] Rosenau, M. J., and Anderson, John F.: A study of the cause of sudden death following the injection of horse serum. Pub. Health and Mar.-Hosp. Serv., Hyg. Lab. Bull. No. 29, 1906.

———: A new toxic action of horse serum. Journ. med. research, Vol. 15, No. 1 (n. s., Vol. 10, No. 1), July, 1906, pp. 179–208.

Anderson, John F.: 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. Pub. Health and Mar.-Hosp. Serv., Hyg. Lab. Bull. No. 30, 1906, and Journ. med. research, Vol. 15, No. 2 (n. s., Vol. 10, No. 2), Sept., 1906, pp. 241–260.

[c] Rosenau, M. J., and Anderson, John F.: Hypersusceptibility. Journ. Am. Med. Assn., Vol. 42, No. 13, Sept. 29, 1906, pp. 1007–1010.

conviction that this phenomenon of hypersusceptibility has an important bearing on the prevention and cure of certain infectious processes. Our work this year upon the hypersusceptibility produced by the bacterial proteids strengthens this belief, for our results prove that the phenomenon of hypersusceptibility to certain proteid substances extracted from the bacterial cell is followed by a definite immunity against infection by the micro-organism.

Since our studies last year several papers have been published which, in the main, have corroborated our findings.

McClintock and King[a] gave ten guinea pigs from $\frac{1}{20}$ to 1 c. c. of horse serum by the stomach and thirteen days later 6 c. c. of serum, either subcutaneously or intraperitoneally, without causing symptoms in any of them. They conclude that the sensitizing action of horse serum given by the mouth is not nearly so great as when given subcutaneously or intraperitoneally. This is in confirmation of our reported experiments.

Currie[b] has studied the effect of repeated injections of horse serum in persons admitted for treatment in the city of Glasgow Fever and Smallpox Hospital at Belvidere. He concludes that it is apparent from the facts detailed by him that repeated injections of horse serum induce symptoms of supersensitation in man, but it is also apparent that the same facts lend no countenance to the suggestion that the death of persons suffering from diphtheria is to be apprehended as the result of repeated injections of antidiphtheric serum.

Besredka and Steinhardt[c] studied with much care certain features of hypersusceptibility to horse serum in guinea pigs; they note that the French serums are much less toxic than those used by Otto in Frankfurt and the serums used by us. Besredka and Steinhardt had a mortality of about 25 per cent when 5 c. c. of serum was given intraperitoneally at the second injection, whereas death was the rule in our experiments under similar conditions. Most of their work was done with doses of 0.05 to 0.25 c. c. given directly into the brain, which either killed or caused grave symptoms in susceptible guinea pigs. Besredka and Steinhardt lay stress upon the production of "anti-anaphylaxis," which we termed "immunity." They found that a single injection of serum given into the peritoneum of a sensitized guinea pig conferred immunity to a subsequent injection of 0.25 c. c. into the brain; in one case the anti-anaphylaxis was present one and a

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[a] McClintock, Charles T., and King, Walter E.: The oral administration of antitoxins for prevention of diphtheria, tetanus, and possibly sepsis. Journ. infec. diseases, Vol. 3, No. 5, Oct., 1906, pp. 700–720.

[b] Currie, J. R.: On the supersensitation of persons suffering from diphtheria by repeated injections of horse serum. Journ. hyg., Vol. 7, No. 1, Jan., 1907, pp. 35–60.

[c] Besredka, A., and Steinhardt, Edna: De l'anaphylaxie et de l'anti-anaphylaxie vis-à-vis du sérum de cheval. Ann. de l'Inst. Pasteur, Vol. 21, No. 2, Feb. 25, 1907, pp. 117–127.

half hours after the injection into the abdominal cavity. They were unable to demonstrate any protective properties in various organs of immune guinea pigs, confirming our work along the same lines.

Nicolle[a] found that guinea pigs were not susceptible to the necrotic action induced by repeated injections of horse serum, as is the case in rabbits; this corresponds with our observations. He also found that daily injections or "spaced" injections, after the method of Arthus, did not induce a high degree of hypersusceptibility in guinea pigs.

Besredka[b] questions whether we should not consider this toxic property of horse serum, as well as its antitoxic power. He suggests that a serum, 0.05 c. c. of which when given into the brain will kill or cause grave symptoms in a sensitive guinea pig, should be considered as above the average toxicity and ought to be excluded from use in man.

The work of Otto[c] on the "Theobald Smith Phenomenon," and of von Pirquet and Schick[d] upon "the serum disease" has been previously referred to.

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[a] Nicolle, Maurice: Contribution à l'étude du phénomène d'Arthus. Ann. de l'Inst. Pasteur, Vol. 21, No. 2, Feb. 25, 1907, pp. 128–136.

[b] Besredka, A.: De la toxicité des sérums thérapeutiques et dumoyen de la doser. Comp. rend. soc. biol., Vol. 62, No. 10, Mar. 22, 1907, pp. 477–478.

[c] Otto, R.: Das Theobald Smithschen Phänomen der Serum-Ueberempfindlichkeit. v. Leuthold-Gedenkschr., Bd. 1.

[d] von Pirquet, C. Frh., and Schick, B.: Die Serumkrankheit. Leipzig and Wien, 1905, 144 p. 8°.

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Part I.

THE SENSITIZING SUBSTANCE.

We ventured the suggestion in our former publication that the substance that sensitizes the guinea pig is the same as that which later poisons it; profound chemical changes perhaps in the central nerve cells, are probably produced by the first injection. Our subsequent work has produced nothing to alter this working hypothesis.

Vaughan[a] advances the theory that the first injection of the strange proteid is broken up into components, one of which is toxic, but that the animal is not poisoned because this breaking up takes place slowly. The cells, however, learn from this lesson how to break up the complex molecule, so that when more of the strange proteid is introduced at the second injection it is violently rent asunder, quickly liberating large quantities of the toxic principle of the complex molecule.

Vaughan and Wheeler[b] have elaborated this explanation by further studies upon egg-white and bacterial proteids split into poisonous and nonpoisonous portions. These authors believe that when egg-white, or the nonpoisonous portion of egg-white, is injected into a fresh animal certain cells of the body are so influenced that they elaborate a new ferment, which, in the form of zymogen, remains in the cell until activated by the second injection, when it is set free and splits up the egg-white in a manner similar to that used by Vaughan in the laboratory. Vaughan and Wheeler believe that the effect induced in the animal is the same as that caused by the poisonous portions of egg-white as they have split it up in the retort.

Currie[c] suggests that the first injection of serum results after an interval in the formation of an antibody. When the second injection of serum is given, after at least ten days from the first, the antibody-producing substance of the second injection of serum and the antibody

[a] Vaughan, V. C.: Discussion of "Hypersusceptibility," by M. J. Rosenau and J. F. Anderson. Journ. Am. Med. Assn., Vol. 47, No. 13, Sept. 29, 1906, p. 1009.

[b] Vaughan, Victor C., and Wheeler, May: Effects of egg-white and its split products upon animals. A study of susceptibility and immunity. Abstract of papers to be read at twenty-second annual meeting of Assn. Am. Physicians, Washington, May 7–9, 1907, p. 9.

[c] Currie, J. R.: On the supersensitation of persons suffering from diphtheria by repeated injections of horse serum. Journ. Hygiene, Vol. 7, No. 1, Jan., 1907, pp. 35–60.

produced by the first injection come in contact without delay; their union is rapid; the whole charge of the poisonous substance is quickly set free and the toxic symptoms are sudden and severe.

Besredka and Steinhardt[a] had, as a working hypothesis, the following: The sensitized guinea pig which appears in good health has, in spite of its apparent well-being, perhaps a latent lesion of the brain. A second injection of serum, made into the peritoneal cavity twelve days later, is able to awaken this nervous lesion, resulting in grave symptoms or even death.

In view of these theoretical considerations it is important to make further studies upon the sensitizing substance in horse serum and other proteid substances.

The following experiments show that the filtrate from horse serum after precipitation with ammonium sulphate renders guinea pigs sensitive. The filtrate contains most of the serum albumen and very little of the globulins. It is exceedingly weak in antitoxic strength.

G. P. No. 400. Six c. c. antitoxic horse serum (Natl. VIII., 18), intraperitoneally. Marked symptoms.

[Previous treatment: 38 days prior, 5 c. c. filtrate of antitoxic serum, precipitated (NYBH). Subcutaneously.]

This filtrate was kindly furnished us by Dr. W. H. Park from some antidiphtheric serum undergoing the Gibson process of refining.

The following experiments show that formaldehyd does not destroy the sensitizing property of horse serum:

G. P. No. 390W. Six c. c. normal horse (roan) serum, intraperitoneally. Severe symptoms.

[Previous treatment: 47 days prior, 6 c. c. antitoxic horse serum (Natl. XVIII)+1 per cent formalin, 23 hours exposure. Subcutaneously.]

G. P. No. 500W. Six c. c. normal horse (roan) serum, intraperitoneally. Very severe symptoms.

[Previous treatment: 29 days prior, 3 c. c. normal horse (roan) serum+5 per cent formalin, 4 hours 30 minutes exposuure. Subcutaneously.]

The results with formaldehyd have a special significance in view of the fact that this active reducing agent is capable of destroying the poisonous properties of tetanus and diphtheria toxines.

We have shown before that the sensitizing and poisonous principles in horse serum are not dialyzable through parchment paper. From the following limited experiments it would seem that the sensitizing principle is not dialyzable through a collodion sac when placed in the peritoneal cavity of the animal.

G. P. No. B. Six c. c. normal horse (No. 15) serum, subcutaneously. No symptoms.

[Previous treatment: 32 days prior, collodion sac containing about 3 c. c. normal horse (No. 15) serum placed in peritoneal cavity.]

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[a] Besredka, A., and Steinhardt, Edna: De l'anaphylaxie et de l'anti-anaphylaxie vis-à-vis du sérum de cheval. Ann. de l'Inst. Pasteur, Vol. 21, No. 2, Feb. 25, 1907, pp. 117–127.

G. P. No. Cx. Collodion sac containing about 3 c. c. normal horse (No. 15) serum placed in peritoneal cavity.

28 days later, 5 c. c. normal horse (roan) serum, subcutaneously. No symptoms.

1 day later, sac removed.

23 days after removal of sac, 5 c. c. normal horse (roan) serum, subcutaneously. Mild symptoms.

By reference to page 62 it will be seen that guinea pigs may be sensitized by injecting horse serum directly into the heart.

Further work upon the action of the sensitizing substance is in progress and will be reported later.

Part II.

THE TOXIC PRINCIPLE.

We added a number of different ferments, alkaloids, and simpler chemical substances to horse serum in order to modify, destroy, or neutralize its toxic action. All these attempts have so far been found unavailing, as will be seen by the following experiments:

FERMENTS.

The ferments were added to the horse serum and allowed to stand at 15° C. over night.

Taka diastase.

G. P. No. 5417. Six c. c. antitoxic horse serum (Natl. IX., 19)+Taka diastase (PDCo.), subcutaneously. Dead, 90 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Pancreatin.

G. P. No. 5413. Six c. c. same serum+pancreatin (PDCo.), subcutaneously. Dead, 40 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 9+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Rennin.

G. P. No. 5405. Six c. c. same serum+rennin (Hansen's junket tablet), subcutaneously. Dead, 50 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Myrosin.

G. P. No. 5416. Six c. c. same serum+myrosin (from white mustard seed), subcutaneously. Dead, 25 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{700}$ c. c. antitoxic horse serum (A. A 208).]

Invertin.

G. P. No. 5375. Six c. c. same serum+invertin (yeast), subcutaneously. Dead, 80 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{1000}$ c. c. antitoxic horse serum (NYBH 305)].

Emulsin.

G. P. No. 5373. Six c. c. same serum+emulsin (from almonds), subcutaneously. Severe symptoms.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (NYBH 305).]

Pepsin in acid solution.

G. P. No. 5409. Six c. c. same serum+pepsin (Wyeth's) rendered acid with HCl, subcutaneously. Severe symptoms.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Pepsin in alkaline solution.

G. P. No. 5412. Six c. c. same serum+pepsin (Wyeth's) rendered alkaline, subcutaneously. Severe symptoms. (Died 18 hours later.)

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Ingluvin.

G. P. No. 5418. Six c. c. same serum+ingluvin (Warren), subcutaneously. Severe symptoms. (Died 18 hours later.)

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum A. A 208).]

Malt.

G. P. No. 5411. Six c. c. same serum+malt (from corn), subcutaneously. Severe symptoms.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Papain.

G. P. No. 5414. Six c. c. same serum+papain (Merck's), subcutaneously. Severe symptoms. (Died 18 hours later.)

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{600}$ c. c. antitoxic horse serum (A. A 208).]

ALKALOIDS.

Atropin.

G. P. No. 5408. Six c. c. antitoxic horse serum (Natl. IX, 19)+0.002 atropin sulphate, subcutaneously. Dead, 27 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{570}$ c. c. antitoxic horse serum (A. A 208).]

Strychnin.

G. P. No. 5420. Six c. c. same serum+0.001 gm. strychnin sulphate, subcutaneously. Dead, 25 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{600}$ c. c. antitoxic horse serum (A. A 208).]

Morphin.

G. P. No. 5406. Six c. c. same serum+0.002 gm. morphin sulphate, subcutaneously. Dead, 35 minutes.

[Previous treatment: 27 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{500}$ c. c. antitoxic horse serum (A. A 208).]

Caffein.

G. P. No. 5377. Six c. c. antitoxic horse serum (Natl. IX, 19)+0.01 gm. caffein citrate, subcutaneously. Dead, 55 minutes.

[Previous treatment: 28 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{1000}$ c. c. antitoxic horse serum (NYBH 305).]

SALTS.

Calcium chlorid.

G. P. No. 5342. Six c. c. same serum+0.5 c. c. calcium chlorid, subcutaneously. Severe symptoms.

[Previous treatment: 35 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{350}$ c. c. antitoxic horse serum (A. A 201).]

Sodium nitrate.

G. P. No. 5376. Six c. c. same serum+0.3 c. c. 1 per cent sodium nitrate, subcutaneously. Severe symptoms.

[Previous treatment: 28 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{3000}$ c. c. antitoxic horse serum (NYBH 305).]

Sodium chlorid.

G. P. No. 5341. Six c. c. same serum+0.5 gm. sodium chlorid, subcutaneously. Severe symptoms.

[Previous treatment: 35 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{240}$ c. c. antitoxic horse serum (A. A 201).]

Magnesium sulphate.

G. P. No. 5364. Six c. c. same serum+0.2 gm. magnesium sulphate, subcutaneously. Dead, 38 minutes.

[Previous treatment: 35 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{1000}$ c. c. antitoxic horse serum (NYBH 306).]

Ammonium sulphate.

G. P. No. 5384. Six c. c. same serum+0.1 gm. ammonium sulphate, subcutaneously. Dead at once.

[Previous treatment: 28 days prior, 0.139 c. c. toxine No. 5+$\frac{1}{1200}$ c. c. antitoxic horse serum (NYBH 305).]

G. P. No. 7847. Six c. c. normal horse (roan) serum+0.1 gm. ammonium sulphate, subcutaneously. Dead, 23 minutes.

[Previous treatment: 59 days prior, 0.24 c. c. toxine No. 9+$\frac{1}{300}$ c. c. antitoxic horse serum (A 192).]

MISCELLANEOUS SUBSTANCES.

Ox bile.

G. P. No. 7616. Six c. c. normal horse (No. 15) serum+heated ox bile, equal parts, intraperitoneally 3 hours after mixing. Dead, 11 minutes.

[Previous treatment: 45 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{300}$ c. c. antitoxic horse serum (A. A 248).]

Animal charcoal.

G. P. No. 7614. Six c. c. normal horse (No. 15) serum+animal charcoal; shaken up well, filtered, let stand for 3 hours; intraperitoneally. Dead, 20 minutes.

[Previous treatment: 45 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{380}$ c. c. antitoxic horse serum (A. A 248).]

Yeast cells.

G. P. No. 7615. Six c. c. normal horse (No. 15) serum+ground yeast cells; let stand 3 hours; intraperitoneally. Dead, 35 minutes.

[Previous treatment: 45 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{380}$ c. c. antitoxic horse serum (A. A 245).]

FORMALDEHYD.

In view of the fact that formaldehyd has a destructive action upon such "haptin" substances as tetanus and diphtheria toxines, and in further view of the fact that the sensitizing and toxic principles of horse serum seem to belong to the haptin group of substances in the sense used by Ehrlich, it became interesting to determine what effect formaldehyd would have upon hypersusceptibility produced by horse serum.

Normal serum+5 per cent formalin:

G. P. No. 442. Six c. c. normal horse (roan) serum+5 per cent formalin, subcutaneously. Dead, 12 minutes.

[Previous treatment: 33 days prior, 0.0006 gm. tetanus toxine A+$\frac{1}{1000}$ c. c. antitoxic horse serum (Hoechst), subcutaneously.]

G. P. No. 7542. Six c. c. same serum+5 per cent formalin; 21 hours exposure; subcutaneously. Dead, 60 minutes.

[Previous treatment: 35 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{170}$ c. c. antitoxic horse serum (Mul 2100).]

Control G. P. Six c. c. same serum+5 per cent formalin; 21 hours exposure; subcutaneously. Severe symptoms of formaldehyd poisoning.

Antitoxic serum+1 per cent formalin:

G. P. No. 7501. Six c. c. antitoxic horse serum (Natl. IX)+1 per cent formalin; 22 hours exposure; subcutaneously. Dead, 60 minutes.

[Previous treatment: 41 days prior, 0.24 c. c. toxine No. 9+$\frac{1}{500}$ c. c. antitoxic horse serum (Led 4D).]

G. P. No. 7198. Five c. c. same mixture; 4 days exposure; subcutaneously. Marked symptoms.

[Previous treatment: 59 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{250}$ c. c. antitoxic horse serum (NYBH 305).]

G. P. No. 7589. Five c. c. same mixture; 4 days exposure; subcutaneously. Mild symptoms.

[Previous treatment: 37 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{1700}$ c. c. antitoxic horse serum (Park ppt. 305–306).]

Normal serum+5 per cent formalin:

G. P. No. 7776. Six c. c. normal horse (roan) serum+5 per cent formalin; 4 hours 20 minutes exposure; subcutaneously. Mild symptoms.

[Previous treatment: 46 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{340}$ c. c. antitoxic horse serum (S 1351).]

G. P. No. 7629. Six c. c. same mixture and exposure; subcutaneously. Slight symptoms.

[Previous treatment: 64 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{350}$ c. c. antitoxic horse serum (A. ppt. 31).]

G. P. No. 7689. Six c. c. same mixture; 4 hours exposure; subcutaneously. Slight symptoms.

[Previous treatment: 56 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{330}$ c. c. antitoxic horse serum (PDCo 080235).]

While the above three pigs showed slight and mild symptoms of hypersusceptibility, they all showed severe symptoms of formaldehyd poisoning.

These results plainly show that formaldehyd, in the strength and time stated, does not apparently appreciably influence the toxicity of horse serum. We have seen before that it also has no effect upon the sensitizing action, page 12.

CALCIUM CHLORID.

Netter[a] has shown that when 1 gram of calcium chlorid is given on the day of injection and on the two following days the number of

[a] Netter, Arnold: Efficacité de l'ingestion de chlorure de calcium comme moyen préventif des éruptions consécutives aux injections de sérum. Compt. rend. soc. biol., tome 60, No. 6, Feb. 16, 1906, p. 279.

———: Influence des quantités de sérum injectées et du nombre des injections sur les éruptions sériques. Nécessité d'augmenter les quantités de sels de chaux dans les cas d'injections répétées ou supérieures à quarante centimètres cubes. Idem, p. 281.

children showing eruption following the injection of serum is greatly reduced. We thought perhaps this salt might have some influence upon the phenomenon produced in guinea pigs by two injections of horse serum. Three series of experiments were made with this object in view.

The effect of 0.1 gram of $CaCl_2$ by mouth for three consecutive days before the second injection of horse serum.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
6065	0.14 c.c. toxine $5+\frac{1}{8}\frac{1}{5}\frac{0}{0}$ c. c. antitoxic horse serum (NYHD. 310), subcutaneously; then for 3 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	65	3 c. c. normal horse (roan) serum, intraperitoneally.	Marked symptoms.
6063	.....do.....	65	.....do.....	Dead, 40 minutes.
6064	.....do.....	65	6 c. c. normal horse (roan) serum, intraperitoneally.	Dead, 3 minutes.
6057	.....do.....	65	3 c. c. normal horse (roan) serum, intraperitoneally.	Mild symptoms.
6062	0.14 c. c. toxine $5+\frac{1}{7}\frac{1}{2}\frac{0}{0}$ c. c. antitoxic horse serum (NYHD. 310), subcutaneously; then for 3 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	65	.....do.....	Dead, 20 minutes.

Effect of 0.1 gram $CaCl_2$ daily for twenty days before the sensitizing inoculation was given, and every other day until the second injection of horse serum, fourteen days later.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
.....	0.1 gm. $CaCl_2$ by mouth daily for 20 days; then 0.14 c. c. toxine $5+\frac{1}{2}\frac{1}{5}\frac{0}{0}$ c. c. antitoxic horse serum (S. 1500), subcutaneously; then $CaCl_2$ every other day till second injection.	14	3 c. c. normal horse (roan) serum, intraperitoneally.	Dead, 30 minutes.
.....	.....do.....	14	.....do.....	Severe symptoms.
.....	.....do.....	14	.....do.....	Marked symptoms.
.....	.....do.....	14	.....do.....	Severe symptoms.
.....	.....do.....	14	.....do.....	Severe symptoms.

Effect of 0.1 gram $CaCl_2$ for fifteen consecutive days before the second injection of horse serum, 104 days after the sensitizing injection.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
5556	0.14 c. c. toxine 5+$\frac{1}{500}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	6 c. c. normal horse (roan) serum, intraperitoneally.	Dead, 35 minutes.
5550	0.14 c. c. toxine 5+$\frac{1}{300}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	.....do	Dead, 25 minutes.
5557	0.14 c. c. toxine 5+$\frac{1}{500}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	.....do	Dead, 15 minutes.
5553	0.14 c. c. toxine 5+$\frac{1}{400}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	3 c. c. normal horse (roan) serum, intraperitoneally.	Dead, 18 minutes.
5555	0.14 c. c. toxine 5+$\frac{1}{500}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	.....do	Dead, 65 minutes.
5552	0.14 c. c. toxine 5+$\frac{1}{400}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	.....do	Severe symptoms.
5551	0.14 c. c. toxine 5+$\frac{1}{300}$ c. c. antoxine horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	104	.....do	Very severe symptoms.

Effect of 0.1 gram $CaCl_2$ for fifteen consecutive days before the second injection of horse serum, 104 days after the sensitizing injection—Continued.

No. G. P.	First injection.	Interval.	Second injection.	Result.
5554	0.14 c. c. toxine 5+$\frac{1}{500}$ c. c. antitoxic horse serum (NYBH. 310), subcutaneously; then daily for 15 days before second injection, 0.1 gm. $CaCl_2$ by mouth.	*Days.* 104	6 c. c. normal horse (roan) serum, intraperitoneally.	Marked symptoms.

As will be seen from the above, the guinea pigs which received 0.1 gm. of $CaCl_2$ for three days previous to the second injection of serum reacted in the usual manner, 2 of them dying in a few minutes, and the other two had severe symptoms.

Of those which received 0.1 gm. $CaCl_2$ daily for twenty days before being given their sensitizing dose and then every other day for fourteen days before they were given the second dose of serum, none showed any marked resistance.

Of those that were sensitized first and then given 0.1 gm. $CaCl_2$ for the fourteen days previous to the second dose, 5 out of 8 died in spite of the fact that 4 received only 3 c. c. of serum.

It is, therefore, quite plain that, while $CaCl_2$ may modify the occurrence of rashes in children following a single injection of serum, it does not influence to any marked extent the toxic effect in guinea pigs of a second injection of serum given fourteen days after the first injection.

FREEZING DOES NOT DESTROY THE TOXIC PRINCIPLE.

Some normal horse serum (roan) was frozen hard at 15° F., then thawed at room temperature, and found toxic when injected into a sensitized guinea pig. For example:

No. G. P.	First injection.	Interval.	Second injection.	Result.
5329	0.22 c. c. toxine no. 7+$\frac{1}{500}$ c. c. antitoxic horse serum (Natl., XIV), subcutaneously.	*Days.* 13	6 c. c. normal horse (roan) serum, frozen hard in brine, then thawed at room temperature, intraperitoneally.	Severe symptoms.

Part III.

IS THE TOXIC PRINCIPLE SPECIFIC?

The toxic action is quantitatively specific so far as various blood serums are concerned. That is, a guinea pig sensitized with horse serum is more susceptible to a subsequent injection of horse serum than to a subsequent injection of the blood serum of cattle, sheep, cats, dogs, hogs, etc. The specific character of the hypersusceptibility is more apparent when proteid substances of quite different origin are used at the first and second injections. For example, guinea pigs sensitized with horse serum do not react at all to subsequent injections of peptone, vegetable proteid extracts, egg albumen or milk.

Horse serum versus other proteid substances.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
7030	0.142 c. c. toxine 5+ $\frac{1}{580}$ c. c. antitoxic horse serum (Ld. 4B), subcutaneously.	52	6 c. c. 3 per cent peptone, intraperitoneally.	No symptoms.
7039	0.142 c. c. toxine 5+ $\frac{1}{1000}$ c. c. antitoxic horse serum (Ld. 5H), subcutaneously.	52	.....do	No symptoms.
7038	0.142 c. c. toxine 5+ $\frac{1}{800}$ c. c. antitoxic horse serum (Ld.5H), subcutaneously.	52	6 c. c. watery extract of peas kept at 15° C. 24 hours, filtered through porcelain intraperitoneally.	No symptoms.
7033	0.142 c. c. toxine 5+ $\frac{1}{740}$ c. c. antitoxic horse serum (Ld. 34), subcutaneously.	52	.....do	No symptoms.
7036	0.142 c. c. toxine 5+ $\frac{1}{1000}$ c. c. antitoxic horse serum (Ld.5H), subcutaneously.	52	6 c. c. egg albumen, saturated solution in salt water (not filtered), intraperitoneally.	No symptoms.
7041	0.142 c. c. toxine 5+ $\frac{1}{780}$ c. c. antitoxic horse serum (Ld.5C), subcutaneously.	52	.....do	No symptoms.
7032	0.142 c. c. toxine 5+ $\frac{1}{580}$ c. c. antitoxic horse serum (Ld. 34), subcutaneously.	52	6 c. c. bottom milk unfiltered, intraperitoneally.	No symptoms.
7035	0.142 c. c. toxine 5+ $\frac{1}{800}$ c. c. antitoxic horse serum (Ld.5H), subcutaneously.	52	.....do	No symptoms.

It naturally occurred to us to determine whether guinea pigs sensitized with injections of albuminous substances, such as are contained in milk, the white of eggs, peas, etc., are sensitive to subsequent injections of horse serum.

The following experiments plainly indicate that animals sensitized with milk, egg albumen, peptone, or the albuminous substance extracted from peas, do not react when subsequently injected with horse serum.

Other proteid substances versus horse serum.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
705	0.1 c. c. fresh whole milk, subcutaneously.	31	6 c. c. normal horse (No. 15) serum, intraperitoneally.	No symptoms.
736	.....do.....	31	.....do.....	No symptoms.
738	.....do.....	31	.....do.....	No symptoms.
703	$\frac{1}{250}$ gm. peptone, subcutaneously.	31	.....do.....	No symptoms.
730	1 c. c. egg albumen in salt solution, subcutaneously.	21	.....do.....	No symptoms.
731	.....do.....	21	.....do.....	No symptoms.
742	1 c. c. watery extract peas, subcutaneously.	21	10 c. c. normal horse (No. 15) serum, intraperitoneally.	No symptoms.
743	0.5 c. c. watery extract peas, subcutaneously.	21	.....do.....	No symptoms.
744	0.1 c. c. watery extract peas, subcutaneously.	21	.....do.....	No symptoms.
484	1 c. c. normal horse (roan) serum, subcutaneously.	64	6 c. c. hemoglobin, horse (roan), intraperitoneally.	Mild symptoms.
485	6 c. c. normal horse (roan) serum, subcutaneously.	64	.....do.....	Mild symptoms.
486	.....do.....	64	.....do.....	Slight symptoms.
487	$\frac{1}{250}$ c. c. normal horse (roan) serum, subcutaneously.	64	.....do.....	Slight symptoms.

Part IV.

OTHER BLOOD SERUMS AND OTHER ALBUMINOUS SUBSTANCES ARE ALSO TOXIC.

So much of our work has been done with horse serum that we desire to record some further experiments with the blood serums of other animals. We confirm and extend our previous work that the same reactions may be induced in the guinea pig with the blood serums of various animals, such as the dog, ox, sheep, cat, and hog.

Other blood serums.

No. G. P.	First injection.	Interval.	Second injection.	Result.
	Subcutaneously, $\frac{1}{250}$ c. c. serum of—	*Days.*	Intraperitoneally, 6 c. c. serum of—	
461	Ox	37	Ox	Dead, 120 minutes.
462	do	37	do	Marked symptoms.
463	do	37	do	Severe symptoms.
465	do	37	do	Severe symptoms.
466	Sheep	37	Sheep	Slight symptoms.
467	do	37	do	Dead, 110 minutes.
468	do	37	do	Severe symptoms.
469	do	37	do	Severe symptoms.
470	do	37	do	Dead, 12 hours.
471	Hog	37	Hog	Mild symptoms.
472	do	37	do	Dead, 12 hours.
473	do	37	do	Dead, 1 hour.
474	do	37	do	Severe symptoms.
475	do	37	do	Severe symptoms.
476	Dog	70	Dog	Dead, 60 minutes.
477	do	70	do	Dead, 120 minutes.
478	do	70	do	Dead, 20 minutes.
479	do	70	do	Dead, 65 minutes.
480	do	70	do	Dead, 70 minutes.
481	Cat	70	Cat	Dead, 120 minutes.
482	do	70	do	Dead, 50 minutes.
483	do	70	do	Dead, 120 minutes.
484	do	70	do	Dead, 50 minutes.
485	do	70	do	Dead, 65 minutes.

OTHER ALBUMINOUS SUBSTANCES.

As soon as we concluded that it is probably the proteid substance in horse serum that is chiefly concerned in sensitizing and poisoning the guinea pigs, we thought of other proteid substances obtained from widely different sources.

We have found that hemoglobin, egg albumen, milk, and extract of peas are quite as active as horse serum. Peptone seems to have slight sensitizing and poisonous properties; leucin and tyrosin none at all. The reaction following the second injection of proteid matter in the guinea pigs appears, then, to be common to all the higher forms of albuminous substances, no matter from what source. It occurs to us that this phenomenon of hypersusceptibility in the guinea pig may be used as a physiological test to distinguish true proteid substances from the lower forms of nitrogenous compounds. It would

be interesting to determine whether the synthetic peptids and polypeptids of Fisher sufficiently approach the true proteid molecular structure to induce hypersusceptibility in the guinea pig.

From our work with other proteid substances it was but a logical step to the albuminous content of the bacterial cell, which is dealt with in another part of this work.

Hemoglobin versus hemoglobin.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
411	0.1 c. c. hemoglobin (washed 20 times) subcutaneously.	22	6 c. c. hemoglobin, intraperitoneally.	Marked symptoms.
412	0.5 c. c. hemoglobin (washed 20 times) subcutaneously.	22	.....do	Severe symptoms.
413	1 c. c. hemoglobin (washed 20 times) subcutaneously.	22	.....do	Dead, 5 minutes.
414	3 c. c. hemoglobin (washed 20 times) subcutaneously.	22	.....do	Slight symptoms.
415	5 c. c. hemoglobin (washed 20 times) subcutaneously.	22	.....do	Very severe symptoms.

The hemoglobin was obtained by dissolving the washed red corpuscles of a normal horse in distilled water. The red corpuscles for the hemoglobin solution used at the first injection, in order to sensitize the guinea pigs, was washed and centrifuged 20 times in order to surely wash away all traces of serum, the smallest remaining quantities of which might have confused the results. The hemoglobin used at the second injection was dissolved from red corpuscles washed four times.

Egg albumen versus egg albumen.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
406	0.1 c. c. egg albumen, subcutaneously.	22	6 c. c. saturated solution of egg albumen in salt solution, intraperitoneally.	Dead, 30 minutes.
407	0.5 c. c. egg albumen, subcutaneously.	22	.....do	Dead, 18 minutes.
408	1 c. c. egg albumen, subcutaneously.	22	.....do	Dead, 25 minutes.
409	3 c. c. egg albumen, subcutaneously.	22	.....do	Dead, 25 minutes.
410	5 c. c. egg albumen, subcutaneously.	22	.....do	Dead, 20 minutes.
732	1 c. c. egg albumen + salt solution, subcutaneously.	21	6 c. c. egg albumen + salt solution equal quantities, intraperitoneally.	Severe symptoms.

Milk versus milk.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
401	3 c. c. milk filtered through porcelain, subcutaneously.	26	10 c. c. bottom milk, intraperitoneally.	Slight symptoms.
402	1 c. c. milk filtered through porcelain, subcutaneously.	26	.....do	Slight symptoms.
403	0.5 c. c. milk filtered through porcelain, subcutaneously.	26	.....do	Slight symptoms.
404	0.1 c. c. milk filtered through porcelain, subcutaneously.	26	.....do	Slight symptoms.
706	0.5 c. c. fresh whole milk, subcutaneously.	31	10 c. c. fresh whole milk, intraperitoneally.	Dead, 20 minutes.
707	1 c. c. fresh whole milk, subcutaneously.	31	.....do	Very severe symptoms.
708	3 c. c. fresh whole milk, subcutaneously.	31	.....do	Very severe symptoms.
709	5 c. c. fresh whole milk, subcutaneously.	31	.....do	Very severe symptoms.
737	0.1 c.c. fresh whole milk, subcutaneously.	31	.....do	Very severe symptoms.
Control: 6 c. c. fresh whole milk, intraperitoneally.....				No symptoms.

Peas versus peas.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
416	0.1 c. c. watery extract peas, 24 hours at 15° C. (acid), filtered through porcelain, subcutaneously.	26	10 c. c. watery extract of peas, 3 days at 15° C., filtered through porcelain intraperitoneally.	Marked symptoms.
417	0.5 c. c. watery extract peas, 24 hours at 15° C. (acid), filtered through porcelain, subcutaneously.	26	.....do	Dead, 7 hours and 30 minutes.
418	1 c. c. watery extract peas, 24 hours at 15° C. (acid), filtered through porcelain, subcutaneously.	26	.....do	Marked symptoms.
419	3 c. c. watery extract peas, 24 hours at 15° C. (acid), filtered through porcelain, subcutaneously.	26	.....do	Dead, 4 hours.
420	5 c. c. watery extract peas, 24 hours at 15° C. (acid), filtered through porcelain, subcutaneously.	26	.....do	Dead, 2 hours.
Control: 10 c. c. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.				No symptoms.
Do.....				No symptoms.

Peptone versus peptone.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
492	0.5 c. c. half-saturated solution peptone, subcutaneously.	21	6 c. c. saturated solution of peptone, intraperitoneally.	Slight symptoms.
490	1 c. c. half-saturated solution peptone, subcutaneously.	21	.....do	Mild symptoms.
491	3 c. c. half-saturated solution peptone, subcutaneously.	21	.....do	Marked symptoms.
488	0.1 c. c. half-saturated solution peptone, subcutaneously.	21	.....do	Marked symptoms.
748	0.1 c. c. heated, half-saturated solution peptone, subcutaneously.	21	6 c. c. half-saturated solution of peptone, intraperitoneally.	Marked symptoms.
749	0.5 c. c. heated, half-saturated solution peptone, subcutaneously.	21	.....do	No symptoms.
750	1 c. c. heated, half-saturated solution peptone, subcutaneously.	21	.....do	Marked symptoms.
751	3 c. c. heated, half-saturated solution, peptone, subcutaneously.	21	6 c. c. half-saturated solution of peptone, intraperitoneally.	Marked symptoms.
752	5 c. c. heated, half-saturated solution peptone, subcutaneously.	21	6 c. c. half-saturated solution of peptone, subcutaneously.	Marked symptoms.
Control: 6 c. c. half-saturated solution of peptone, heated, subcutaneously.				No symptoms.

Tyrosin versus tyrosin.

No. G. P.	First injection.	Interval.	Second injection.	Results.
		Days.		
739	0.002 gm. watery solution of tyrosin, subcutaneously.	21	6 c. c. tyrosin (0.1 gm. + 50 c. c.) watery solution, intraperitoneally.	No symptoms.
740	0.01 gm. watery solution of tyrosin, subcutaneously.	21	20 c. c. tyrosin (0.1 gm. + 50 c. c.) watery solution, intraperitoneally.	No symptoms.
741	0.02 gm. watery solution of tyrosin, subcutaneously.	21	10 c. c. tyrosin (0.1 gm. + 50 c. c.) watery solution, intraperitoneally.	No symptoms.
Control: 6 c. c. tyrosin (0.1 gm. + 50 c. c.) watery solution, intraperitoneally, into a fresh guinea pig.				No symptoms.

Leucin versus leucin.

No. G. P.	First injection.	Interval.	Second injection.	Results.
		Days.		
745	0.02 gm. leucin in watery solution, subcutaneously.	17	20 c. c. (0.2 gm. + 50 c. c.) watery solution intraperitoneally.	No symptoms.
746	0.02 gm. leucin in watery solution, subcutaneously.	17	6 c. c. (0.2 gm. + 50 c. c.) watery solution intraperitoneally.	No symptoms.
747	0.02 gm. leucin in watery solution, subcutaneously.	17	10 c. c. (0.2 gm. + 50 c. c.) watery solution intraperitoneally.	No symptoms.
Control: 6 c. c. leucin (0.2 gm. + 50 c. c.) watery solution, intraperitoneally, into a fresh guinea pig.				No symptoms.

Part V.

HYPERSUSCEPTIBILITY AND IMMUNITY PRODUCED BY BACTERIAL PROTEIDS.

Experimental studies with the bacterial proteids are of the greatest importance on account of the practical uses to which results along this line may lead. Our conviction that the phenomenon of hypersusceptibility which we have been studying in the guinea pig has a deep significance in general pathology, especially in the problem of immunity, induced us to undertake an extensive series of experiments with proteid extracts obtained from bacterial cell masses. Some of this work is sufficiently advanced for us to record our results in part.

Hypersusceptibility may easily be induced in guinea pigs with proteid extracts obtained from the bacterial cell. The first injection of most of the extracts used by us seems comparatively harmless to the animal. A second injection of the same extract shows, however, that profound physiological changes have taken place. A definite period must elapse between the first and the second injection. The symptoms presented by the guinea pigs as a result of the second injection resemble those caused by horse serum.

The phenomenon induced by a second injection is followed (in certain cases) by an immunity to the corresponding infection.

These results strengthen our belief that the phenomenon of hypersusceptibility has a practical significance in the prevention and cure of certain infectious processes. It gives a possible explanation to the period of incubation of some of the communicable diseases. Is it a coincidence that the period of incubation of a number of infectious diseases is about ten to fourteen days, which corresponds significantly with the time required to sensitize animals with a strange proteid? In certain infectious diseases with short periods of incubation, such as pneumonia, the crisis which commonly appears about the tenth day may find a somewhat similar explanation. It is evident that disease processes produced by soluble toxines, such as diphtheria and tetanus, do not belong to the category now under consideration.

EXTRACT OF COLON BACILLUS.

The extract from the colon bacillus in the following experiments was obtained as follows:

A 2-day-old culture of *B. coli communis* in Dunham's solution was used to heavily inoculate the surface of 84 large agar plates. These

plates were grown at 37° C. for four days and the surface growth collected.

The bacterial mass was frozen forty-eight hours at about 15° F., thawed at room temperature, and then ground with sand by hand in a mortar and pestle for five hours, shaken vigorously half an hour, and again frozen eighteen hours. After again thawing, the fluid was diluted with salt solution and filtered through a Berkefeld filter. The clear filtrate gave a distinct coagulum with heat and acetic acid.

All the other extracts were obtained by a similar process. In the case of the tubercle bacillus the bacterial mass was first washed three days in running water to eliminate the soluble tuberculin as much as possible.

B. coli.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
817	5 c. c. colon extract, subcutaneously.	35	6 c. c. colon extract, intraperitoneally.	Marked symptoms.
819	1 c. c. colon extract, subcutaneously.	35	.....do.....	Mild symptoms.
818	.....do.....	35	6 c. c. colon extract, subcutaneously.	Marked symptoms
820	.....do.....	35	.....do.....	Slight symptoms.
821	.....do.....	35	6 c. c. colon extract, intraperitoneally.	Mild symptoms.
822	0.5 c. c. colon extract, subcutaneously.	35	6 c. c. colon extract, subcutaneously.	Mild symptoms.
823	0.1 c. c. colon extract, subcutaneously.	35	6 c. c. colon extract, intraperitoneally.	Slight symptoms.
824	0.01 c. c. colon extract, subcutaneously.	35	6 c. c. colon extract, subcutaneously.	Marked symptoms.
825	0.005 c. c. colon extract, subcutaneously.	35	6 c. c. colon extract, intraperitoneally.	Severe symptoms.

The hypersusceptibility induced by the colon extracts manifested itself by symptoms resembling those already described in the case of horse serum. The guinea pigs scratched at the mouth with their hind legs. Most of them showed evidences of respiratory embarrassment by quickened, labored, or irregular breathing. Many of the pigs lay over on their sides, which is a very common symptom. A few developed jerky movements, but in no case was convulsion noted. The pigs looked quite sick and ill at ease, but gradually recovered, so that by next morning they seemed normal.

Ten days following the second injection of the extract all the above pigs were given 5 c. c. of a heavy emulsion of colon bacillus from 24-hour old agar slants, but showed no symptoms, and remain in good condition. Three controls received the same injection and died in twelve hours.

YEAST.

The manifestations of hypersusceptibility produced by the proteid extract from yeast cells are restlessness, scratching, irregular respirations; the guinea pigs lie down and look sick; sometimes jerky movements are seen and, in one instance, convulsions.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
755	1 c. c. extract yeast cells, subcutaneously.	22	6 c. c. extract yeast cells, intraperitoneally.	Very severe symptoms.
754	0.5 c. c. extract yeast cells, subcutaneously.	22	6 c. c. extract yeast cells, subcutaneously.	Slight symptoms.
864	0.1 c. c. extract yeast cells, subcutaneously.	19	5 c. c. extract yeast cells, subcutaneously.	Slight symptoms.
803	1 c. c. extract yeast cells, subcutaneously.	27	6 c. c. extract yeast cells, intraperitoneally.	Mild symptoms.
807	.....do.....	27	.....do.....	Mild symptoms.
804	.....do.....	27	.....do.....	Marked symptoms.
809	.....do.....	27	.....do.....	Dead, 2 hours 10 minutes.
802	.....do.....	27	6 c. c. extract yeast cells, subcutaneously.	Slight symptoms.
805	.....do.....	27	.....do.....	Slight symptoms.
810	.....do.....	27	.....do.....	Marked symptoms.
806	.....do.....	27	.....do.....	Very severe symptoms.
815	0.005 c. c. extract yeast cells, subcutaneously.	27	5 c. c. extract yeast cells, subcutaneously.	No symptoms.
814	0.01 c. c. extract yeast cells, subcutaneously.	27	.....do.....	Slight symptoms.
813	0.02 c. c. extract yeast cells, subcutaneously.	27	.....do.....	Mild symptoms.
812	0.1 c. c. extract yeast cells, subcutaneously.	27	.....do.....	Severe symptoms.
811	0.5 c. c. extract yeast cells, subcutaneously.	27	.....do.....	Very severe symptoms.
801	5 c. c. extract yeast cells, subcutaneously.	27	.....do.....	Mild symptoms.
800	10 c. c. extract yeast cells, subcutaneously.	27	.....do.....	Very severe symptoms.

HAY BACILLUS.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
869	1 c. c. extract subtilis, subcutaneously.	14	7 c. c. extract subtilis, intraperitoneally.	Slight symptoms.
864	10 c. c. extract subtilis, subcutaneously.	25	6 c. c. extract subtilis, intraperitoneally.	Marked symptoms.

HAY BACILLUS—Continued.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
865	8 c. c. extract subtilis, subcutaneously.	25	6 c. c. extract subtilis, intraperitoneally.	No symptoms.
866	6 c. c. extract subtilis, subcutaneously.	25	.....do	Marked symptoms.
868	2 c. c. extract subtilis, subcutaneously.	25	.....do	Marked symptoms.
870	0.5 c. c. extract subtilis, subcutaneously.	25	.....do	Marked symptoms.
871	0.1 c. c. extract subtilis, subcutaneously.	25	.....do	Slight symptoms.
872	0.01 c. c. extract subtilis, subcutaneously.	25	.....do	Slight symptoms.
873	0.001 c. c. extract subtilis, subcutaneously.	25	.....do	Slight symptoms.

ANTHRAX.

Indications of hypersusceptibility produced by anthrax are scratching, rapid respirations; pigs frequently fall over on their sides and look sick. None of the pigs coughed or had convulsions.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
842	10 c. c. extract of anthrax, subcutaneously.	21	6 c. c. extract anthrax, subcutaneously.	Marked symptoms.
843	5 c. c. extract of anthrax, subcutaneously.	21	.....do	Mild symptoms.
844	1 c. c. extract of anthrax, subcutaneously.	21	.....do	Mild symptoms.
845	.....do	21	.....do	Slight symptoms.
846	.....do	21	.....do	Severe symptoms.
847	.....do	21	.....do	Mild symptoms.
848	0.5 c. c. extract of anthrax, subcutaneously.	21	.....do	Mild symptoms.
849	0.1 c. c. extract of anthrax, subcutaneously.	21	.....do	Slight symptoms.
850	0.01 c. c. extract of anthrax, subcutaneously.	21	.....do	Slight symptoms.
851	0.005 c. c. extract of anthrax, subcutaneously.	21	.....do	Slight symptoms.
852	1 c. c. extract of anthrax, subcutaneously, daily 7 days.	11	.....do	No symptoms.
853	.....do	11	.....do	No symptoms.
854	.....do	11	4 c. c. extract anthrax, subcutaneously.	Mild symptoms.
855	.....do	11	6 c. c. extract anthrax, subcutaneously.	Slight symptoms.
856	.....do	11	.....do	No symptoms.

All the above guinea pigs were subsequently inoculated with a virulent culture of anthrax. They all died in a few days with the usual lesions.

A number of guinea pigs were given the extract from anthrax bacilli before infection; some were given a single injection, some two injections, and others daily injections for twenty days. Other guinea pigs were given the extract used as a vaccine, both in single and repeated injections, after being infected with anthrax bacilli. The extract did not seem to have any influence on the course of the disease, whether given before or after the infection.

TUBERCULOSIS.

The indications of hypersusceptibility induced by extract of tubercle bacilli are restlessness, scratching, irregular respiration, tremor; most of the pigs lie down on their sides and look sick.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
827	0.1 c. c. extract human tubercle bacilli, subcutaneously.	32	6 c. c. extract human tubercle bacilli, subcutaneously.	Mild symptoms.
828	1 c. c. extract human tubercle bacilli, subcutaneously.	32	.....do	No symptoms.
829	2 c. c. extract human tubercle bacilli, subcutaneously.	32	.....do	Mild symptoms.
830	3 c. c. extract human tubercle bacilli, subcutaneously.	32	.....do	Mild symptoms.
831	4 c. c. extract human tubercle bacilli, subcutaneously.	32	.....do	Mild symptoms.
832	5 c. c. extract human tubercle bacilli, subcutaneously.	32	6 c. c. extract human tubercle bacilli, intraperitoneally.	Severe symptoms.
833	6 c. c. extract human tubercle bacilli, subcutaneously.	32	.....do	Slight symptoms.
834	7 c. c. extract human tubercle bacilli, subcutaneously.	32	10 c. c. extract human tubercle bacilli, intraperitoneally.	Slight symptoms.
835	8 c. c. extract human tubercle bacilli, subcutaneously.	32	6 c. c. extract human tubercle bacilli, intraperitoneally.	Mild symptoms.
836	9 c. c. extract human tubercle bacilli, subcutaneously.	32	6 c. c. extract human tubercle bacilli, subcutaneously.	No symptoms.

The guinea pigs which have reacted to two injections of proteid extract obtained from the tubercle bacillus are now being tested for immunity to infection with tubercle cultures.

TYPHOID.

The indications of hypersusceptibility induced by two injections of typhoid extract manifest themselves by rapid respirations; most of the pigs lie down on their sides. The symptoms presented by this series of pigs were mild.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
857	10 c. c. typhoid extract, subcutaneously.	34	10 c. c. typhoid extract, subcutaneously.	Slight symptoms.
858	5 c. c. typhoid extract, subcutaneously.	34	.....do	Slight symptoms.
859	1 c. c. typhoid extract, subcutaneously.	34	.....do	Slight symptoms.
860	.....do	34	6 c. c. typhoid extract, intraperitoneally.	Slight symptoms.
862	0.5 c. c. typhoid extract, subcutaneously.	34	10 c. c. typhoid extract, subcutaneously.	Slight symptoms.
863	0.1 c. c. typhoid extract, subcutaneously.	34	.....do	Slight symptoms.

Nine days following the second injection of the extract, five pigs of the above series, which had received 10 c. c. of the typhoid extract at the second injection, resisted a large dose of a virulent typhoid culture. Two controls died in eighteen hours. One or two of the pigs which had received the extract were slightly sick the following day, but the next day had fully recovered and have remained so. A definite immunity was, therefore, conferred by the two injections of extract from the typhoid bacillus.

TYPHO-LYSIN.

Along somewhat the same lines efforts to obtain the phenomenon of hypersusceptibility with the dissolved typhoid bacilli (natural lysis) failed.

G. P. 480W and 550W:
September 11 to 20, fed dead typhoid culture daily.
September 26 to 28, fed live typhoid culture daily.
December 14 to 28, 1 c.c. bouillon culture of typhoid daily, subcutaneously.
January 15, 10 c.c. heavy old bouillon culture of typhoid, intraperitoneally. Killed 1 hour later. Peritoneal contents collected. Then peritoneal cavity washed with salt solution.

G. P. 100T and 109T:
September 11 to 20, fed dead typhoid bacilli daily.
September 28 to November 9, fed live typhoid bacilli daily.
December 17 to 28, 1 c.c. bouillon culture of typhoid daily, subcutaneously.
February 19, 6 c.c. heavy old bouillon culture of typhoid, intraperitoneally. Killed 1 hour later. Peritoneal contents collected. Then peritoneal cavity washed with salt solution.

Peritoneal contents and saline washings from the peritoneal cavities of the above pigs used as follows:

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
114	0.5 c. c. peritoneal contents of G. P. 480 and 550 containing dissolved typhoid bacilli, subcutaneously.	35	6 c. c. saline washings from peritoneal cavities of 10 pigs (100T to 109T) containing dissolved typhoid bacilli intraperitoneally.	No symptoms.
115	0.75 c. c. peritoneal contents of G. P. 480 and 550 containing dissolved typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
116	.....do	35	.....do	No symptoms.
117	2 c. c. peritoneal contents of G. P. 480 and 550 containing dissolved typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
118	0.5 c. c. saline washings from peritoneal cavities of G. P. 480 and 550, containing dissolved typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
119	1 c. c., saline washings from peritoneal cavities of G. P. 480 and 550, containing dissolved typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
120	1.5 c. c. saline washings from peritoneal cavities of G. P. 480 and 550, containing dissolved typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
121	6 c. c. saline washings from peritoneal cavities of G. P. 480 and 550, containing dissolved typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
111T	0.5 c. c. bouillon culture of typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
112T	1 c. c. bouillon culture of typhoid bacilli, subcutaneously.	35	.....do	No symptoms.
113T	.....do	35	.....do	No symptoms.

Part VI.

COMPARATIVE TOXICITY OF UNTREATED AND REFINED ANTITOXIC SERUM.

It has long been known that diphtheria antitoxin is precipitated from the serum with the globulins, and many attempts have been made to separate the antitoxin from the nonantitoxic substances contained in the serum.

Gibson[a] has evolved a practical method of concentrating and refining diphtheria antitoxic serum. Part of the process consists in placing the one-half saturation of ammonium sulphate precipitate derived from the antitoxic serum in saturated sodium chlorid solution. This dissolves a portion of the globulins with all the antitoxin. In this way the nucleoproteids and insoluble globulins present in the first precipitate are eliminated. The soluble globulins are precipitated by acetic acid, filtered, partially dried, and finally placed in a sack of parchment membrane and dialyzed in running water. This antitoxic solution of soluble globulins is then rendered neutral and sufficient sodium chlorid added to make it isotonic.

Park and Throne[b] find, from a comparative study of 100 cases, that the removal of a considerable portion of the non-antitoxic globulins from the serum by the Gibson method eliminates much of the deleterious matter from the serum, so that severe rashes, joint complications, fever, and other constitutional disturbances are less likely to occur from the antitoxic globulins than from the antitoxic serum from which they were obtained.

We asked ourselves the question whether the precipitated and refined serum is less toxic to sensitized guinea pigs than the untreated serum from which it was made. Doctor Park kindly furnished us some of the precipitated serum and the corresponding untreated serum from which it was made in order to carry out these tests.

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[a] Gibson, R. B. Journ. biolog. chemistry. Vol. 1, Nos. 2 and 3, 1906.

[b] Park, William H., and Throne, Binford: The results of the use of "refined diphtheria antitoxin," Gibson's "globulin preparation," in the treatment of diphtheria. Trans. Assn. Am. Physicians, Vol. 21, 1906, pp. 259–267.

Comparative toxicity of untreated and refined antitoxic serum.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
7491	0.24 c. c. toxine 9+$\frac{1}{180}$ c. c. antitoxic horse serum (PD. 08004), subcutaneously.	25	1 c. c. antitoxic horse serum, Gibson precipitated (NYBH. 305–6), intraperitoneally.	Dead, 25 minutes.
7480	0.24 c. c. toxine 9+$\frac{1}{320}$ c. c. antitoxic horse serum (S. 68D), subcutaneously.	25	.....do	Dead, 30 minutes.
7493	0.24 c. c. toxine 9+$\frac{1}{300}$ c. c. antitoxic horse serum (PD. 08516), subcutaneously.	25	.....do	Dead, 40 minutes.
7489	0.24 c. c. toxine 9+$\frac{1}{120}$ c. c. antitoxic horse serum (PD. 08004), subcutaneously.	25	.....do	Dead, 42 minutes.
7478	0.24 c. c. toxine 9+$\frac{1}{180}$ c. c. antitoxic horse serum (S. 69H), subcutaneously.	25	1 c. c. antitoxic horse serum (NYBH. 305–6), untreated.	Dead, 12 minutes.
7490	0.24 c. c. toxine 9+$\frac{1}{150}$ c. c. antitoxic horse serum (PD. 08004), subcutaneously.	25	.....do	Very severe symptoms.
7477	0.24 c. c. toxine 9+$\frac{1}{150}$ c. c. antitoxic horse serum (S. 69H), subcutaneously.	25	.....do	Very severe symptoms.
7505	0.24 c. c. toxine 9+$\frac{1}{170}$ c. c. antitoxic horse serum (Hb. 27), subcutaneously.	25	2 c. c. antitoxic horse serum, Gibson precipitated (NYBH. 305–6), subcutaneously.	Marked symptoms.
7503	0.24 c. c. toxine 9+$\frac{1}{300}$ c. c. antitoxic horse serum (Hb. 27), subcutaneously.	25	.....do	Severe symptoms.
7502	0.24 c. c. toxine 9+$\frac{1}{230}$ c. c. antitoxic horse serum (Hb. 26), subcutaneously.	25	.....do	Marked symptoms.
7504	0.24 c. c. toxine 9+$\frac{1}{370}$ c. c. antitoxic horse serum (Hb. 26), subcutaneously.	25	2 c. c. antitoxic horse serum (NYBH. 305–6), untreated.	Marked symptoms.
7506	0.24 c. c. toxine 9+$\frac{1}{220}$ c. c. antitoxic horse serum (Hb. 27), subcutaneously.	25	.....do	Severe symptoms.
7507	0.24 c. c. toxine 9+$\frac{1}{270}$ c. c. antitoxic horse serum (Hb. 27), subcutaneously.	25	.....do	Severe symptoms.

Comparative toxicity of untreated and refined antitoxic serum.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
7195	0.142 c. c. toxine 5+$\frac{1}{10}$ c. c. antitoxic horse serum (NYBH. 305), subcutaneously.	41	1 c. c. antitoxic horse serum, Gibson precipitated (NYBH. 305-6), subcutaneously.	Severe symptoms.
7190	0.142 c. c. toxine 5+$\frac{1}{1000}$ c. c. antitoxic horse serum (NYBH. 305), subcutaneously.	41	.....do	Severe symptoms.
7194	0.142 c. c. toxine 5+$\frac{1}{100}$ c. c. antitoxic horse serum (NYBH. 305), subcutaneously.	41	.....do	Very severe symptoms.
7189	0.142 c. c. toxine 5+$\frac{1}{1000}$ c. c. antitoxic horse serum (NYBH. 305), subcutaneously.	41	1 c. c. antitoxic horse serum (NYBH. 305-6), untreated.	Very severe symptoms.
7193	0.142 c. c. toxine 5+$\frac{1}{100}$ c. c. antitoxic horse serum (NYBH. 305), subcutaneously.	41	.....do	Very severe symptoms.
7196	0.142 c. c. toxine 5+$\frac{1}{10}$ c. c. antitoxic horse serum (NYBH. 305), subcutaneously.	41	.....do	Very severe symptoms.

We think it evident from the above that refined antitoxic serum precipitated and dialyzed in accordance with the Gibson method is quite as toxic, bulk for bulk, as the untreated serum from which it has been obtained. We must, however, consider that the treatment to which the serum is subjected in accordance with the Gibson method concentrates its antitoxic power about twice. There is, therefore, a distinct advantage gained, so far as bulk is concerned, in giving a corresponding number of antitoxic units; for, the serum reaction in children depends partly upon the quantity of serum given.

Part VII.

COMPARATIVE TOXICITY OF DIFFERENT HORSE SERUMS.

Besredka and Steinhardt[a] believe that the French horse serums are much less toxic than those used by Otto[b] and the serums used by us. Besredka and Steinhardt had a mortality of about 25 per cent when 5 c. c. of serum was injected intraperitoneally at the second injection, whereas Otto's and our percentage under similar conditions was much higher. Besredka kindly sent us a quantity of "serum antidiphterique" prepared at the Pasteur Institute and this serum was injected into a series of guinea pigs in order to compare its toxicity with the normal horse serum of our roan horse that we have used so much in these experiments.

Toxicity of "serum antidiphterique" (Pasteur Institute).

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
7768	0.142 c. c. toxine 5+$\frac{1}{3\,2\,0}$ c. c. antitoxic horse serum (S. spl. 1351), subcutaneously.	76	5 c. c. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 11 minutes.
7774	0.142 c. c. toxine 5+$\frac{1}{3\,5\,0}$ c. c. antitoxic horse serum (S. spl. 1351), subcutaneously.	76	.....do	Dead, 10 minutes.
7723	0.142 c. c. toxine 5+$\frac{1}{5\,8\,0}$ c. c. antitoxic horse serum (PD. 08022), subcutaneously.	83	.....do	Dead, 15 minutes.
7726	0.142 c. c. toxine 5+$\frac{1}{5\,6\,0}$ c. c. antitoxic horse serum (PD. 08022), subcutaneously.	83	.....do	Dead, 9 minutes.
7849	0.142 c. c. toxine 5+$\frac{1}{2\,9\,0}$ c. c. antitoxic horse serum (A. 192), subcutaneously.	58	.....do	Dead, 19 minutes.
440	0.0006 gm. tetanus toxine A+$\frac{1}{3\,0\,0}$ c. c. antitoxic horse serum (Hoechst), subcutaneously.	56	.....do	Dead, 10 minutes.
451	0.0006 gm. tetanus toxine A+$\frac{1}{3\,0\,0}$ c. c. antitoxic horse serum (Parke), subcutaneously.	56	.....do	Dead, 10 minutes.

[a] Besredka, A., and Steinhardt, Edna: De l'anaphylaxie et de l'anti-anaphylaxie vis-à-vis du sérum de cheval. Ann. de l'Inst. Pasteur, Vol. 21, No. 2, Feb. 25, 1907, pp. 117–127.

[b] Otto, R.: Das Theobald Smithschen Phänomen der Serum-Ueberempfindlichkeit. Leuthold-Gedenkschr., Bd. 1, 1905.

Toxicity of normal horse serum (our roan).

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
7845	0.24 c. c. toxine 9+$\frac{1}{3\,1\,0}$ c. c. antitoxic horse serum (A. 192), subcutaneously.	58	5 c. c. normal horse (roan) serum intraperitoneally.	Dead, 20 minutes.
7850	0.24 c. c. toxine 9+$\frac{1}{2\,0\,0}$ c. c. antitoxic horse serum (A. 142), subcutaneously.	58	.....do	Dead, 18 minutes.
7725	0.24 c. c. toxine 9+$\frac{1}{5\,0\,0}$ c. c. antitoxic horse serum (PD. 08022), subcutaneously.	80	.....do	Dead, 35 minutes.
444	0.0006 gm. tetanus toxine A+$\frac{1}{1\,2\,0\,0}$ c. c. antitoxic horse serum (Hoechst), subcutaneously.	56	.....do	Very severe symptoms.
447	0.0006 gm. tetanus toxine A+$\frac{1}{5\,0\,0}$ c. c. antitoxic horse serum (M. 2122), subcutaneously.	56	.....do	Very severe symptoms.

It is perfectly evident from the above that our results upon the comparative toxicity of the French and American serums do not agree with those reported by Besredka and Steinhardt. With us, the French serums are perhaps somewhat more toxic than our own. We believe these contradictory results are due to other causes than the relative toxicity of the different serums. It is not likely that these differences are due to varying susceptibility of the different breeds of guinea pigs. We have found little difference between guinea pigs obtained from five or six different sources. Further, we have sometimes been struck with the fact that guinea pigs from our own stock and raised under precisely similar conditions show striking differences of degree in the reaction to the second injection. For instance, all the guinea pigs sensitized with toxine-antitoxin mixtures upon a certain date will subsequently prove exceedingly sensitive, and most of them will die at the second injection, whereas another lot of guinea pigs similarly sensitized at another time will prove much less susceptible at the second injection. So far as we are able to judge, this difference of toxicity depends upon something connected with the sensitizing action and not with the variety of horse serum given at the second injection.

NOTE.—When *toxine* is mentioned in the tables diphtheria toxine is meant unless otherwise stated.

Part VIII.

THE IMMUNITY TO HYPERSUSCEPTIBILITY OR "ANTI-ANAPHYLAXIS."

The immunity produced against the toxic action by repeated injections of horse serum has been called anti-anaphylaxis by Besredka and Steinhardt.[a] From our subsequent work we learn that this immunity is relatively not quite as lasting and definite as many instances of active immunity seen in the laboratory against bacterial infections. Guinea pigs that have received a number of prior injections of horse serum may again show symptoms when reinjected with large amounts. The symptoms in such cases are usually mild, and death has never occurred in an "immunized" guinea pig as a result of subsequent injections with horse serum.

G. P. No. 410:

January 15, 1906, 1 c. c. antitoxic horse serum (Natl. VIII, 17), subcutaneously. No symptoms.

January 23, 1906, 1 c. c. antitoxic horse serum (Natl. VIII, 17), subcutaneously. No symptoms.

February 8, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 17), subcutaneously. No symptoms.

February 14, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 17), subcutaneously. No symptoms.

February 23, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 17), subcutaneously. No symptoms.

March 29, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 18), subcutaneously. No symptoms.

April 18, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 19), subcutaneously. No symptoms.

May 16, 1906, 6 c. c. normal horse (roan) serum, subcutaneously. No symptoms.

May 18 to June 27, 1906, normal horse (roan) serum, 1 c. c. daily, subcutaneously. No symptoms.

September 7, 1906, 6 c. c. normal horse (roan) serum, intraperitoneally. No symptoms.

G. P. No. 430:

May 18 to June 27, 1906, 1 c. c. normal horse (roan) serum, daily, subcutaneously. No symptoms.

February 27, 1907, 6 c. c. normal horse (roan) serum, intraperitoneally. Severe symptoms.

G. P. No. 427:

May 18 to June 27, 1906, 1 c. c. normal horse (roan) serum, daily, subcutaneously. No symptoms.

February 27, 1907, 6 c. c. normal horse (roan) serum, intraperitoneally. Severe symptoms.

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[a] Ann. de l'Inst. Pasteur, Vol. 21, No. 2, Feb. 25, 1907, pp. 117–127.

G. P. No. 4426:

January 10, 1906, 0.002 c. c. toxine No. 5, subcutaneously.

January 18, 1906, 6 c. c. normal horse (No. 15) serum, intraperitoneally. No symptom.

February 14, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 17), intraperitoneally. Symptoms (?).

February 23, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 17), intraperitoneally. No symptoms.

March 29, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 17), intraperitoneally. Mild symptoms.

April 18, 1906, 6 c. c. antitoxic horse serum (Natl. VIII, 18), intraperitoneally. No symptoms.

May 16, 1906, 6 c. c. normal horse (roan) serum, intraperitoneally. Severe symptoms.

May 18 to June 27, 1906, 1 c. c. normal horse (roan) serum, intraperitoneally, daily, except Sunday; 22 injections. No symptoms.

January 25, 1907, 6 c. c. normal horse (No. 15) serum, intraperitoneally. No symptoms.

March 26, 1907, 6 c. c. normal horse (roan) serum, intraperitoneally. No symptoms.

For other instances of this variation of susceptibility see Part IX, pages 59 to 62, Bulletin 29, Hygienic Laboratory, United States Public Health and Marine-Hospital Service.

Part IX.

MATERNAL TRANSMISSION OF HYPERSUSCEPTIBILITY AND IMMUNITY.

In our previous work we showed that hypersusceptibility to the toxic effects of horse serum may be transmitted from the mother guinea pig to her young. Later, one of us (Anderson) showed that the female guinea pig may transmit hypersusceptibility to horse serum and immunity to diphtheria toxine at the same time. On account of certain analogies between the reaction to tuberculin and the toxic action of horse serum, we have made further studies along these lines. In this bulletin we shall refer only to our studies upon the transmission of hypersusceptibility and immunity to the toxic action of horse serum, leaving related studies with tuberculosis and tuberculin for a future publication.

Our present studies corroborate the fact that hypersusceptibility to the toxic action of horse serum is always transmitted from the mother guinea pig to her young. This function is solely maternal; the male takes no part whatever in the transmission of these acquired properties. Whether this maternal transmission is hereditary or congenital can not be definitely stated.

We are able to exclude the milk as a factor in transmitting the hypersusceptibility to the toxic action of horse serum by a series of exchange experiments, which are given in detail below.

"Exchange" experiments consist in at once placing guinea pigs born of a susceptible mother to nurse with an untreated female and, in exchange, the young of the untreated female are at the same time placed to nurse with the susceptible female. From these "exchange" experiments we learn that the hypersusceptibility is not transmitted to the young in the milk.

We also learn from our experiments that hypersusceptibility may be transmitted from mother to young whether the mother is sensitized before or after conception. The fact that this influence may take place after conception might be taken to indicate that the transmission is congenital and not hereditary.

GROUP A.

FAMILY NO. 1.

(Sensitized female; untreated male.)

Female (G. P. No. 610). October 20, 1906. Six c. c. antitoxic horse serum. (Natl. IX, 17) intraperitoneally. Dead, 30 minutes.

[Previous treatment: 151 days prior, 0.15 c. c. toxine No. 9+$\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (G. P. No. 102). June 8, 1906. Untreated. Put in cage with above female after female was sensitized.

Offspring. August 24, 1906. Four young born. Two tested as follows:

G. P. No. 610a. October 20. 59 days old. Three c. c. antitoxic horse serum (Natl. IX, 17) intraperitoneally. Dead, 4 minutes.

(G. P. No. 610b.) Ditto. Dead, 60 minutes.

FAMILY NO. 2.

(Sensitized female; untreated male.)

Female (G. P. No. 612). January 25, 1907. Six c. c. normal horse serum (horse No. 15) intraperitoneally. Very severe symptoms.

[Previous treatment: 245 days prior, 0.15 c. c. toxine $9+\frac{1}{2}\frac{1}{5}\frac{1}{0}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (untreated). June 8, 1906. Placed in cage with above female after she was sensitized.

Offspring. One young born January 10, 1907, and tested as follows:

(G. P. No. 612a.) January 25. 15 days old. Two c. c. horse serum (horse No. 15) intraperitoneally. Marked symptoms.

FAMILY NO. 3.

(Sensitized female; untreated male.)

Female (G. P. No. 611). September 7, 1906. Six c. c. normal horse serum (roan) intraperitoneally. Dead, 120 minutes.

[Previous treatment: 106 days prior, received 0.15 c. c. toxine $9+\frac{1}{2}\frac{1}{5}\frac{1}{0}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (untreated). Paired 17 days after treatment of female.

Offspring. One young. Tested as follows:

(G. P. No. 611a.) September 7, 1906. 2 days old. One c. c. normal horse serum (roan) intraperitoneally. Dead, 20 minutes.

FAMILY NO. 4.

(Sensitized female; untreated male. Exchange.)

Female (G. P. No. 613). February 27, 1907. Six c. c. normal horse (roan) serum intraperitoneally. Very severe symptoms.

[Previous treatment: 282 days prior 0.15 c. c. toxine $9+\frac{1}{2}\frac{1}{5}\frac{1}{0}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (G. P. No. 10x). June 8, 1906. Untreated. Put into cage 18 days after female was sensitized.

Offspring. January 22, 1907. Two young born and at once put to nurse with untreated female. Tested as follows:

(G. P. No. 613a.) February 27, 1907. 36 days old. Two c. c. normal (roan) horse serum intraperitoneally. Very severe symptoms.

(G. P. No. 613b.) Ditto. Dead, 45 minutes.

Two young, born of untreated female, put to nurse with above sensitized female (No. 613). Tested as follows:

(G. P. No. P-68a.) February 27, 1907. 36 days old. Two c. c. normal horse (roan) serum intraperitoneally. No symptoms.

(G. P. No. P-68b.) Ditto. No symptoms.

FAMILY NO. 5.

(Sensitized female; untreated male. Exchange.)

Female (G. P. No. 614). March 26, 1907. Six c. c. normal horse (roan) serum intraperitoneally. Dead, 38 minutes.

[Previous treatment; 309 days prior, 0.15 c. c. toxine No. 9+$\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (G. P. No. 10). June 8, 1906. Untreated. Put in cage after female was sensitized.

Offspring. February 17, 1907. Two young born, at once put to nurse with untreated female, and tested as follows:

(G. P. No. 614a). March 26, 1907. 38 days old. Two c. c. normal horse (roan) serum intraperitoneally. Dead, 25 minutes.

(G. P. No. 614b). Ditto. Dead, 20 minutes.

Two young, born of untreated female and nursed with above sensitized female (614), tested as follows:

(G. P. No. P–20). March 26, 1907. 38 days old. Two c. c. normal horse (roan) serum intraperitoneally. No symptoms.

(G. P. No. P–20a). Ditto. No symptoms.

GROUP B.

FAMILY NO. 6.

(Sensitized female; sensitized male. Exchange.)

Female (G. P. No. 601). October 23, 1906. Six c. c. antitoxic horse serum (Natl. IX, 18) intraperitoneally. Dead, 15 minutes.

[Previous treatment: 153 days prior, 0.15 c. c. toxine No. 9 + $\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (G. P. No. 606x). Paired after both were sensitized. September 7, 1906. Six c. c. normal horse (roan) serum intraperitoneally. Dead, 17 minutes.

[Previous treatment: 106 days prior, 0.15 c. c. toxine No. 9 + $\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Offspring. Two young, born August 28, 1906. Immediately nursed with an untreated female.

(G. P. No. 601a.) October 23, 1906. 56 days old. Three c. c. antitoxic horse serum (Natl. IX, 18) intraperitoneally. Severe symptoms.

(G. P. No. 601b.) Ditto. Severe symptoms.

Three untreated young pigs placed to nurse with the above mother (No. 601).

(G. P. No. X.) October 23. 56 days old. Three c. c. antitoxic horse serum intraperitoneally. No symptoms.

(G. P. No. Y.) Ditto. No symptoms.

(G. P. No. Z.) Ditto. No symptoms.

(G. P. mother of X, Y, and Z.) October 23. Six c. c. same serum intraperitoneally. No symptoms.

FAMILY NO. 7.

(Sensitized female; sensitized male. Exchange.)

Female (G. P. No. 603). February 27, 1907. Six c. c. normal horse (roan) serum intraperitoneally. Dead, 45 minutes.

[Previous treatment: 282 days prior, 0.15 c. c. toxine No. 9+$\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Male (G. P. No. 606y). June 8, 1906. Placed in cage with above female after both were sensitized.

Offspring. January 17, 1907. Two young born, nursed with untreated female, and tested as follows:

(G. P. No. 603a.) February 27, 1907. 41 days old. Two c. c. normal horse (roan) serum intraperitoneally. Marked symptoms.

(G. P. No. 603b.) Ditto. Very severe symptoms.

Three young, born of untreated female and nursed with above sensitized female (603), tested as follows:

(G. P. No. 66a.) February 27, 1907. 41 days old. Two c. c. normal horse (roan) serum intraperitoneally. No symptoms.

(G. P. No. 66b.) Ditto. No symptoms.

(G. P. No. 66c.) Ditto. No symptoms.

GROUP C.

FAMILY NO. 8.

(Immune female; immune male.)

Female (G. P. No. 428). January 25, 1907. Six c. c. normal horse serum (horse No. 15) intraperitoneally. Slight symptoms.

[Previous treatment: May 18 to June 27, 1906, 1 c. c. normal horse (roan) serum daily (except Sunday), subcutaneously, 22 injections.]

Male (G. P. No. 4530). June 8, 1906. Immune. Placed in cage with above female. For history of this male see Family No. 14.

Offsping. December 28, 1906. Two young born and nursed with own mother. Tested as follows:

(G. P. No. 428a.) January 25, 1907. 28 days old. Two c. c. normal horse serum (horse No. 15) intraperitoneally. Marked symptoms.

(G. P. No. 428b.) Ditto. Marked symptoms.

FAMILY NO. 9.

(Immune female; immune male. Exchange.)

Female (G. P. No. 4426). January 10, 1906. 0.002 c. c. toxine 7. Severe reaction, slough.

January 18, 1906. Six c. c. normal horse serum (horse No. 15) intraperitoneally. No symptoms.

February 14, 1906. Six c. c. antitoxic horse serum (Natl. VIII, 17) intraperitoneally. Symptoms.

February 23, 1906. Ditto. No symptoms.

March 29, 1906. Ditto. Mild symptoms.

April 18, 1906. Ditto (Natl. VIII, 18). No symptoms.

May 16, 1906. Six c. c. normal (roan) horse serum intraperitoneally. Severe symptoms.

May 18 to June 27, 1906. One c. c. normal horse (roan) serum subcutaneously daily (except Sunday), 22 injections. No symptoms.

January 25, 1907. Six c. c. normal (No. 15). No symptoms.

March 26, 1907. Six c. c. normal (roan). No symptoms.

Male (G. P. No. 4530). June 8, 1906. Immune. Placed in cage with above female.

Previous treatment:

January 12, 1906. 0.19 c. c. toxine 7 + 1 unit antitoxic horse serum (B27).

January 22, 1906. Six c. c. antitoxic horse serum (Natl. VIII, 17) intraperitoneally. Definite symptoms.

February 6, 1906. Ditto. Symptoms.

February 8, 1906. Ditto. No symptoms.

February 14, 1906. Ditto. No symptoms.

February 23, 1906. Ditto. No symptoms.

March 25, 1906. Ditto (Natl. VIII, 18). No symptoms.

April 18, 1906. Ditto. Ditto. No symptoms.

May 16, 1906. Six c. c. normal horse (roan) serum intraperitoneally. No symptoms.

May 18 to June 27, 1906. One c. c. same serum subcutaneously daily (except Sunday), 22 injections. No symptoms.

January 25, 1907. Six c. c. normal horse serum (horse No. 15). Very slight symptoms.

Offspring, first litter. One young born December 26, 1906. As soon as born, placed to nurse with untreated female (pen 120), whose young were placed to nurse with G. P. No. 4426.

(G. P. No. 4426a.) January 25, 1907. Two c. c. normal horse (No. 15) serum intraperitoneally, when 30 days old. No symptoms.

Young of untreated female P. 120.

(G. P. No. P. 120a.) January 25, 1907. 35 days old. Two c. c. normal horse (No. 15) serum intraperitoneally. No symptoms.

February 18, 1907. 24 days later. Six c. c. same serum. Very severe symptoms.

(G. P. No. P. 120b.) January 25, 1907. Same as G. P. No .P. 120a. No. symptoms.

February 18, 1907. Ditto. Dead, 70 minutes.

Second litter. Three young born March 1, 1907, and not exchanged.

(G. P. No. 4426b.) March 26, 1907. 25 days old. Two c. c. normal horse (roan) serum intraperitoneally. No symptoms.

(G. P. No. 4426c.) Same treatment. No symptoms.

(G. P. No. 4426d.) Same treatment. No symptoms.

These three pigs were again tested April 18 with 4 c. c. of normal horse (roan) serum into the peritoneal cavity. All three showed slight symptoms, thus proving that immunity, or "antianaphylaxis," was not transmitted from the mother.

FAMILY NO. 10.

(Immune female; immune male. Exchange.)

Female (G. P. No. 429). January 25, 1907. Six c. c. normal horse (No. 15) serum intraperitoneally. Slight symptoms.

[Previous treatment: May 18 to June 27, 1906, 1 c. c. normal horse (roan) serum daily except Sunday, 22 injections.]

Male (G. P. No. 4530). June 8, 1906. Placed in cage with female.

For history of this male, see Family No. 14.

Offspring. Three young, born December 29, 1906, and nursed with untreated G. P. No. P. 50. Tested as follows:

(G. P. No. 429a.) January 25, 1907. 27 days old. Two c. c. normal horse serum (horse No. 15) intraperitoneally. Marked symptoms.

(G. P. No. 429b.) Ditto. Marked symptoms.

(G. P. No. 429c.) Ditto. Marked symptoms.

Two young of untreated G. P. No. P. 50 nursed with immune female No. 429, and tested as follows:

(G. P. No. P. 50a.) January 25, 1907. 27 days old. Two c. c. normal horse serum (horse No. 15) intraperitoneally. No symptoms.

(G. P. No. P. 50b.) Ditto. No symptoms.

GROUP D.

FAMILY NO. 11.

(Immune female; untreated male.)

Female (G. P. No. 410). January 15, 1906. One c. c. antitoxic horse serum (Natl. VIII, 17). No symptoms.

January 23, 1906. Ditto. No symptoms.

February 8, 1906. Six c. c. same serum. No symptoms.

February 14, 1906. Ditto. No symptoms.

February 23, 1906. Ditto. No symptoms.

March 29, 1906. Six c. c. antitoxic horse serum (Natl. VIII, 18). No symptoms.

April 18, 1906. Ditto. No symptoms.

May 16, 1906. Six c. c. normal horse (roan) serum. No symptoms.

May 18 to June 27, 1906, daily. One c. c. same serum. No symptoms.

September 7, 1906. Six c. c. normal horse (roan) serum intraperitoneally. No symptoms.

All injections subcutaneously.

Male (G. P. No. 410m). June 8, 1906. Untreated. Put in cage after female was immunized.

Offspring. July 20, 1906. Three young born, and tested as follows:

(G. P. No. 410a.) September 7, 1906. 18 days old. Two c. c. normal horse (roan) serum intraperitoneally. No symptoms.

(G. P. No. 410b.) Ditto. No symptoms.

(G. P. No. 410c.) Ditto. No symptoms.

FAMILY NO. 12.

(Immune female; untreated male.)

Female (G. P. No. 430). February 27, 1907. Six c. c. normal horse (roan) serum intraperitoneally. Severe symptoms.

[Previous treatment: 245 days prior (May 18 to June 27). One c. c. normal horse (roan) serum subcutaneously daily.]

Male (G. P. No. —). June 8, 1906. Untreated. Put in cage during period of immunization.

Offspring. January 22, 1907. Two young born and nursed with mother. Tested as follows:

(G. P. No. 430a). February 27, 1907. 36 days old. Two c. c. normal horse (roan) serum intraperitoneally. Marked symptoms.

(G. P. No. 430b.) Ditto. Marked symptoms.

(G. P. No. 430c.) Ditto. Mild symptoms.

FAMILY NO. 13.

(Immune female; untreated male.)

Female (G. P. No. 110). February 8 to 20, 1906. One c. c. Daily subcutaneous injections 1 c. c. antitoxic horse serum (Natl. IX, 19). No symptoms.

March 29, 1906. Six c. c. antitoxic horse serum (Natl. VIII, 18). Mild symptoms.

April 4 to 17, 1906. One c. c. daily injections antitoxic horse serum (Natl. IX, 17). No symptoms.

April 23, 1906. Six c. c. normal horse (roan) serum. Very slight symptoms.

May 15, 1906. Ditto. Very slight symptoms.

May 18 to June 27, 1906. One c. c. daily, same serum. No symptoms.

September 7, 1906. Six c. c. normal horse (roan) serum, intraperitoneally. Very slight symptoms.

Male (G. P. No. —). June 8, 1906. Untreated. Put in cage after immunization of female was well advanced.

Offspring. August 10, 1906. One young born, and tested as follows:

(G. P. No. 110a.) September 7, 1906. 27 days old. Two c. c. normal horse (roan) serum intraperitoneally. Very slight symptoms.

FAMILY NO. 14.

(Immune female; untreated male.)

Female (G. P. No. 426). September 7, 1906. Six c. c. normal horse (roan) serum, intraperitoneally. Slight symptoms.

[Previous treatment: May 19 to June 27, 1906, 1 c. c. daily subcutaneous injections of normal horse (roan) serum.]

Male (G. P. No. 426m). June 8, 1906. Untreated. Put in cage during period of immunization of female.

Offspring. July 24, 1906. Two young born, and tested as follows:

(G. P. No. 426a.) September 7, 1906. 41 days old. Two c. c. normal horse (roan) serum, intraperitoneally. Slight symptoms.

(G. P. No. 426b.) Ditto. Slight symptoms.

FAMILY NO. 15.

(Immune female; untreated male. Exchange.)

Female (G. P. No. 427). February 27, 1907. Six c. c. normal horse (roan) serum intraperitoneally. Severe symptoms.

[Previous treatment: 245 days prior (May 18 to June 26) 1 c. c. normal horse (roan) serum subcutaneously daily.] No symptoms.

Male (G. P. No. 42601). June 8, 1906. Normal. Put into cage during period of immunization of female.

Offspring. January 22, 1907. Two young born. At once placed to nurse with untreated female. Tested as follows:

(G. P. No. 427a.) February 27, 1907. 36 days old. Three c. c. normal horse (roan) serum intraperitoneally. Severe symptoms.

(G. P. No. 427b.) Ditto. Dead, 62 minutes.

Two young born of untreated female, but nursed with above female No. 427, tested as follows:

(G. P. No. P. 108a.) February 27, 1907. 36 days old. Three c. c. normal horse (roan) serum intraperitoneally. No symptoms.

(G. P. No. P. 108b.) Ditto. No symptoms.

GROUP E.

FAMILY NO. 16.

(Untreated female; sensitized male.)

Female (G. P. No. 606). September 7, 1906. Six c. c. horse serum intraperitoneally. No prior treatment. No symptoms.

Male (G. P. No. 606x). September 9, 1906. Six c. c. horse serum (roan) intraperitoneally. Dead, 17 minutes.

[Previous treatment: 106 days prior received 0.15 c. c. tox. $9+\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII, 18).]

Offspring. Two young born August 4, 1906, and tested as follows:

(G. P. No. 606a.) September 9, 1906. 1 month old. Two c. c. normal horse serum into peritoneum. No symptoms.

(G. P. No. 606b.) September 9, 1906. 1 month old. Two c. c. normal horse serum (roan) into peritoneum. No symptoms.

FAMILY NO. 17.

(Untreated female; sensitized male.)

Females (G. P. No. 609). January 25, 1907. Six c. c. normal horse serum (horse No. 15), intraperitoneally. No prior treatment. No symptoms.

(G. P. No. 607). Ditto. No prior treatment. No symptoms.

Male (G. P. No. 4527). January 25, 1907. Six c. c. normal horse serum (horse No. 15), intraperitoneally. Dead, 37 minutes.

[Previous treatment: 1 year 13 days prior, 0.19 c. c. toxine No. 7+1 umt B27.]

June 8, 1906. Placed with untreated females Nos. 609 and 607.

Offspring. December 26, 1906. Three young born to G. P. No. 609. September 6, 1906. One young born to G. P. No. 607.

Young tested as follows:

(G. P. No. 609a). January 25, 1907. 30 days old. Two c. c. normal horse serum (horse No. 15) intraperitoneally. No symptoms.

(G. P. No. 609b). Ditto. No symptoms.

(G. P. No. 609c). Ditto. No symptoms.

(G. P. No. 607a). Ditto (141 days old). No symptoms.

We conclude from these experiments that—

1. Hypersusceptibility to the toxic action of horse serum is transmitted through the female guinea pig; the male has no influence.

2. The susceptibility is not transmitted through the milk.

3. Maternal transmission of hypersusceptibility succeeds, whether the female guinea pig is sensitized before or after conception.

Part X.

THE RELATION OF HYPERSUSCEPTIBILITY TO VARIOUS INFLUENCES.

We have already shown that hypersusceptibility to the action of horse serum in the guinea pig has no evident relation to hemolysis or precipitins. We offer the following experiments, planned with the object of correlating hypersusceptibility with other phenomena:

RELATION TO AGGRESSINES.

The work of Bail upon aggressine induced us to try whether a similar action may explain hypersusceptibility. The following experiments indicate that no relation exists between the two phenomena.

PERITONEAL FLUID FROM NORMAL GUINEA PIG INTO SENSITIZED GUINEA PIG.

Eight c. c. of normal horse (No. 15) serum was injected into the peritoneal cavity of a normal guinea pig. This produced no symptom. Two hours later the animal was chloroformed and about 6 c. c. of fluid was taken from the peritoneal cavity and injected into the following sensitized guinea pig:

G. P. No. 7050. Six c. c. of above fluid into peritoneal cavity. Dead, 20 minutes.
[Previous treatment: 57 days prior, 0.142 c. c. toxine No. $5+\frac{1}{200}$ c. c. antitoxic horse serum (S. 063H), subcutaneously.]

PERITONEAL FLUID FROM SENSITIZED GUINEA PIG INTO NORMAL GUINEA PIG.

Six c. c. of normal horse (No. 15) serum was injected into the peritoneal cavity of G. P. No. 7051, which had been sensitized fifty-seven days previously with 0.142 c. c. toxine No. $5+\frac{1}{200}$ c. c. axtitoxic horse serum (S. 063H). The guinea pig developed typical symptoms and died in fifteen minutes as a result of the second injection. Fifteen minutes after the death of this guinea pig about 4 c. c. of the peritoneal contents were withdrawn and injected into the peritoneal cavity of a normal guinea pig. This caused no symptoms.

PERITONEAL FLUID FROM SENSITIZED GUINEA PIG INTO SENSITIZED GUINEA PIG.

Six c. c. normal horse (No. 15) serum was injected into the peritoneal cavity of a sensitized guinea pig (No. 7068) which had received fifty-five days previously a subcutaneous injection of 0.142 c. c. toxine No. $5+\frac{1}{100}$ c. c. antitoxic horse serum (Natl. V, 7). As a result of the second injection of horse serum the guinea pig had characteristic symptoms and died in thirty minutes. Fifteen minutes after the

death of this guinea pig about 3 c. c. of the peritoneal contents were collected and injected into the following sensitized guinea pig:

G. P. No. 7026. Three c. c. of above fluid into peritoneal cavity. Very severe symptoms.

[Previous treatment: 57 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{2}\frac{1}{0}\frac{1}{0}$ c. c. antitoxic horse serum (Ld. 8), subcutaneously.]

PERITONEAL FLUID FROM SENSITIZED GUINEA PIGS INTO NORMAL GUINEA PIGS.

The peritoneal contents were collected immediately after the death from 9 sensitized guinea pigs that had received 6 c. c. normal horse serum (No. 15) each; 3.5 c. c. of this fluid was injected into the peritoneal cavity of a normal guinea pig, but produced no symptoms.

After withdrawing the fluid of the peritoneal cavities of the above 9 guinea pigs the peritoneal cavities were washed with sterile salt solution and this fluid injected into the following normal guinea pigs:

G. P. No. A.—6 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

B.—5 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

C.—7.5 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

D.—10 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

E.—20 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

F.—6 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

G.—6 c. c. saline washings from above 9 guinea pigs, intraperitoneally. No symptoms.

RELATION TO METHEMAGLOBIN POISONING.

The symptoms in the guinea pig somewhat resemble methemaglobin poisoning. We are indebted to Assistant Surgeon A. M. Stimson for comparative spectroscopic studies of the blood of normal guinea pigs and of the blood of susceptible guinea pigs immediately after death caused by a second injection of horse serum. No methemaglobin was found. Only the bands corresponding to oxyhemaglobin were seen in the blood of the guinea pigs examined immediately after death.

OXYGEN HAS NO INFLUENCE UPON THE SYMPTOMS.

A sensitized guinea pig was inoculated with horse serum and at once placed in an almost pure atmosphere of oxygen. Another sensitized guinea pig (not reinoculated) was placed under the same bell jar as a control. The inoculated pig developed symptoms and was dead in thirty-five minutes. The control animal showed no unusual manifestations after thirty minutes in the atmosphere of oxygen. It was then given an injection of horse serum and immediately replaced under the

bell jar. It developed characteristic symptoms and died in fifteen minutes.

G. P. No. 7028. Six c. c. antitoxic horse serum (Natl. IX, 17), intraperitoneally; immediately placed in an atmosphere of almost pure oxygen. Dead, 35 minntes.

[Previous treatment: 54 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{4}\frac{1}{0}$ c. c. antitoxic horse serum (Led. 8).]

Control G. P. Kept 30 minutes in same atmosphere. No symptoms.

[Previous treatment: 52 days prior, 0.142 c. c. toxine No. 5+$\frac{1}{2}\frac{1}{0}$ c. c. antitoxic horse serum (PDCo. 07555A).]

At the end of 30 minutes in oxygen atmosphere, given 6 c. c. antitoxic horse serum (Natl. IX, 17), intraperitoneally. Dead, 15 minutes.

THE INFLUENCE OF DIPHTHERIA TOXINE UPON HYPERSUSCEPTIBILITY.

The question was raised by both Otto and us as to the influence of the diphtheria toxine in accentuating the phenomenon of hypersusceptibility.

While guinea pigs may be sensitized with fresh normal horse serum alone, it seemed to us and also to Otto that a greater degree of hypersusceptibility is produced when sensitized with a mixture of diphtheria toxine and antitoxic horse serum than when the horse serum alone is given at the first injection. It seems, however, that this is by no means always the case.

All the guinea pigs in the following series were sensitized with $\frac{1}{2}\frac{1}{0}$ c. c. of antitoxic horse serum (Alex. A., 228) precipitated and dialyzed according to Gibson's method. One-half the animals received, in addition, 0.2 c. c. toxine No. 7 (MLD = 0.006). After thirty-one days interval all the guinea pigs were tested with 3 c. c. normal horse serum (roan) into the peritoneal cavity.

[All tested by injecting 3 c. c. normal horse (No. 15) serum intraperitoneally.]

No. G. P.	Previous treatment, 31 days prior.	Result.
720	$\frac{1}{2}\frac{1}{0}$ c. c. precipitated antitoxic horse serum (Alex. A., 228), subcutaneously.	Dead, 28 minutes.
721	.....do.....	Dead, 12 minutes.
722	.....do.....	Very severe symptoms.
723	.....do.....	Dead, 44 minutes.
724	.....do.....	Dead, 10 minutes.
725	.....do.....	Very severe symptoms.
726	.....do.....	Very severe symptoms.
727	.....do.....	Very severe symptoms.
728	.....do.....	Very severe symptoms.
729	.....do.....	Very severe symptoms.
710	Ditto + 0.2 c. c. toxine No. 7.....	Dead, 10 minutes.
711	.....do.....	Very severe symptoms.
712	.....do.....	Dead, 10 minutes.
713	.....do.....	Dead, 43 minutes.
714	.....do.....	Dead, 31 minutes.
715	.....do.....	Very severe symptoms.
716	.....do.....	Dead, 27 minutes.
717	.....do.....	Severe symptoms.
718	.....do.....	Very severe symptoms.
719	.....do.....	Dead, 92 minutes.

THE INFLUENCE OF TETANUS TOXINE UPON HYPERSUSCEPTIBILITY.

Besredka and Steinhart[a] intimate that guinea pigs sensitized with a mixture of tetanus toxine and antitetanic serum are not sensitive to subsequent injections of horse serum. These scientists, however, suggest that their failures on this point may have been due to the small amount of horse serum used at the first injection, viz, $\frac{1}{10000}$ and $\frac{1}{100000}$ c. c.

We tested some of our used tetanus guinea pigs to determine this point and found that tetanus toxine does not apparently influence the phenomenon of hypersusceptibility to horse serum. The guinea pigs were sensitized with 0.0006 gm. of a dried tetanus toxine, which represents 100 minimal lethal doses, plus various amounts of antitetanic serum. All those tested reacted to a second injection of horse serum.

Tetanus toxine.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
438	0.0006 gm. tetanus toxine A + $\frac{1}{500}$ c. c. antitoxic horse serum (Hoechst) subcutaneously.	17	6 c. c. normal horse (roan) serum, intraperitoneally.	Dead, 30 minutes.
439	0.0006 gm. tetanus toxine A + $\frac{1}{700}$ c. c. antitoxic horse serum (Hoechst) subcutaneously.	17	.....do	Dead, 30 minutes.
444	0.0006 gm. tetanus toxine A + $\frac{1}{1200}$ c. c. antitoxic horse serum (Hoechst) subcutaneously.	28	.....do	Very severe symptoms.
447	0.0006 gm. tetanus toxine A + $\frac{1}{500}$ (M 2122) c. c. antitoxic horse serum subcutaneously.	28	.....do	Very severe symptoms.
451	0.0006 gm. tetanus toxine A + $\frac{1}{500}$ (Pk) c. c. antitoxic horse serum subcutaneously.	28	6 c. c. normal horse (roan) serum, intraperitoneally (Pasteur Institute).	Dead, 10 minutes.
440	0.0006 gm. tetanus toxine A + $\frac{1}{800}$ (Hoechst) c. c. antitoxic horse serum subcutaneously.	28	.....do	Dead, 10 minutes.

THE RELATION OF THE SPLEEN AND THYROID TO HYPERSUSCEPTIBILITY.

While we believe that this reaction is probably localized in the central nervous system, several experiments were undertaken to determine what influence the spleen or the thyroid gland may have upon the hypersusceptibility produced by the injection of horse serum.

[a] Ann. Pasteur Inst., Vol. 21, No. 2, 1907, pp. 117–127.

The removal of the thyroid gland or the spleen either before or after an injection of horse serum does not prevent the phenomenon of hypersusceptibility.

It is of interest to note that the guinea pigs upon which splenectomy was performed lost much hair and became reduced in weight, although the appetite seemed to remain good.

THYROIDECTOMY.

G. P. No. 7017. 0.142 c. c. toxine No. 5+$\frac{1}{100}$ c. c. antitoxic horse serum (Mem. A1103), subcutaneously.

55 days later, thyroid removed.

80 days from sensitizing inoculation and 25 days after thyroidectomy, given 6 c. c. normal horse (No. 15) serum, subcutaneously. Dead, 10 minutes.

G. P. No. 7018. O. 142 c. c. toxine No. 5+$\frac{1}{250}$ c. c. antitoxic horse serum (Mem. 1103).

55 days later, thyroid removed.

125 days from sensitizing inoculation and 70 days after thyroidectomy, given 6 c. c. normal horse (No. 15) serum, intraperitoneally. Dead, 30 minutes.

G. P. No. X. Untreated. Thyroid removed.

11 days after thyroidectomy, given 0.15 c. c. toxine No. 9 + $\frac{1}{300}$ c. c. antitoxic horse serum (PD., 08516), subcutaneously.

17 days after sensitizing inoculation and 28 days after thyroidectomy, given 6 c. c. normal horse (No. 15) serum, intraperitoneally. Dead, 28 minutes.

G. P. No. 425W. Untreated. Thyroid removed.

11 days later, 0.15 c. c. toxine No. 9+$\frac{1}{300}$ c. c. antitoxic horse serum (PD .08516).

69 days after sensitizing inoculation and 58 days after thyroidectomy, 6 c. c. normal horse (No. 15) serum, intraperitoneally. Dead, 5 minutes.

SPLENECTOMY.

G. P. No. 7044. 0.142 c. c. toxine No. 5+$\frac{1}{250}$ c. c. antitoxic horse serum (Hb. 21A), subcutaneously.

55 days later, spleen removed.

80 days from sensitizing inoculation and 25 days after splenectomy, 6 c. c. normal horse (No. 15) serum, intraperitoneally. Very severe symptoms.

G. P. No. 7024. 0.142 c. c. toxine No. 5+$\frac{1}{250}$ c. c. antitoxic horse serum (Mem 1103), subcutaneously.

55 days later, spleen removed.

80 days from sensitizing inoculation and 25 days after splenectomy, 6 c. c. normal horse (No. 15) serum, intraperitoneally. Very severe symptoms.

G. P. No. 380W. Spleen removed.

69 days later, 6 c. c. normal horse (No. 15) serum, intraperitoneally. No symptoms.

G. P. No. XY. Spleen removed.

11 days later, 0.15 c. c. toxine No. 9+$\frac{1}{300}$ c. c. antitoxic horse serum (PD. 08516), subcutaneously.

28 days after splenectomy and 17 days after sensitizing, 6 c. c. normal horse (No. 15) serum, intraperitoneally. Severe symptoms.

G. P. No. 410W. Spleen removed.

11 days later, 0.15 c. c. toxine No. 9+$\frac{1}{300}$ c. c. antitoxic horse serum (PD. 08516), subcutaneously.

58 days after sensitizing and 69 days after splenectomy, 6 c. c. normal horse (No. 15) serum, intraperitoneally. Marked symptoms.

Part XI.

MISCELLANEOUS.

FEEDING EXPERIMENTS WITH COOKED MEAT.

In our former work we showed that guinea pigs may be sensitized by feeding them blood serum or meat. We know that heating blood serum to 100° C. for fifteen minutes is sufficient to destroy its toxic action. We then asked ourselves the question, Would the heating of meat prevent the sensitizing action? The following experiments indicate that heat does destroy this property of meat, as far as the guinea pig is concerned.

In these experiments well-cooked horse meat was used. It was heated in the hot air sterilizer at 110° C. for thirty minutes. Two grams of it was fed to the animals daily from June 15 to June 30. Each guinea pig, therefore, received 32 gm. of the cooked meat. None of them showed symptoms when injected eighteen days later with 6 c. c. of normal horse serum.

No. G. P.	First treatment.	Interval.	Second treatment.	Result.
		Days.		
496	Fed 2 gm. horse meat, heated to 110° C. (hot air) 30 minutes, daily for 16 days.	18	6 c. c. normal horse (roan) serum, intraperitoneally.	No symptoms.
497	.....do.....	18	.....do.....	No symptoms.
498	.....do.....	18	.....do.....	No symptoms.
499	.....do.....	18	.....do.....	No symptoms.
500	.....do.....	18	.....do.....	No symptoms.

FEEDING EXPERIMENTS WITH RAW BEEF.

We know that guinea pigs fed with horse serum or horse meat are susceptible to subsequent injections of horse serum, and we are now able to show, by the following series of experiments, that guinea pigs fed with beef are susceptible to subsequent injections of cattle serum:

No. G. P.	First treatment.	Interval.	Second treatment.	Result.
		Days.		
501	Fed 2 gm. dried beef daily for 23 days.	19	6 c. c. cattle serum, intraperitoneally.	Mild symptoms.
502	.....do.....	19	.....do.....	Mild symptoms.
503	.....do.....	19	.....do.....	Severe symptons.

RESULT OF CARDIAC INJECTIONS.

We have shown that guinea pigs may be readily sensitized by subcutaneous or intraperitoneal inoculations, and that the second injection produces symptoms when the serum is injected either under the skin or into the peritoneal cavity.

Besredka and Steinhardt have shown that the injection of serum into the brain of a sensitized guinea pig is very poisonous. We are able to confirm this observation. We then asked ourselves the question, Can guinea pigs be sensitized by injecting the horse serum directly into the heart? And can sensitized guinea pigs be poisoned by such injections directly into the circulation? We are now enabled to answer these questions affirmatively, in view of the following experiments:

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
7077	0.142 c. c. toxine 5+$\frac{1}{2}\frac{1}{5}\frac{1}{0}$ c. c. antitoxic horse serum (NYBH. 17), subcutaneously.	131	1.5 c. c. normal horse (roan) serum, into heart.	Dead, 3 minutes.
7692	0.142 c. c. toxine 5+$\frac{1}{4}\frac{1}{4}\frac{1}{0}$ c. c. antitoxic horse serum (A. 247), subcutaneously.	35	0.01 c. c. normal horse (roan) serum, into heart.	Dead, 55 minutes.
7630	0.142 c. c. toxine 5+$\frac{1}{3}\frac{1}{0}\frac{1}{0}$ c. c. antitoxic horse serum (A. ppt. 31), subcutaneously.	44	1 c. c. normal horse (roan) serum, into heart.	Dead, 3½ minutes.
7632	0.142 c. c. toxine 5+$\frac{1}{2}\frac{1}{5}\frac{1}{0}$ c. c. antitoxic horse serum (A. ppt. 31), subcutaneously.	44	.....do	Dead, 3 minutes.
7691	0.142 c. c. toxine 5+$\frac{1}{4}\frac{1}{4}\frac{1}{0}$ c. c. antitoxic horse serum (A. 247), subcutaneously.	35	.....do	Dead, 3 minutes.
Control.	2 c. c. normal horse (roan) serum, into heart.	.....	.....	No symptoms.
	Same guinea pig	20 days later.	6 c. c. normal horse (roan) serum, intraperitoneally.	Marked symptoms.

These experiments indicate that the endothelial cells lining the peritoneal cavity or the connective cells of the subcutaneous tissue do not necessarily play a rôle in the phenomenon we are studying.

THE GUINEA PIG REMAINS SUSCEPTIBLE A VERY LONG TIME.

That the guinea pig remains susceptible to the toxic action of horse serum a very long time is indicated in the following experiments, in

which 378 days elapsed between the first treatment and the second injection.

No. G. P.	First injection.	Interval.	Second injection.	Result.
		Days.		
4515	0.19 c. c. toxine 7+1 unit antitoxic horse serum (B. 27), subcutaneously.	238	6 c. c. normal horse (roan) serum, intraperitoneally.	Dead, 30 minutes.
4527	.....do.....	378	6 c. c. normal horse (No. 15) serum intraperitoneally.	Dead, 37 minutes.
4495	.....do.....	365	.....do.....	No symptoms.
			Ditto, 2 days later..	Severe symptoms.
	Two young, born of above female (4495), tested as follows:			
4495a	When 7 days old, 1 c. c. normal horse (No. 15) serum, intraperitoneally.			Dead, 13 minutes.
4495b	When 9 days old, 1 c. c. normal horse (No. 15) serum, intraperitoneally.			Dead, 12 minutes.

The above guinea pig (No. 4495) showed no symptoms at all after receiving 6 c. c. of horse serum into the peritoneal cavity 365 days after the first injection. It was then given the same quantity of serum two days following and showed severe symptoms. We have had several such instances following intraperitoneal injections, and can only explain it by the fact that sometimes the serum enters the lumen of the intestine instead of the peritoneal cavity. We called attention to this probability in Hygienic Laboratory Bulletin No. 29, page 63.

THE EFFECT OF FIRST INJECTIONS OF HORSE SERUM INTO GUINEA PIGS.

Theobald Smith[a] stated that guinea pigs which have received no preliminary doses of serum may die of a first injection; of 58 guinea pigs receiving 3 to 5 c. c. of diphtheria antitoxin with no preliminary treatment, 9 died and 49 showed no effect, making 15.5 per cent of the guinea pigs reacting to the first injection of horse serum. In reply to a question, Smith stated that he did not know whether or not the animals were the young of guinea pigs that had been treated.

We have injected many guinea pigs with horse serum and have never noted symptoms of death to follow the first injection, and can not help but believe that the results obtained by Smith are explained by our studies upon the maternal transmission of hypersusceptibility.

[a] Smith, Theobald: Discussion of Rosenau and Anderson's paper on "Hypersusceptibility." Journ. Am. Med. Assn., Vol. 27, No. 13, Sept. 29, 1906, p. 1010.

Part XII.

SUMMARY AND CONCLUSIONS.

Profound chemical changes, perhaps in the central nervous system, are probably produced by the first injection of a strange proteid.

Guinea pigs may be sensitized with horse serum by injections directly into the heart. From this it appears that the cells lining the peritoneal cavity or the connective tissue cells of the subcutaneous tissue do not necessarily play a part in the phenomenon of hypersusceptibility.

Guinea pigs may be sensitized with the filtrate obtained from horse serum after precipitation with ammonium sulphate.

Formaldehyd does not appreciably influence the toxicity of horse serum and has no effect upon the sensitizing action.

The sensitizing substance is not dialyzable through a collodion sac.

The toxic principle is not altered by various ferments, such as taka-diastase, pancreatin, rennin, myrosin, invertin, emulsin, pepsin, ingluvin, malt, or papain, nor by certain alkaloids, such as atropin, strychnin, morphin, or caffein; it is also not altered by calcium chlorid, sodium nitrate, sodium chlorid, magnesium sulphate, or ammonium sulphate.

Guinea pigs sensitized with horse serum do not react to the second injection of other proteid substances such as peptone, extract of peas, egg albumen, and milk. Conversely, guinea pigs sensitized with subcutaneous injections of these substances do not react to a subsequent injection of horse serum.

Guinea pigs show quite as high a degree to susceptibility to cattle, sheep, hog, dog, and cat serum as they do to horse serum.

Guinea pigs are quite susceptible to injections of hemoglobin, egg albumen, milk, or the extract of peas when given two injections with an interval of at least ten days. Simpler albuminous substances, such as peptone, seem to have slight sensitizing and poisonous properties, while lower nitrogenous compounds such as leucin and tyrosin possess none at all.

The reaction following a second injection of proteid matter in the guinea pig appears to be common to all the higher forms of albuminous substances, no matter from what source.

This phenomenon of hypersusceptibility in the guinea pig may be useful as a physiological test to distinguish true proteid substances from the lower forms of nitrogenous compounds.

The refined antitoxic serum, bulk for bulk, is quite as toxic to sensitized guinea pigs as the untreated serum from which it was precipitated and dialyzed. There is, however, a distinct advantage gained in using the concentrated serum, as the same number of units may be given in half the bulk, and it is well known that the serum reaction in man depends partly upon the quantity of serum given.

Serum from one horse appears quite as toxic as serum from other horses. The apparent differences seem to depend upon something connected with the sensitizing action.

The immunity produced by repeated injections, termed "anti-anaphylaxis" by Besredka and Steinhardt, appears to be relatively not quite as lasting and definite as many instances of active immunity against bacterial infections.

Hypersusceptibility to the toxic action of horse serum is transmitted through the female guinea pig. The male has no influence.

The susceptibility is not transmitted through the milk.

Maternal transmission of hypersusceptibility succeeds whether the female guinea pig is sensitized before or after conception.

The phenomenon of hypersusceptibility appears to have no relation to aggressins.

Methemaglobin is not present in the blood of guinea pigs dead of a second injection of horse serum.

Oxygen has no influence upon the symptoms.

Neither diphtheria toxine nor tetanus toxine appreciably influence the phenomenon of hypersusceptibility produced by horse serum.

The removal of the spleen or thyroid gland does not influence hypersusceptibility in the guinea pig.

Guinea pigs fed upon beef are susceptible to a subsequent injection of cattle serum.

Guinea pigs fed with cooked meat are not susceptible to subsequent injections of serum.

When a second injection of horse serum is given directly into the heart of a susceptible guinea pig the symptoms are manifested with promptness and virulence. Under these circumstances, 0.01 c. c. (injected directly into the heart) in one instance was sufficient to cause the death of a sensitized guinea pig.

Guinea pigs remain susceptible a very long time. There is no diminution in the susceptibility of a guinea pig to subsequeut injections of horse serum for at least one year. The longest period we have observed is 480 days.

We have never seen symptoms resulting from the first injection of horse serum in a guinea pig born of an untreated mother.

The problem of hypersusceptibility has an important bearing upon the question of immunity. Our work indicates that hypersusceptibility is either an essential element or one stage in the process of

resistance to a certain class of diseases. We can not escape the conviction that further studies upon the phenomenon of hypersusceptibility will have an important bearing upon the prevention and cure of certain infectious processes. The hypersusceptibility obtained by bacterial proteids and the subsequent immunity furnishes data for this belief.

From our work upon the proteid substances of animal and vegetable origin, it was but a step to the albuminous content of the bacterial cell. Experimental studies upon the bacterial proteids are of the greatest importance on account of the practical uses to which results along this line may lead.

Hypersusceptibility may easily be induced in guinea pigs with proteid extracts obtained from the bacterial cell. The first injection of the extracts used by us seems comparatively harmless to the animal. A second injection of the same extract shows, however, that profound physiological changes have taken place. A definite period must elapse between the first and the second injection. The symptoms presented by the guinea pig as a result of the second injection resemble those caused by horse serum.

The phenomenon induced by a second injection is followed, in certain cases, by an immunity to the corresponding infection.

These results give a possible explanation of the period of incubation in some of the communicable diseases. Is it a coincidence that the period of incubation in a number of infectious diseases is about ten to fourteen days, which corresponds significantly with the time required to sensitize animals with a strange proteid? In certain infectious diseases with a short period of incubation, such as pneumonia, the crisis, which commonly appears in about ten days, may find a somewhat similar explanation. It is evident that diseased processes produced by soluble toxines, such as diphtheria and tetanus, do not belong to the category now under consideration.

The phenomenon of hypersusceptibility has been produced in the guinea pig by extracts obtained from the colon bacillus, yeast, hay bacillus, anthrax, tubercle bacillus, and the typhoid bacillus. The hypersusceptibility produced by the colon and typhoid bacillus was followed by a definite immunity to the corresponding infections. In the case of anthrax, however, immunity does not follow hypersusceptibility to the anthrax proteid. We are, therefore, not dealing with a general law applicable to all infections, but with certain limitations as in the case of antitoxic immunity.

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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 States 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.

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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 toxin. II. Maternal transmission of immunity to diphtheria toxin 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 horse-hair 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.—Studies upon hypersusceptibility and immunity. 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. ——.

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