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A study of the  
cause of sudden death  
following the injection of  
horse serum.

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M. J. Rosenau.

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HYGIENIC LABORATORY.—BULLETIN No. 29.  
M. J. ROSENAU, Director.  
APRIL, 1907.

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A STUDY OF THE CAUSE OF SUDDEN DEATH  
FOLLOWING THE INJECTION OF  
HORSE SERUM.

BY

M. J. ROSENAU  
AND  
JOHN F. ANDERSON.



WASHINGTON:  
GOVERNMENT PRINTING OFFICE:  
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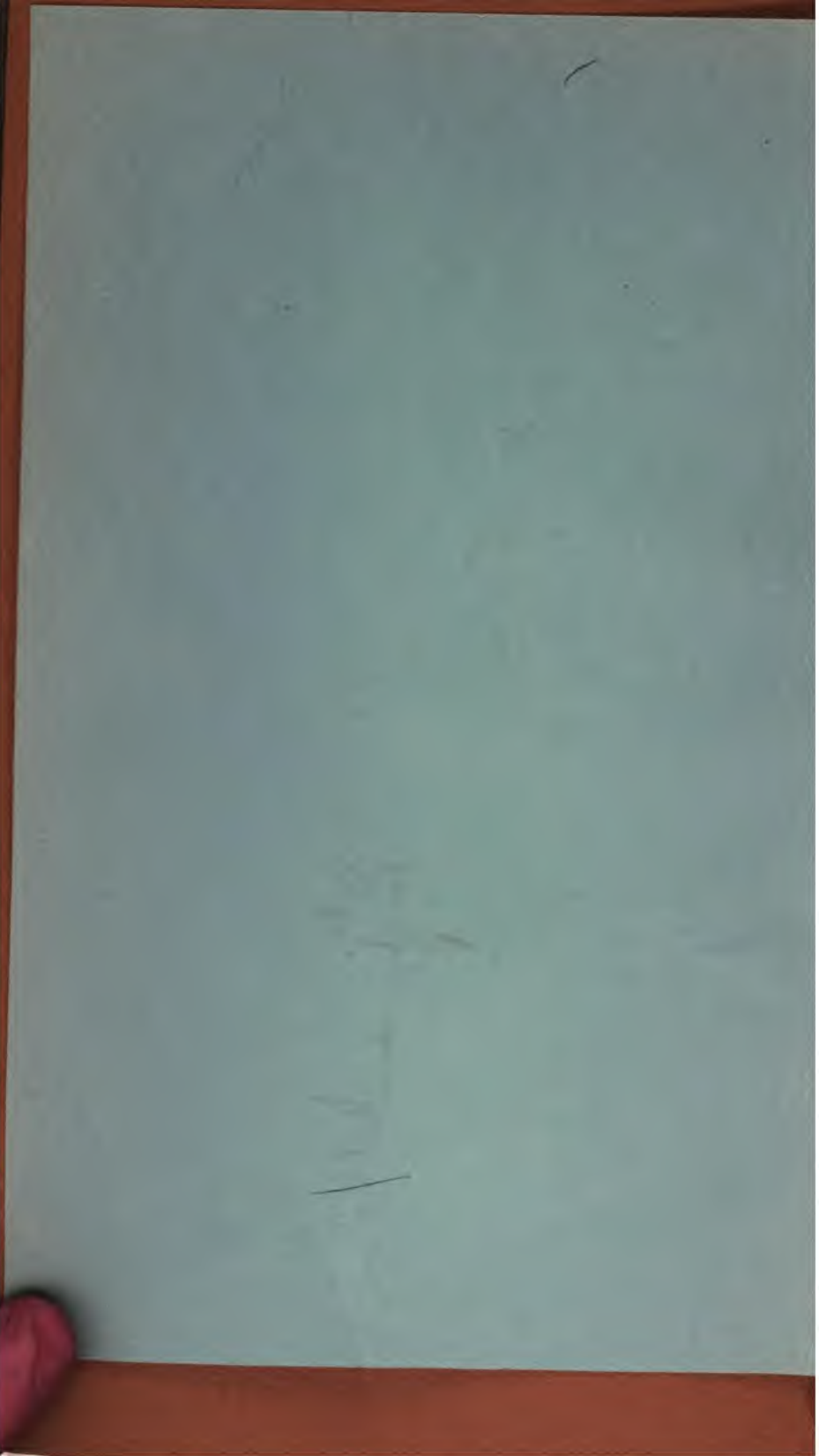
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## A STUDY OF THE CAUSE OF SUDDEN DEATH FOLLOWING THE INJECTION OF HORSE SERUM.

---

By MILTON J. ROSENAU,

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---

It has long been known that the blood of certain animals is poisonous when transfused or injected into certain other species.

Many instances might be cited showing that the blood serum of one animal has poisonous properties when injected into an animal of another species. But the blood serum of the horse apparently lacks such poisonous action. Very large quantities of the blood serum of the horse may be injected into man, rabbits, guinea pigs, and many other animals without serious inconvenience, except occasionally a slight reaction at the site of inoculation.

In a certain proportion of cases the injection of horse serum into man is followed by urticarial eruptions, joint pains, fever, swelling of the lymph nodes, edema, and albuminuria. This reaction, which appears after an incubation period of eight to thirteen days, has been termed by Pirquet and Schick the "serum disease."

In exceptional instances sudden death has followed an injection of horse serum in man.

These studies were taken up in October, 1905, in order to throw light upon the cause of this unfortunate accident. We have shown that ordinarily horse serum is a comparatively bland and harmless substance when injected into certain animals; but these animals may be rendered so susceptible that an injection of horse serum may produce sudden death or severe symptoms. For example, large quantities of horse serum may be injected subcutaneously or into the peritoneal cavity of a guinea pig without apparently causing the animal the least inconvenience. However, if a guinea pig is injected with a small quantity, say  $\frac{1}{2}$  c. c., of horse serum and after the

expiration of a certain interval is again injected with horse serum the result will probably be fatal. The first injection of horse serum has sensitized the animal in such a way as to render it very susceptible to a toxic principle in horse serum. It is probable that when the guinea pig is injected with the first, or sensitizing, quantity of serum the strange proteid contained in the horse serum develops in the body of the guinea pig "antibodies" which, when brought into contact with more horse serum given at a second injection, produce either a union or a reaction, which causes the toxic action.

A certain time is necessary to elapse between the first and second injections of horse serum before this toxic action is able to manifest itself. This "period of incubation" is from ten to twelve days, and corresponds suggestively with the period of incubation of the serum disease which Pirquet and Schick place at eight to thirteen days.

Guinea pigs may be sensitized with exceedingly small quantities of horse serum. In most of our work we used quantities less than  $\frac{1}{250}$  c. c. and we found in one instance that  $\frac{1}{1,000,000}$  c. c. of horse serum was sufficient to render a guinea pig susceptible.

It also requires very small quantities of horse serum, when given in a second injection, to produce poisonous symptoms. One-tenth c. c. injected into the peritoneal cavity is sufficient to cause the death of a half-grown guinea pig. One-tenth c. c. of horse serum injected subcutaneously is sufficient to produce serious symptoms. The fact that this toxic action may be developed by such small quantities of serum and the fact that exceedingly small quantities are sufficient to produce symptoms and death upon a second injection, a priori places both the sensitizing and the toxic principle in the horse serum in the "haptin group" of substances in the sense used by Ehrlich.

A still further indication that the side-chain theory in its broadest sense may be applicable is the further fact that immunity may be produced against the toxic action by multiple injections of the serum.

While at first we thought that diphtheria antitoxin had some relation to this action, we are now able to state positively that it has nothing whatever to do with the poisonous action of horse serum; further, that diphtheria antitoxin in itself is absolutely harmless. The toxic action which we have studied is caused by a principle in normal horse serum and is entirely independent of the antitoxic properties of the serum.

## Part I.

### CONTROL EXPERIMENTS.

#### ACTION OF NORMAL HORSE SERUM UPON NORMAL GUINEA PIGS.

Normal horse serum when injected into normal guinea pigs causes no symptoms. Large amounts, such as 6 or 10 c. c., may be injected into the peritoneal cavity of a guinea pig without any apparent inconvenience to the animal. When normal horse serum is injected subcutaneously into the guinea pig it is sometimes either absorbed very slowly or there is a slight local reaction, as indicated by edema and induration of the subcutaneous tissue at the site of injection.

- G. P. No. 2.<sup>a</sup> Six c. c. normal horse serum (roan horse) injected into the peritoneal cavity. No symptoms.
- G. P. No. 3. Ten c. c. normal horse serum (roan horse) injected into the peritoneal cavity. No symptoms.
- G. P. No. 63. Six c. c. normal horse serum (Sp. horse) injected into the peritoneal cavity. No symptoms.
- G. P. No. 64. Six c. c. normal horse serum (Jane horse) injected into the peritoneal cavity. No symptoms.
- G. P. No. 66. Six c. c. normal horse serum (Sam horse) injected into the peritoneal cavity. No symptoms.
- G. P. No. 65. Six c. c. normal donkey serum (donkey No. 58) injected into the peritoneal cavity. No symptoms.
- G. P. No. 296. Six c. c. normal donkey serum injected into the peritoneal cavity. No symptoms.
- G. P. No. 297. Do.
- G. P. No. 298. Six c. c. normal horse serum (Sp.) injected into the peritoneal cavity. No symptoms.
- G. P. No. 299. Do.

Specimens of serum from each one of the normal horses used in this work appear in the above series of guinea pigs, which serve as controls.

Donkey serum is also harmless for normal guinea pigs and is just as toxic as horse serum to a sensitized guinea pig.

Uhlenhuth (*Zur Kenntniss der giftigen Eigenschaften des Blutserums*, *Ztschr. f. Hyg.*, v. 26, 1897, p. 384) found that normal horse serum, when injected intravenously into rabbits, is harmless. He injected as large quantities as 60 c. c. per 1 kilogram of rabbit and found that this caused no reaction at all. On the other hand he found that 11 c. c. of sheep serum, 12 c. c. of hog serum, or 6 c. c. of cattle serum per 1 kilogram of rabbit, when injected intravenously, caused the death of the animal.

The nontoxic action of horse serum on animals was further shown by Hermann Pfeiffer (*Ueber die nekrotisirende Wirkung normaler*

<sup>a</sup>G. P., abbreviation for guinea pig. The numbers refer to the laboratory serial numbers.



Seren, *Ztschr. f. Hyg. u. Infektionskrankh.*, v. 51, (2), pp. 181-196, 1905), who first confirmed the above work of Uhlenhuth and then showed that the subcutaneous inoculation of large quantities of horse serum into guinea pigs produced at most a slight local reaction. He found on the other hand that the serum of certain other animals, e. g., cattle, hog, man, when injected subcutaneously into guinea pigs, produced a local reaction and in large quantities caused necrosis.

We took the temperature of a number of guinea pigs twice daily for eighteen days following the injection of large quantities of horse serum subcutaneously, in order to determine whether a febrile reaction followed. No marked deviation from the normal temperature was noted.

#### ACTION OF ANTITOXIC HORSE SERUM UPON NORMAL GUINEA PIGS.

It is a well-known fact that large quantities of horse serum containing high antitoxic potency may be injected subcutaneously or into the peritoneal cavity of a normal guinea pig without causing symptoms. We use this method of testing the diphtheria antitoxin bought upon the open market to determine its freedom from bacterial and toxic impurities. Of the many guinea pigs thus inoculated none has shown untoward effects except in the case of bacterial or toxic contaminations. This method of testing the purity of antitoxin is also used by the manufacturers almost daily. We may therefore state that diphtheria antitoxic serum when injected into a normal guinea pig causes no symptoms.

However, we present the following experimental data, which serve as controls for this work, because the normal guinea pigs in the following series have been inoculated into the peritoneal cavity with large quantities of the antitoxic horse serums subsequently used in this work.

- G. P. No. 300. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. No symptoms.  
 G. P. No. 301. Do.  
 G. P. No. 302. Six c. c. antitoxic horse serum (Natl. V. 14) injected into the peritoneal cavity. No symptoms.  
 G. P. No. 303. Do.  
 G. P. No. 304. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity.  
 No symptoms.  
 G. P. No. 305. Do.  
 G. P. No. 306. Six c. c. antitoxic horse serum (NYBH 10B, Gibson) injected into the peritoneal cavity. No symptoms.  
 G. P. No. 307. Six c. c. antitoxic horse serum (NYBH 13C, Gibson) injected into the peritoneal cavity. No symptoms.  
 G. P. No. 308. Six c. c. antitoxic horse serum (Alex. A 211) injected into the peritoneal cavity. No symptoms.  
 G. P. No. 309. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.  
 G. F. No. 310. Do.

## Part II.

### HORSE SERUM IS POISONOUS TO A "TREATED" OR "USED" GUINEA PIG.

It has long been known by some of the manufacturers of diphtheria antitoxin that when antidiphtheric serum is inoculated into a used guinea pig the result is almost invariably death. By a "used" or "treated" guinea pig we mean one that has recovered from the effects of an inoculation of the toxine-antitoxin mixture used in testing the potency of diphtheria antitoxin. For example:

G. P. No. 4191. Six c. c. antitoxic horse serum (Alex. 192) injected into the peritoneal cavity. Death in 15 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{388}$  c. c. antitoxic horse serum (PD&Co 08033).]

G. P. No. 4103. Nine c. c. antitoxic horse serum (Alex. 193) injected into the peritoneal cavity. Dead in 5 minutes.

[Previous treatment: 31 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{210}$  c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 4330. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into peritoneal cavity. Dead in 2 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{210}$  c. c. antitoxic horse serum (Alex. 185).]

G. P. No. 6B. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into peritoneal cavity. Dead in 27 minutes.

[Previous treatment: 86 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 + 1 unit antitoxic horse serum (Standard B25).]

G. P. No. 4381. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 11 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{310}$  c. c. antitoxic horse serum (Natl. V. 10).]

G. P. No. 4336. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 12 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{388}$  c. c. antitoxic horse serum (PD&Co 07635).]

G. P. No. 4369. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{400}$  antitoxic horse serum (Cutter 1461).]

G. P. No. 4377. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{388}$  c. c. antitoxic horse serum (Natl. V. 7).]

- G. P. No. 4526. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 30 minutes.  
[Previous treatment: 17 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 4491. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 41 minutes.  
[Previous treatment: 18 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 4494. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 37 minutes.  
[Previous treatment: 19 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 75. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 3 hours.  
[Previous treatment: 15 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{2}$  $\frac{1}{10}$  c. c. antitoxic horse serum (Natl. IX. 17).]
- G. P. No. 88. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 15 days prior, inoculated subcutaneously with 0.006 c. c. toxine No. 7+1 unit antitoxic horse serum (Natl. IX. 17).]
- G. P. No. 4522. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 20 minutes.  
[Previous treatment: 20 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 4500. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 21 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 4471. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 8 minutes.  
[Previous treatment: 22 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{2}$  $\frac{1}{10}$  c. c. antitoxic horse serum (Natl. IX. 17).]
- G. P. No. 4503. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.<sup>a</sup>  
[Previous treatment: 22 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 4499. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 20 minutes.  
[Previous treatment: 24 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]
- G. P. No. 4465. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 40 minutes.  
[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{2}$  $\frac{1}{10}$  c. c. antitoxic horse serum (Natl. VIII. 17).]
- G. P. No. 4387. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 28 days prior, inoculated subcutaneously with 0.2 c. c. toxine No. 58+1 unit antitoxic horse serum (Standard B27).]

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<sup>a</sup> Explanation of occasional irregularities, page 63.

G. P. No. 4509. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 143. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{30}$  c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 73. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 57 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+250 units antitoxic horse serum (Natl. IX. 17).]

G. P. No. 82. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 57 days prior, inoculated subcutaneously with 0.006 c. c. toxine No. 7+ $\frac{1}{30}$  c. c. antitoxic horse serum (Natl. IX. 17).]

G. P. No. 139. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 49 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{30}$  c. c. antitoxic horse serum (Stearns 1351).]

All of the above pigs were first given the toxine-antitoxin mixtures in order to test the strength of diphtheria antitoxic serum bought on the open market in accordance with the law of July 1, 1902.

Horse serum is also toxic when injected subcutaneously and in small quantities, as will be seen from the following experiments:

G. P. No. 4951. Six c. c. normal horse serum (roan) inoculated subcutaneously. Dead in 35 minutes.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{30}$  c. c. antitoxic horse serum (NYBH 10B).]

G. P. No. 5047. Same injection. Dead in 40 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{40}$  c. c. antitoxic horse serum (Alex. A205).]

G. P. No. 4950. Same injection. Symptoms; recovered.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{30}$  c. c. antitoxic horse serum (NYBH 10B).]

G. P. No. 5046. Same injection. Dead in 40 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{40}$  c. c. antitoxic horse serum (Alex. A204).]

G. P. No. 4524. Six c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{30}$  c. c. antitoxic horse serum (Alex. 192).]

G. P. No. 4623. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{30}$  c. c. antitoxic horse serum (Alex. 192).]

G. P. No. 4512. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Dead in 81 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4502. Six c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4514. Two-tenths c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

#### SYMPTOMS CAUSED BY THE INJECTION OF HORSE SERUM INTO A SUSCEPTIBLE GUINEA PIG.

Very characteristic symptoms are produced by horse serum, either normal or antitoxic, when injected into a susceptible guinea pig. The symptoms are apparently the same whether the injection is made subcutaneously or into the peritoneum, or whether normal or antitoxic horse serum is used. In five or ten minutes after injection the pig manifests indications of respiratory embarrassment by scratching at the mouth, coughing, and sometimes by spasmodic, rapid, or irregular breathing; the pig becomes restless and agitated. This stage of exhilaration is soon followed by one of paresis or complete paralysis. The pig is unable to stand or, if it attempts to move, falls upon its side; when taken up it is limp. Spasmodic, jerky, and convulsive movements now supervene.

Pigs in this stage with complete paralysis may fully recover, but usually convulsions appear, and are almost invariably a forerunner of death. Symptoms appear about ten minutes after the injection has been given; occasionally in pigs not very susceptible they are delayed thirty to forty-five minutes. Only in one or two instances of the several hundred pigs which we have observed have the symptoms developed after one hour. Pigs developing symptoms as late as this are not very susceptible and do not die. The chain of symptoms is exceedingly characteristic. The symptoms do not always follow in the order given. Death usually occurs within an hour and frequently in less than thirty minutes.

#### THE POISONOUS ACTION OF HORSE SERUM ACTS UPON THE RESPIRATORY CENTERS.

Judging from the symptoms produced by the injection of horse serum into a susceptible guinea pig we assumed that the poison acted upon the nervous system. Autopsies done immediately after the death of the guinea pigs showed invariably that the heart continued to beat long after respiration had ceased. In some instances the heart would continue to beat a full hour when exposed. This would seem to indicate that we were dealing with a poison which caused death through the nervous control of the respiration, and the following experiment shows that this effect is certainly not local.

- P. No. 4526. Six c. c. antitoxic horse serum (National VIII. 17) were injected into the peritoneal cavity. Death in 30 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with 0.19 c. c. of toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

- Immediately after death the phrenic nerve was exposed and stimulated high up, causing contractions of the diaphragm both upon making and breaking the galvanic current. The contractions were also caused with the induced current. The contractions of the diaphragm caused by stimulating the phrenic nerve in this way were produced with a weaker current than those required to cause similar contractions in a normal control guinea pig.



### Part III.

#### THE TOXIC ACTION BEARS NO RELATION TO DIPHTHERIA.

##### THE POISON IS NOT TOXONE.

It occurred to us that possibly diphtheria antitoxic serum may contain free toxone which, when injected into a "used" or "treated" guinea pig, might produce acute effects upon the nervous system of such a pig, which, on account of the previous effects of diphtheria toxine, had been rendered peculiarly susceptible. It is well known that the toxone does not cause the acute death of a guinea pig, but only produces a local edema and late paralysis. This fact, first demonstrated by Ehrlich, may readily be demonstrated by inoculating guinea pigs with toxine and antitoxin mixed in such proportions so that the toxine is but partly neutralized. We were further led to believe that the poisonous effects of horse serum upon a treated guinea pig might be a manifestation of the toxones because the symptoms so plainly point to the nervous system. The pigs almost invariably show paresis or paralysis.

The fact that neither toxone nor any other constituent in the toxine plays any rôle in the poisonous action of horse serum is demonstrated in the following experiment:

G. P. No. 4161. 0.1 c. c. toxine No. 15 (MLD=.01) was injected into the peritoneal cavity.

No symptoms. Completely paralyzed at the time, resulting from previous treatment.

[Previous treatment: 31 days previously inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{8}$  c. c. diphtheria antitoxic serum (Mulford's No. 942).]

One hour 50 min. later this same pig was inoculated with 6 c. c. diphtheria antitoxic serum (P. D. & Co. No. 08022) into the peritoneal cavity. Death in 10 minutes.

It is well known that overpowering doses of toxine injected either subcutaneously or into the peritoneal cavity\* of a guinea pig require about twenty hours to kill the animal. These large doses of toxine do not cause immediate symptoms.

It occurred to us, however, that while toxine alone may not have this power, nevertheless when it is mixed with antitoxic horse serum and when large doses of the mixture are injected into a guinea pig there may be a sufficient liberation of toxone or other poisonous substances capable of producing symptoms and sudden death.



The following mixture of toxine and antitoxin represents these two substances in the proportion of the L<sup>o</sup> dose:

G. P. 4483. 1.6 c. c. toxine No. 7+11.5 units antitoxic serum (standard B27) injected subcutaneously. No symptoms.

18 days later 6 c. c. antitoxic horse serum (National VIII, 17) injected into the peritoneal cavity of the same pig. Death 30 minutes.

In a similar way the effects of mixing large doses of the diphtheria toxine with normal horse serum were tested, as follows:

G. P. No. 40. Six c. c. normal horse serum (roan horse)+0.3 c. c. diphtheria toxine No. 5 mixed in vitro and injected into the peritoneal cavity. No immediate symptoms. Died 2 days later.

**GUINEA PIGS CAN NOT BE RENDERED SUSCEPTIBLE BY PRIOR INFECTIONS WITH THE BACILLUS DIPHTHERIÆ.**

When this work was first undertaken it was thought to have a bearing upon sudden deaths in children suffering with diphtheria. The work was begun in order to discover, if possible, the cause of this accident. We thought that possibly by injecting small quantities of a virulent culture of the Klebs-Löffler bacillus into guinea pigs, in that way imitating mild cases of diphtheria, some of these pigs could be rendered susceptible to subsequent inoculations of antitoxic horse serum. This, however, is not the case for guinea pigs, as is plainly seen in the following experiments, in which a number of guinea pigs were treated with sufficient quantities of a culture of the Klebs-Löffler bacillus to cause slight symptoms and at varying intervals of time were inoculated again into the peritoneal cavity with large quantities of antitoxic horse serum. In no instance did the guinea pigs show any ill effects from this latter treatment.

G. P. No. 41. Six c. c. antitoxic horse serum (Nat. VIII, 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 4 days prior, inoculated subcutaneously with 0.1 c. c. suspension.]

G. P. No. 9. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: 7 days prior, inoculated subcutaneously with 1 gtt. of 24-hour suspension.]

G. P. No. 51. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: 7 days prior, inoculated subcutaneously with 0.1 c. c. of suspension.]

G. P. No. 42. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: 9 days prior, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

G. P. No. 56. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: 9 days prior, inoculated subcutaneously with 0.1 c. c. suspension.]

G. P. No. 43. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: 12 days prior, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

G. P. No. 45. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: 14 days prior, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

- G. P. No. 58. Six c. c. same serum and injection as g. p. 41. No symptoms.  
[Previous treatment: 21 days prior, inoculated subcutaneously with 0.1 c. c. suspension.]
- G. P. No. 44. Six c. c. same serum and injection as g. p. 41. No symptoms.  
[Previous treatment: 24 days prior, inoculated subcutaneously with 1 gtt. 24-hour suspension.]
- G. P. No. 52. Six c. c. same serum and injection as g. p. 41. No symptoms.  
[Previous treatment: 27 days prior, inoculated subcutaneously with 0.1 c. c. 24-hour suspension.]
- G. P. No. 50. Six c. c. same serum and injection as g. p. 41. No symptoms.  
[Previous treatment: 32 days prior, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

#### DIPHTHERIA TOXINE CAN NOT RENDER GUINEA PIGS SUSCEPTIBLE.

For the same reason that guinea pigs were treated with cultures of the *Bacillus diphtheriæ* we also attempted to render them susceptible by the injection of small quantities of diphtheria toxine. On account of the great importance of this subject a large number of guinea pigs were used to demonstrate the rôle which the diphtheria toxine may play in the subsequent action of horse serum. Our experiments demonstrate conclusively that diphtheria toxine alone plays absolutely no rôle in rendering guinea pigs susceptible to the toxic action of horse serum; for in no instance, as will be seen in the following series, does the guinea pig show the slightest effects when subsequently injected with large quantities of serum.

- G. P. No. 4584. Six c. c. antitoxic horse serum (Nat. VIII. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: 2 days prior, inoculated subcutaneously with 0.001 c. c. toxine No. 7.]
- G. P. No. 4593. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 3 days prior, as g. p. 4584.]
- G. P. No. 4534. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 4 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]
- G. P. No. 4550. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 5 days prior, as g. p. 4534.]
- G. P. No. 4548. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 6 days prior, as g. p. 4534.]
- G. P. No. 4585. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 6 days prior, as g. p. 4584.]
- G. P. No. 4432. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 7 days prior, as g. p. 4534.]
- G. P. No. 4434. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 7 days prior, inoculated subcutaneously with 0.004 c. c. toxine No. 7.]
- G. P. No. 4426. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 8 days prior, as g. p. 4534.]
- G. P. No. 4583. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: 9 days prior, as g. p. 4534.]

- G. P. No. 4579. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *10 days prior*, as g. p. 4534.]
- G. P. No. 4582. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *11 days prior*, as g. p. 4534.]
- G. P. No. 4430. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *12 days prior*, as g. p. 4534.]
- G. P. No. 4572. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *12 days prior*, as g. p. 4534.]
- G. P. No. 4568. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *13 days prior*, as g. p. 4534.]
- G. P. No. 4576. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *14 days prior*, as g. p. 4534.]
- G. P. No. 4577. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *16 days prior*, as g. p. 4534.]
- G. P. No. 4535. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *17 days prior*, as g. p. 4534.]
- G. P. No. 4547. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *18 days prior*, as g. p. 4534.]
- G. P. No. 4580. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *19 days prior*, as g. p. 4534.]
- G. P. No. 4556. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *20 days prior*, as g. p. 4534.]
- G. P. No. 4558. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *21 days prior*, as g. p. 4534.]
- G. P. No. 4545. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *25 days prior*, as g. p. 4534.]
- G. P. No. 4555. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *24 days prior*, as g. p. 4534.]
- G. P. No. 4541. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *26 days prior*, as g. p. 4534.]
- G. P. No. 4404. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *27 days prior*, inoculated subcutaneously with 0.002 c. c. toxine No. 5.]
- G. P. No. 4454. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *27 days prior*, inoculated subcutaneously with 0.01 c. c. toxine No. 19.]
- G. P. No. 4565. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *27 days prior*, as g. p. 4534.]
- G. P. No. 4554. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *30 days prior*, as g. p. 4534.]
- G. P. No. 4578. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *31 days prior*, as g. p. 4534.]
- G. P. No. 4542. Six c. c. same serum and injection as g. p. 4584. No symptoms.  
[Previous treatment: *33 days prior*, as g. p. 4534.]
- G. P. No. 4529. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: *46 days prior*, as g. p. 4534.]
- G. P. No. 4428. Do.
- G. P. No. 4427. Do.
- G. P. No. 4431. Do.

G. P. No. 4567. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 65 days prior, as g. p. 4534.]

G. P. No. 4554. Do.

G. P. No. 4553. Do.

#### DIPHTHERIA ANTITOXIN PLAYS NO RÔLE IN THIS ACTION.

Before we realized that the diphtheria toxine or antitoxin played no rôle in rendering guinea pigs susceptible to subsequent injections of horse serum we prepared the following series of guinea pigs, in order to eliminate the antitoxin as a factor. These pigs were injected subcutaneously with a mixture of 1 c. c. of normal horse serum and a small quantity of diphtheria toxine, slightly less than a minimal lethal dose. This was done for comparison with the susceptibility produced by the toxine-antitoxin mixtures.

G. P. No. 91. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms. Died 12 hours later.

[Previous treatment: 65 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 296. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 60 minutes.

[Previous treatment: 71 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 96. Do. Dead in 40 minutes.

G. P. No. 93. Do. Symptoms; recovered.

G. P. No. 90. Do.

It will be seen from our experiments that guinea pigs are quite as sensitive when previously treated with toxine-normal serum as with the toxine-antitoxin mixtures.

In order to compare the toxic action of normal serum with antitoxic serum upon pigs so treated, four animals of the above series were inoculated with antitoxic horse serum, as follows:

G. P. No. 92. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms. Died 12 hours later.

[Previous treatment: 15 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 98. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 53 minutes.

[Previous treatment: 58 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 95. Do. Dead in 43 minutes.

G. P. No. 94. Same injection. Symptoms; recovered.

[Previous treatment: 65 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

It is evident from the above that fresh normal serum is quite as poisonous to a sensitized guinea pig as is antitoxic horse serum.

In order further to eliminate the antitoxin as a factor in this problem, the following series of used or treated guinea pigs were injected with normal horse serum:

- G. P. No. 4100. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 15 minutes.  
 [Previous treatment: 36 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{2}\frac{1}{10}$  c. c. antitoxic horse serum (Alex. 190).]
- G. P. No. 4105. Same injection. Dead in 30 minutes.  
 [Previous treatment: 36 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Alex. 189).]
- G. P. No. 4106. Same injection. Dead in 5 minutes.  
 [Previous treatment: 36 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Alex. 189).]
- G. P. No. 4331. Same injection. Dead in 90 minutes.  
 [Previous treatment: 25 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{2}\frac{1}{10}$  c. c. antitoxic horse serum (Alex. 185).]
- G. P. No. 4351. Same injection. Dead in 40 minutes.  
 [Previous treatment: 18 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{4}\frac{1}{10}$  c. c. antitoxic horse serum (PD&Co 08021).]
- G. P. No. 3950. Six c. c. normal horse serum (Sam) injected into peritoneal cavity. Dead in 10 minutes.  
 [Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{2}\frac{1}{10}$  c. c. antitoxic horse serum (Hubbert 1).]
- G. P. No. 3951. Six c. c. normal horse serum (Jane) injected into the peritoneal cavity. Dead in 30 minutes.  
 [Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{2}\frac{1}{10}$  c. c. antitoxic horse serum (Hubbert 1).]
- G. P. No. 3952. Six c. c. normal horse serum (Sp.) injected into the peritoneal cavity. Dead in 38 minutes.  
 [Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{2}\frac{1}{10}$  c. c. antitoxic horse serum (Hubbert 1).]
- G. P. No. 3953. Six c. c. normal donkey serum (No. 58) injected into the peritoneal cavity. Dead in 60 minutes.  
 [Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Hubbert 1).]
- G. P. No. 4109. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.  
 [Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{4}\frac{1}{10}$  c. c. antitoxic horse serum (Alex. 188).]
- G. P. No. 4945. Six c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.  
 [Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{2}\frac{1}{10}$  c. c. antitoxic horse serum (Wellcome 478).]
- G. P. No. 4934. Six c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.  
 [Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Wellcome 479).]
- G. P. No. 4930. Six c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.  
 [Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Wellcome 475).]

G. P. No. 4931. Six c. c. normal horse serum (roan) inoculated subcutaneously. Dead in 5 minutes.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{8}$  c. c. antitoxic horse serum (Wellcome 475).]

G. P. No. 4941. Six c. c. normal horse serum (roan) inoculated subcutaneously. Dead in 20 minutes.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{8}$  c. c. antitoxic horse serum (Wellcome 474).]

All of the above animals and most of those which appear upon the subsequent pages of this bulletin were first used to test the strength of the diphtheria antitoxin bought on the open market in accordance with the law of July 1, 1902.

The following series of guinea pigs were given small quantities of antitoxic horse serum, some subcutaneously and some intraperitoneally, in order to compare the sensitizing action by these two methods of inoculation. At the second injection they were all given 6 c. c. of normal horse serum into the peritoneal cavity.

G. P. No. 276. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 25 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with  $\frac{1}{2}\frac{1}{8}$  c. c. antitoxic horse serum (Natl. V. 14).]

G. P. No. 277. Do. Dead in 30 minutes.

G. P. No. 278. Do. Dead in 55 minutes.

G. P. No. 279. Do. Symptoms; recovered.

G. P. No. 280. Do. Symptoms; recovered.

G. P. No. 281. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 17 days prior,  $\frac{1}{2}\frac{1}{8}$  c. c. antitoxic horse serum (Natl. V. 14) injected into the peritoneal cavity.]

G. P. No. 282. Do. Dead in 23 hours.

G. P. No. 283. Do. Symptoms; recovered.

G. P. No. 284. Do. Dead in 60 minutes.

G. P. No. 285. Do. Symptoms; recovered.

From the above series it will be seen that guinea pigs may be rendered as susceptible by intraperitoneal as by subcutaneous inoculations.

Finally, in order to demonstrate without doubt that neither antitoxin nor diphtheria toxine plays any part in this action, and to show that this action is a property of normal horse serum, we treated a number of guinea pigs giving them at both the first and second injection fresh normal horse serum, with the following results:

G. P. No. 101. Three c. c. normal (roan) horse serum injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 55 days prior, inoculated subcutaneously with  $\frac{1}{2}\frac{1}{8}$  c. c. normal horse serum (Jane).]

G. P. No. 105. One c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: As above.]

G. P. No. 104. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: As above.]

G. P. No. 102. Three c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: As above.]

G. P. No. 286. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 25 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with  $\frac{1}{2}$  c. c. normal horse serum (roan).]

G. P. No. 287. Do. Dead in 50 minutes.

G. P. No. 288. Do. Symptoms; recovered.

G. P. No. 289. Do. do do

G. P. No. 290. Do. do do

## Part IV.

### THE TOXIC PRINCIPLE.

At one time we made efforts to isolate the active principle in horse serum which causes the symptoms, but as soon as we realized that the toxic principle present in horse serum exerts its action in quantities so minute as to place it almost in the category of the ferments, and, further, when we concluded from our work that this toxic principle is doubtless one of those highly organized and complex proteid substances belonging to the "haptin" group, we recognized how hopeless it would be with present technique to isolate this substance. Nevertheless, we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eliminating or neutralizing this toxic principle from horse serum is at once evident.

### IS THE POISON SPECIFIC?

Whether the poisonous substance in horse serum is specific may be determined in several ways. We first endeavored to determine whether guinea pigs treated with horse serum are susceptible to the serums of other animals; and, secondly, whether guinea pigs treated with the serums of other animals are susceptible to subsequent injections of horse serum.

The following is a list of animals treated with horse serum and subsequently tested with the blood serums of other animals.

It will be noticed that in this list some of the guinea pigs were treated with donkey serum instead of horse serum, but as the donkey serum in our hands has shown itself to act the same as horse serum we have used them interchangeably.



Table showing the effect of alien serums upon guinea pigs sensitized with horse serum.

No. of guinea pig.	Kind and amount of normal serum.	Previous treatment.		Result.
		Days prior.	Inoculated subcutaneously with—	
178	Cattle, 6 c. c. ....	34	$\frac{1}{2}$ c. c. donkey serum.....	Symptoms, recovered.
149	.....do.....	41	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Stearns 1351).	Do.
180	Hog, 6 c. c. ....	34	$\frac{1}{2}$ c. c. donkey serum.....	No symptoms.
138	.....do.....	41	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Stearns 1351).	Symptoms, recovered.
4743	Hog, 1 c. c. ....	41	0.139 c. c. toxine No. 5 + $\frac{1}{2}$ c. c. horse serum (Alex. 192).	Do.
179	Sheep, 6 c. c. ....	34	$\frac{1}{2}$ c. c. donkey serum.....	Do.
152	.....do.....	41	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Stearns 1351).	Do.
177	Cat, 6 c. c. ....	34	$\frac{1}{2}$ c. c. donkey serum.....	Do.
124	.....do.....	41	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Stearns 1351).	No symptoms.
4744	Cat, 1 c. c. ....	41	0.139 c. c. toxine No. 5 + $\frac{1}{2}$ c. c. horse serum (Alex. 192).	Symptoms, recovered.
121	Rabbit, 6 c. c. ....	41	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Stearns 1351).	Do.
4380	.....do.....	19	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Natl. V. 10).	Do.
4374	.....do.....	19	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Natl. V. 7).	Do.
153	Dog, 6 c. c. ....	41	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Stearns 1351).	Do.
4379	.....do.....	19	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Natl. V. 10).	Do.
4742	Dog, 1 c. c. ....	41	0.139 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Alex. 192).	Do.
4375	Guinea pig, 3 c. c. ....	19	0.22 c. c. toxine No. 7 + $\frac{1}{2}$ c. c. horse serum (Natl. V. 7).	No symptoms.

As the above series of guinea pigs previously treated with horse serum showed unmistakable susceptibility to subsequent injections of the blood serum of other animals, we are justified in saying that the action is not specific; but there is a very evident quantitative difference. It will be noticed that none of these guinea pigs died, and it was a matter of observation that, for the most part, they had characteristic but mild symptoms.

We next took up the question as to whether a guinea pig may be sensitized to horse serum with the blood serum of animals other than the horse. The following is a list of guinea pigs which had been treated by the subcutaneous inoculation of small quantities ( $\frac{1}{2}$  c. c.) of blood serum from the rabbit, rat, donkey, cat, dog, hog, sheep, chicken, cattle, and man. Each pig was subsequently injected with 6 c. c. of antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity.

Table showing the results of injecting 6 c.c. of antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity of guinea pigs treated from 25 to 34 days prior with  $2\frac{1}{3}$  c. c. of the blood serum of other animals.

No. of guinea pig.	Previous treatment.		Result.
	Kind of serum.	Days prior.	
171	Rabbit..... $2\frac{1}{3}$ c. c.	25	No symptoms.
172	.....do.....do.....	34	Do.
174	.....do.....do.....	34	Symptoms, recovered.
167	Rat.....do.....	25	No symptoms.
168	.....do.....do.....	34	Do.
169	.....do.....do.....	34	Do.
170	.....do.....do.....	34	Do.
176	Donkey.....do.....	25	Do.
191	Cat.....do.....	25	Do.
192	.....do.....do.....	34	Symptoms, recovered.
193	.....do.....do.....	34	Symptoms (?), recovered.
214	Dog.....do.....	26	No symptoms.
215	.....do.....do.....	35	Do.
212	.....do.....do.....	35	Symptoms, recovered.
185	Hog.....do.....	30	No symptoms.
186	.....do.....do.....	30	Symptoms, recovered.
182	Sheep.....do.....	30	Do.
184	.....do.....do.....	30	No symptoms.
196	Chicken.....do.....	26	Symptoms, recovered.
197	.....do.....do.....	26	Do.
206	Cattle.....do.....	31	No symptoms.
207	.....do.....do.....	31	Do.
210	.....do.....do.....	31	Do.
201	Man.....do.....	31	Symptoms, recovered.
202	.....do.....do.....	31	No symptoms.

As the above series of guinea pigs was tested with antitoxic horse serum, we tested another series, similarly sensitized, with normal horse serum, as shown in the table on the following page, the results in all respects being similar.

Table showing the results of injecting 4 c. c. of normal horse serum (roan) into the peritoneal cavity of guinea pigs from 47 to 56 days prior with  $2\frac{1}{5}$  c. c. of the blood serum of other animals.

No. of guinea pig.	Previous treatment.		Result.
	Kind of serum.	Days prior.	
173	Rabbit.....	$\frac{1}{2}$ c. c.	Symptoms, recovered.
175	.....do.....	do	Do.
195	Cat.....	do	Do.
213	Dog.....	do	Do.
211	.....do.....	do	Do.
187	Hog.....	do	Do.
189	.....do.....	do	Do.
190	Sheep.....	do	No symptoms.
183	.....do.....	do	Symptoms, recovered.
181	.....do.....	do	Do.
199	Chicken.....	do	No symptoms.
198	.....do.....	do	Symptoms (?), recovered.
200	.....do.....	do	Symptoms, recovered.
203	Man.....	do	No symptoms.
204	.....do.....	do	Symptoms, recovered.
205	.....do.....	do	Do.

We think it is plain from the above that this action is not strictly specific for horse serum; that is, guinea pigs treated with horse serum are rendered somewhat susceptible to the subsequent injection of the serum of certain other species and that guinea pigs treated with the serum of other animals are slightly sensitive to the toxic action of horse serum. However, it is also perfectly plain from this series of experiments that the action is quantitatively specific, for it will be noticed that of the many guinea pigs sensitized with the blood serum of animals other than the horse, none died from subsequent injections of large quantities (6 c. c.) of horse serum. The same may be said of the action of these other serums upon guinea pigs previously treated with horse serum. While most of these serums produced symptoms, none of the guinea pigs died.

In other words, there is plainly a distinct quantitative difference, both in the results of the power of horse serum to render guinea pigs susceptible to other serums and in its toxic action upon guinea pigs sensitized by other serums.

The action of horse serum upon other animals than the guinea pig is treated of in another chapter (p. 55).

#### ARE OTHER BLOOD SERUMS EQUALLY TOXIC?

We now know that guinea pigs previously treated with small injections of horse serum become very susceptible to subsequent injections of horse serum, but only slightly susceptible to the blood serum of other animals. It becomes important to determine whether

guinea pigs treated with the serum of other animals (dog, hog, rat, cattle, sheep, cat) become as highly sensitized to a second injection of the homologous serum.

The following series of guinea pigs were all sensitized with 1/250 c. c. of the blood of various animals. Subsequently they were injected with horse serum to which they either did not respond or, after characteristic symptoms, recovered. These guinea pigs were finally injected with large quantities of the same kind of serum as that used at the first injection.

*Dog serum.*

G. P. No. 214. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 26 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. dog serum.]

5 hrs. later 6 c. c. dog serum injected into the peritoneal cavity. Symptoms.

G. P. No. 215. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 35 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. dog serum.]

22 days later 6 c. c. dog serum injected into peritoneal cavity. Severe symptoms.

G. P. No. 212. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Mild symptoms.

[Previous treatment: 35 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. dog serum.]

22 days later 6 c. c. dog serum injected into peritoneal cavity. Severe symptoms.

G. P. No. 211. Four c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 56 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. dog serum.]

1 day later 6 c. c. dog serum injected into the peritoneal cavity. Dead in 35 minutes

*Hog serum.*

G. P. No. 186. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Severe symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. hog serum.]

22 days later 6 c. c. hog serum injected into the peritoneal cavity. Severe symptoms.

G. P. No. 185. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. hog serum.]

22 days later 5 c. c. hog serum injected into the peritoneal cavity. No symptoms.

G. P. No. 187. Four c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 51 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. hog serum.]

1 day later 6 c. c. hog serum injected into the peritoneal cavity. Symptoms.

G. P. No. 189. Same injection and previous treatment. No symptoms.

1 day later 6 c. c. hog serum injected into the peritoneal cavity. Symptoms.

*Cattle serum.*

G. P. No. 206. Six c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 31 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *cattle serum*.]

22 days later 6 c. c. *cattle serum* injected into the peritoneal cavity. Dead in 12 minutes.

G. P. No. 210. Six c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 31 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *cattle serum*.]

22 days later 6 c. c. *cattle serum* injected into the peritoneal cavity. Dead in 45 minutes.

G. P. No. 207. Six c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 31 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *cattle serum*.]

22 days later 6 c. c. *cattle serum* injected into the peritoneal cavity. Dead in 90 minutes.

*Sheep serum.*

G. P. No. 184. Six c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *sheep serum*.]

22 days later 6 c. c. *sheep serum* injected into the peritoneal cavity. Severe symptoms; dead in 23 hours.

G. P. No. 182. Six c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. Severe symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *sheep serum*.]

22 days later 6 c. c. *sheep serum* injected into the peritoneal cavity. Severe symptoms.

G. P. No. 190. Four c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 47 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *sheep serum*.]

1 day later 6 c. c. *sheep serum* injected into the peritoneal cavity. Severe symptoms.

G. P. No. 183. Four c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 51 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *sheep serum*.]

1 day later 6 c. c. *sheep serum* injected into the peritoneal cavity. Dead in 18 hours.

G. P. No. 181. Same injection and previous treatment. Mild symptoms.

1 day later 6 c. c. *sheep serum* injected into the peritoneal cavity. Severe symptoms. Dead in 21 hours.

*Cat serum.*

G. P. No. 192. Six c. c. antitoxic *horse serum* (Natl. IX. 17) injected into the peritoneal cavity. Severe symptoms.

[Previous treatment: 34 days prior, inoculated subcutaneously with  $\frac{1}{250}$  c. c. *cat serum*.]

22 days later 6 c. c. *cat serum* injected into the peritoneal cavity. Severe symptoms.

**P. No. 195.** Four c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 55 days prior, inoculated subcutaneously with  $\frac{1}{30}$  c. c. *cat* serum.]

1 day later 6 c. c. *cat* serum injected into the peritoneal cavity. Dead in 35 minutes.

*Rat serum.*

**P. No. 168.** Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 34 days prior inoculated subcutaneously with  $\frac{1}{30}$  c. c. *rat* serum.]

2 hours later 2 c. c. *rat* serum injected into the peritoneal cavity. Severe symptoms. Dead in 3 hours.

We infer from the above series that guinea pigs treated with the serums of various animals and subsequently injected are much more susceptible to homologous serums than to heterologous serums. We have here another indication that this toxic action of blood serum is quantitatively specific.

We expect to take up the work with the serums of other animals more in detail, and the results will be reported at another time.

THE RELATION OF HEMOLYSIS.

So much work has recently been done indicating that the toxic property of blood serum is closely allied with its hemolytic action that we made some experiments in order to determine what connection exists between the hemolytic and toxic action of horse serum upon guinea pigs. As a result of these studies we believe that we have shown very clearly that blood serum may contain an acute poison entirely independent of any hemolytic action.

Landois <sup>a</sup> in his work on transfusion showed long ago (1875) that the blood serum of many mammals dissolves the red blood cells of other mammals. Landois specifically demonstrated that the red blood corpuscles of rabbits, when mixed with alien blood serum, are readily dissolved, while, on the other hand, the red blood corpuscles of cats and dogs are much more resistant. If large quantities of red blood corpuscles are dissolved, there follows an excretion of hemoglobin through the kidneys, intestines, and also into the serous cavities. If the serum is injected into the blood stream of a rabbit, some of the erythrocytes of the rabbit are dissolved in vivo, as may readily be confirmed microscopically; but before the corpuscles are dissolved there is an agglutination of the blood corpuscles into clumps, by means of which large capillary areas may become plugged. The same process is seen by the further extraction of hemoglobin from the mass of the stroma of the corpuscles which fills the capil-

<sup>a</sup> Landois: Lehrbuch der Physiologie des Menschen, 1896. Die Transfusion des Blutes. Leipzig, 1875.

laries. The dissolved hemoglobin now causes further blocking of the vessels by means of clots, which are formed through the action of the hemoglobin upon the leucocytes, freeing the fibrin ferment. From this somewhat complicated process there may result serious symptoms, as dyspnea, convulsions, etc., in proportion to the amount of blood injected. If the amount of blood is large, death may follow, as Landois has shown, with phenomena of asphyxiation. (Uhlenhuth: Zur Kenntniss der giftigen Eigenschaften des Blutserums. *Zeit. f. Hyg.*, vol. 26, 1897, p. 384.)

Hermann Pfeiffer,<sup>a</sup> confirming the work of Uhlenhuth upon the necrotic action of blood serums when injected subcutaneously into guinea pigs, believes that the toxic substance producing the necrosis is the same as the hemolytic agent found in the blood serum.

In recent years hemolysis has been studied more particularly by Ehrlich, Morgenroth, Bordet, Metchnikoff, and many others. Much of the work done appears to corroborate the early observations of Landois, who believed that the toxic action of blood serum when injected intravenously depends upon a "globulicidal" action upon the red blood cells and the plugging of the capillaries. On the other hand, we have the views of other authors based in part upon experimental data that the toxic action of blood serum is dependent upon albuminous substances independently of the lytic substance present in the serum.

Creite<sup>b</sup> in 1869 was aware of Landois's view concerning hemolysis, but separated the toxic power from the blood serum by means of coagulation (heat).

Also, Weiss<sup>c</sup> in 1896 showed by separating albuminous substances from the blood serum by means of heating or absolute alcohol that the serum has no longer the toxic power and concluded, therefore, that the toxic action is brought about by the albumins.

Albu<sup>d</sup> separated the albuminous substances from blood serum by precipitation with ammonium sulphate, which he dialyzed out, as it is itself poisonous, and came to the same conclusion as Creite and Weiss.

Rummo<sup>e</sup> believed that the toxic substance is a tox-albumin which, according to his views, is formed in the living cells of the respective animals.

<sup>a</sup> Hermann Pfeiffer: Über die nekrotisirende Wirkung normaler Seren. *Zeit. f. Hyg.*, 1905, Band 150, p. 183.

<sup>b</sup> Creite: Versuche über die Wirkung des Serumweisses nach Injection in das Blut. *Zeit. f. rationelle Med.* Bd. 36, S. 90.

<sup>c</sup> Weiss: Ueber die Wirkungen von Blutserum-Injectionen in's Blut. *Pflüger's Arch.*, Bd. 65, 1896.

<sup>d</sup> Albu: Untersuchungen über die Toxicität normaler u. pathol. Serumflüssigkeit. *Virchow's Arch.*, Bd. 149, 3.

<sup>e</sup> Rummo: Ueber die Giftigkeit des Blutserums bei Menschen und Thieren im normalen tande und bei Infectiouskrankheiten. *Wiener med. Woch.*, 1891, nr. 19-20.

Mairet and Bosc <sup>a</sup> were able to distinguish in blood serum a substance capable of producing coagulation and another substance capable of producing poisonous symptoms. They believed both of these substances to be albumins. They were able to eliminate the coagulation-producing substance by heating from 52° to 53° C. or by the addition of sodium chloride and sodium sulphate.

Pearce <sup>b</sup> believes that it appears to be definitely established that red blood corpuscle thrombi occur in the experimental lesions caused by sera possessing hemagglutinative power. He demonstrated that the capillaries of certain areas in the liver are entirely occluded by fused red cells, and he also showed the inability of the products of hemolysis to produce such action and necrotic areas in the liver.

L. Camus and E. Gley <sup>c</sup> first endeavored to separate the hemolytic action from the toxic action of blood serum by means of heating. They found that heat destroys the hemolytic action; but while it diminishes it does not entirely destroy its general toxic action. This work was done with eel serum. In their previous work the two actions seemed about parallel. For instance, eel serum is very hemolytic for rabbits and guinea pigs and equally poisonous for these animals. On the other hand, the red blood cells of the pigeon and those of the porcupine are very resistant to eel serum, and these animals also resist doses which are fatal for the rabbit and the guinea pig. However, these authors finally found an animal, the marmot, for which eel serum had slight hemolytic action but was extremely toxic, and they believed that these researches upon the marmot have permitted them to separate the toxic action of eel serum from its hemolytic action better than did their previous work by means of heating.

We found horse serum to have practically no hemolytic action upon the corpuscles of the guinea pig. This applies with equal force to antitoxic horse serum as to normal horse serum, as may be seen from the following table:

*Hemolysis.*

G. P. No. 2. Normal pig. Serum obtained by coagulation.

G. P. No. 1. Normal pig. Copuscles obtained by centrifugation, etc.

<sup>a</sup> Mairet and Bosc: Recherches sur les causes de la toxicité du serum du sang. Compt. rend., t. 119, 4, p. 292. Toxicité du serum du sang de l'homme sain, Compt. rend., 1897. Des effets de la chaleur sur la toxicité du sérum. Compt. rend. July 7, 1894.

<sup>b</sup> Pearce, Richard M.: A further study of the experimental production of liver necrosis by the injection of hemagglutinative sera. J. M. Research, v. 14, Apr., 1906, p. 541. Pearce, R. M., and Winne, C. K., jr.: Concerning hemagglutins of bacterial origin and their relation to hyalin thrombi and liver necroses. Am. J. M. Sci., v. 128, 1904, p. 669.

<sup>c</sup> Camus, L., and Gley, E.: Comparison between the hemolytic and the toxic action of eel's serum in the marmot (*Arctomys marmota*). Arch. internat. de pharmacodyn. et de therapie, tome 15, fasc. 1 and 2, 1905.



G. P. No. 4517. Serum obtained by coagulation.

[Previous treatment: 35 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. 4510. Corpuscles obtained by centrifugation, etc.

[Previous treatment: 35 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

1 c. c. of—	1 c. c., 5 per cent corpuscles, normal guinea pig No. 1 (washed).		1 c. c., 5 per cent corpuscles, treated guinea pig No. 4510 (washed).		
	Do. +1 gtt. serum normal guinea pig No. 2.	Do. +1 gtt. serum guinea pig No. 4517.	Do. +1 gtt. serum normal guinea pig No. 2.	Do. +1 gtt. serum guinea pig No. 4517.	Do. +1 gtt. serum guinea pig No. 4517.
Normal horse serum (Sam.).....	0	0	0	0	0
Normal donkey serum.....	Faintest trace.	0	0	0	0
Normal horse serum (Sp.).....	0	Faintest trace.	0	0	Faintest trace.
Antitoxic horse serum (Natl. VIII. 17)...	0	0	0	0	0
Antitoxic horse serum (Natl. IX. 19)....	0	0	0	0	0
Antitoxic horse serum (Natl. V. 14).....	0	0	0	0	0
Antitoxic horse serum (Alex. A206, Gibson's method).	Trace.	Trace.	Trace.	Trace.	Trace.
Salt solution (control).....	0	Trace.	0	0	0

First the corpuscles were placed in the test tubes, then the various horse serums, and finally the drop of guinea-pig serum.

The corpuscles were obtained by whipping the blood and washing in the usual way four times and suspended so as to make the so-called 5 per cent suspension. After the corpuscles and the serum were mixed they were well shaken and placed in the incubator, again shaken at the end of one hour, again at the end of two hours, and at the end of three hours taken out of the incubator and placed in the ice chest. The readings were taken the next morning.

This work was repeated several times until we were satisfied that neither the normal horse serum nor the antitoxic horse serum had practically any power of dissolving the hemoglobin from the guinea-pig corpuscles. There can therefore be no doubt that there is a toxic substance in the blood serum of horses entirely independent of any hemolytic action. As a further corroboration we may state that autopsies upon guinea pigs which have died from the results of the injections of horse serum showed none of the usual effects, indicating that hemolysis took place within the body.

Further, horse serum heated to 60° for six hours is as toxic as unheated serum.

As a further study of the relation of hemolysis to the toxic action and also of the relation of the toxic principle to the haptin group we made the following experiments:

G. P. No. 249. Four subcutaneous injections of 1.5 c. c. each on successive days, using normal roan horse corpuscles washed three times. The blood serum of this guinea pig developed a high degree of hemolytic power against horse corpuscles. The blood serum of this guinea pig was mixed with equal quantities of normal horse serum and the mixture injected into the following three guinea pigs:

G. P. No. 4983. Two c. c. of the above mixture injected into the peritoneal cavity. Dead in 40 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.23 c. c. toxine No. 7 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Alex. A206).]

G. P. No. 4988. Same injection. Dead in 30 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.23 c. c. toxine No. 7 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Alex. A202).]

G. P. No. 4992. Same injection. Dead in 35 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Alex. A208).]

The above experiment was repeated as follows:

G. P. No. 248. Four subcutaneous injections of 1.5 c. c. each on successive days were made, using roan corpuscles washed three times. The blood serum of this guinea pig developed a high degree of hemolytic power against horse corpuscles. The serum was mixed with equal quantities of normal horse serum and the mixture injected into the following guinea pigs:

G. P. No. 4913. Four c. c. of the above mixture injected into the peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 41 days prior, inoculated subcutaneously with 0.139 c. c. of toxine No. 5 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Stearns 12D).]

G. P. No. 4914. Two c. c. same serum injected into the peritoneal cavity. Dead in 20 minutes.

[Previous treatment: Same as G. P. No. 4913.]

#### THE RELATION OF PRECIPITIN TO THE TOXIC ACTION.

The precipitins are so specific and so closely related to the cytotoxins that it naturally occurred to us to determine what relation there may exist between the toxic power of the blood serum of the horse and of the guinea pig, and the power of these two serums to precipitate each other.

If we inject horse serum several times into a guinea pig we would expect to find that the blood serum of a guinea pig so treated would have a power of precipitating horse serum. This we found to be the case; but we could not find that the injection of a single small dose of horse serum into the guinea pig developed this power in the guinea pig's blood serum. We might, however, conjecture that the union of the two serums under the conditions named causes a precipitation which is not visible, or causes a chemical union between two highly organized albuminous substances having toxic properties. In order to test this subject more thoroughly we first made some experiments to determine the precipitating action of normal and antitoxic horse serum upon normal guinea-pig serum, as follows:

One c. c. of horse serum in dilution of 1:21 was placed in a small test tube. The 0.1 c. c. of normal guinea-pig serum was added to each tube. These were thoroughly shaken, placed in the incubator, and the results read after twenty-four hours.

Normal G. P. No. 1. Killed the same day with chloroform, blood defibrinated, and the serum separated in the centrifuge.

Normal G. P. No. 2. Killed by chloroform 3 days previously and the serum allowed to separate by coagulation.

(Two tests made of each serum.)

1 c. c. horse serum (1:21).	0.1 c. c. serum normal guinea pig 1.	0.1 c. c. serum normal guinea pig 2.
Normal horse serum (Sam).....	Nil.	Nil.
Normal horse serum (Jane).....	Nil.	Nil.
Antitoxic horse serum (Natl. VIII, 17).....	Nil.	Nil.
Antitoxic horse serum (Hubbert op. 1).....	Nil.	Nil.
Antitoxic horse serum (Natl. IX, 17).....	Slight precipi- tation(?).	Nil.
Antitoxic horse serum (Stearns 1110).....	Nil.	Nil.
	Nil.	Nil.
	Nil.	Nil.
	Nil.	Nil.

From this and other similar experiments we are justified in concluding that neither normal horse serum nor antitoxic horse serum has any power to precipitate normal guinea-pig serum.

We next made some tests to determine whether a guinea pig previously treated with one small injection of horse serum developed precipitins.

One c. c. of horse serum both normal and antitoxic in dilution of 1:21 was placed in small tubes, as before, and 0.1 c. c. of a sensitized guinea-pig serum added to each tube, as shown in the table. They were shaken and placed in the incubator and results read after twenty-four hours.

G. P. No. 4617. [Previous treatment: 35 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (standard B27).]

G. P. No. 4510. [Previous treatment: same.]

1 c. c. horse serum (1:21).	0.1 c. c. normal guinea-pig serum.		0.1 c. c. treated guinea-pig serum.	
	Centrif- ugation.	Coagula- tion.	Guinea pig 4517.	Guinea pig 4510.
Normal horse serum (Sam).....	0	0	0	0
Normal horse serum (Jane).....	0	0	0	0
Normal horse serum (S. P.).....	0	0	0	0
Normal donkey serum.....	0	0	0	0
Antitoxic horse serum (Natl. VIII, 17).....	0	0	0	0
Antitoxic horse serum (Natl. V, 14).....	0	0	0	0
Antitoxic horse serum (Natl. IX, 17).....	+(?)	0	0	0
Antitoxic horse serum (Stearns 1110).....	0	0	0	0
Antitoxic horse serum (Stearns 1429).....	0	0	0	0

It would therefore appear that guinea pigs which have previously been inoculated with one injection of a very small amount of horse serum sufficient to render them highly susceptible to a second injection of horse serum do not contain sufficient precipitin in their

blood serum to cause a visible precipitation when added to horse serum.

The following studies show still more clearly that the specific precipitins have nothing to do with this toxic reaction.

We prepared guinea pigs by injecting them many times at short intervals with horse serum, so as to develop a high degree of precipitin in their blood serum against horse serum. The blood serum of guinea pigs so prepared was then added to horse serum in the proportions of about one of the former to two of the latter and allowed to stand until the precipitating action was complete. This guinea-pig serum was so active that very large quantities of the precipitum settled to the bottom of the test tubes. The mixture was now filtered, so as to separate the precipitum entirely, and the filtrate injected into sensitized guinea pigs and found to be quite as toxic as untreated horse serum.

G. P. No. 107. Seventeen subcutaneous injections of 1 c. c. each normal horse serum (roan) and one peritoneal injection of 6 c. c. antitoxic horse serum (Natl. IX, 19) were given between February 8 and March 11.

Six c. c. normal horse serum (roan) were mixed with 3 c. c. of the serum of this guinea pig and allowed to stand about 3 hours at room temperature. The large amount of precipitum which developed was filtered off and the filtrate injected into the following two guinea pigs:

G. P. No. 4996. Two c. c. of the above filtrate injected into peritoneal cavity. Dead in 20 minutes.

[Previous treatment: 27 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Wellcome 474A).]

G. P. No. 5000. Five c. c. of the above filtrate injected into the peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Wellcome 477B).]

These experiments were repeated under varying conditions, especially allowing the two serums to remain in contact a longer time before filtering, and similar results obtained.

The results of this part of our work are strengthened by the conclusions of Pirquet and Schick, who found that the antibodies, whatever they may be, producing symptoms of the serum disease, are not identical with the precipitins. They found, for instance, that the symptoms of the serum disease appear within eight to thirteen days following the first injection of horse serum into man, whereas it requires about three weeks for precipitins to appear in the blood in children after the injection of horse serum.

The formation of precipitins does not take place as readily in man following the injection of horse serum as it does in rabbits. In fact, Pirquet and Schick found that sometimes even after the injection of 200 c. c. there was no production of precipitins, and, further, that the length of time that the precipitin reaction remains in the

blood of man following the injection is variable. It sometimes disappears in the fourth week, and sometimes is still markedly present in the ninth week.

Finally, we know that the precipitins are strictly specific, whereas the toxic action is only quantitatively specific.

#### EFFECT OF HEAT UPON THE TOXICITY OF HORSE SERUM.

One of the first questions which required solution was whether the toxic substance in blood serum is thermolabile. That it is able to withstand a comparatively high degree of heat for a long time, viz, 60° C. for six hours, is evident from the following experiments:

G. P. No. 4349. Six c. c. antitoxic horse serum (Natl. VIII. 17) heated to 60° C. for 30 minutes, then injected into the peritoneal cavity. Dead in 30 minutes.

G. P. No. 60. One c. c. same serum and injection as g. p. 4349. Dead in 10 minutes.

[Previous treatment: With toxine-antitoxin mixture.]

G. P. No. 61. Do. Dead in 10 minutes.

G. P. No. 62. Do. Symptoms; recovered.

G. P. No. 4897. Injected with 5 c. c. of an extract of normal horse serum (roan) heated to 60° C. for 6 hours. This degree of heat coagulated the serum. About 1 c. c. of the coagulum was added to 9 c. c. of salt solution, thoroughly shaken up, filtered, and the filtrate injected into the peritoneal cavity. Symptoms; death in about 3 hours.

[Previous treatment: 41 days prior, injected subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{8}$  c. c. horse serum (Alex. No. 192).]

G. P. No. 4508. Six c. c. of an extract of antitoxic horse serum (Natl. IX. 19) heated to 100° C. for 15 minutes, injected into the peritoneal cavity. The coagulum was ground up with a few c. c. of salt solution and strained through gauze before being injected. No symptoms.

One and a half hours later about 8 c. c. of the coagulum was ground up and the entire mass, not strained, injected into the peritoneal cavity of the same guinea pig. No symptoms.

G. P. No. 5048. Six c. c. of the coagulum of normal horse serum (roan) heated to 100° C. for 15 minutes and ground up with salt solution (0.85) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 20 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{5}\frac{1}{8}$  c. c. antitoxic horse serum (Alex. A204).]

From the above it is evident that while a temperature of 60° C. continued for six hours has practically no influence upon the toxic substance in horse serum, the toxicity is entirely destroyed by a temperature of 100° C. for fifteen minutes.

#### THE TOXIC PRINCIPLE IN HORSE SERUM IS FILTERABLE THROUGH PORCELAIN.

The following experiment is sufficient to demonstrate that the close-grained pores of a Pasteur-Chamberland filter B do not retain the toxic principle of horse serum:

G. P. No. 4521. Six c. c. of antitoxic horse serum (Natl. VIII. 17) filtered through a Pasteur-Chamberland filter B and injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 32 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

**DRYING DOES NOT INJURE THE TOXIC PRINCIPLE.**

We have on hand a large quantity of dried antitoxic horse serum which has been prepared and preserved under special conditions for the preparation of the standard antitoxic unit. This serum was carefully dried under bacteriological precautions at a temperature below 37° C., as described in Bulletin No. 21.<sup>a</sup>

That such dried and redissolved serum still retains its toxic property is evident from the following experiment:

G. P. No. 4376. Six c. c. antitoxic horse serum (B 1+12) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 19 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{10}$  c. c. horse serum (Natl. V. 7).]

**THE TOXIC PRINCIPLE CAN NOT BE SEPARATED BY PRECIPITATION AND DIALYSIS.**

We made several experiments to determine whether precipitation by ammonium sulphate and subsequent dialysis to remove the sulphate and other inorganic salts would also remove the toxic principle. For this purpose we used antitoxic horse serum prepared and dialysed in accordance with Gibson's method.<sup>b</sup> It is plain from the following experiments that precipitation and dialysis do not remove the toxic principle:

G. P. No. 4104. Six c. c. antitoxic horse serum (NYBH A) injected into the peritoneal cavity. Dead in 5 minutes.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{10}$  c. c. horse serum (Alex. 190).]

G. P. No. 4506. Five c. c. antitoxic horse serum (Alex. A 203/NYBH) injected into the peritoneal cavity. No symptoms.<sup>c</sup>

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4513. Five c. c. antitoxic horse serum (Alex. A 203/NYBH) injected into the peritoneal cavity. Dead in 39 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

**EFFECT OF VARIOUS CHEMICAL SUBSTANCES UPON THE TOXIC PRINCIPLE OF HORSE SERUM.**

Various substances of widely different chemical nature were added to horse serum, in order to see whether any of them had the power of precipitating, oxidizing, or neutralizing the toxic property of the serum. The following substances were used: Succinic acid per-

<sup>a</sup> Rosenau, M. J.: The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum); official standard prepared under the act approved July 1, 1902. Bulletin 21, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service. Washington, Government Printing Office, April, 1905.

<sup>b</sup> Gibson, R. B.: On the practical concentration of diphtheria antitoxin. Am. med., vol. 10, 1905, p. 915.

<sup>c</sup> Explanation of occasional irregularities, p. 63.

oxide (alphozone), butyric acid, hydrogen dioxide, permanganate of potash, alcohol, ammonium sulphate, and citrate of soda.

*Butyric acid.*

G. P. No. 4368. Ten c. c. of a  $\frac{N}{10}$  butyric acid solution were added to 25 c. c. antitoxic horse serum (Natl. IX. 17); the mixture was kept at 15° C. for 40 hours, at the end of which time the fine precipitate which developed was filtered off and 3 c. c. injected into the peritoneal cavity. Dead in 2 hours 10 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{3\frac{1}{2}}$  c. c. horse serum (Cutter 1461).]

*Hydrogen dioxide.*

G. P. No. 4371. Five c. c. of hydrogen dioxide (Oakland) were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours, at the end of which time a very little fine precipitate had formed, but the mixture effervesced in filtering; 3 c. c. of the filtrate was injected into the peritoneal cavity. Symptoms (?); recovery.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{2\frac{1}{2}}$  c. c. horse serum (Cutter 1504).]

G. P. No. 4917. Five c. c. hydrogen dioxide (McKesson & Robbins) were added to 25 c. c. antitoxic horse serum (Natl. IX. 17); the mixture was kept at 15° C. for 40 hours, at the end of which time the fine precipitate which developed was filtered off, and 6 c. c. injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 41 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{1\frac{1}{2}}$  c. c. antitoxic horse serum (Stearns 16H).]

*Permanganate of potash.*

G. P. No. 4370. Three c. c. of a 1 per cent solution of permanganate of potash were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours, and as no precipitate had formed in that time it was not filtered; 3 c. c. of the mixture inoculated into the peritoneal cavity. Dead in 45 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{4\frac{1}{2}}$  c. c. horse serum (Cutter 1461).]

*Succinic peroxide acid (alphozone).*

G. P. No. 4372. Five c. c. of a 1 per cent solution of alphozone were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours; the precipitate which formed was filtered off and 3 c. c. of the filtrate injected into the peritoneal cavity. No symptoms.<sup>a</sup>

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{3\frac{1}{2}}$  c. c. horse serum (Cutter 1504).]

G. P. No. 5A. Ten c. c. of the same filtrate injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 30 days prior, inoculated with 0.22 c. c. toxine No. 7 + 1 unit of horse serum (Standard B27).]

As we found that 2 c. c. of a 1 per cent solution of succinic peroxide acid (alphozone) injected into the peritoneal cavity of a normal guinea pig produced transient irritating effects these experiments were repeated, using smaller quantities of the acid, as follows:

<sup>a</sup> Explanation of occasional irregularities, p. 63.

G. P. No. 4519. One c. c. of a 1 per cent solution of alphozone was added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15 C. for 40<sup>h</sup> hours; the precipitate which formed was filtered off and 6 c. c. of the filtrate injected into the peritoneal cavity. Dead in 24 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4518. Five c. c. added as above and 6 c. c. of the filtrate injected into the peritoneal cavity. Dead in 25 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

#### *Alcohol.*

G. P. No. 4373. Five c. c. of 95 per cent alcohol were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours; the precipitate which formed was filtered off and 3 c. c. of the filtrate injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{3}{37}$  c. c. horse serum (Cutter 1504).]

G. P. No. 5B. Seven and a half c. c. added as above and 3 c. c. of the filtrate injected into the peritoneal cavity. No symptoms.<sup>a</sup>

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 156. Seven and a half c. c. added as above and 6 c. c. of the filtrate injected into the peritoneal cavity. Dead in 90 minutes.

[Previous treatment: 60 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{300}$  c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 4363. Ten c. c. added as above and 3 c. c. of the filtrate injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{330}$  c. c. horse serum (P D & Co .07173-G).]

G. P. No. 4C. Twenty-five c. c. added as above. This caused practically solid coagulation on the serum. The coagulant was vigorously shaken up with salt solution (0.85) and filtered by the aid of a vacuum, and 6 c. c. of the opalescent filtrate injected into the peritoneal cavity. Dead in 4 hours 10 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

#### *Ammonium sulphate.*

We know from the work done with serum precipitated with ammonium sulphate and subsequently dialysed that the ammonium sulphate has no action upon the toxic principle found in horse serum. (See guinea pigs Nos. 4104, 4106, and 4513, p. 39.)

#### *Citrate of soda.*

We know that citrate of soda has no injurious action upon the toxic principle found in horse serum because some of the serum we used was prepared by adding 1 per cent solution of citrate of soda to the whole blood as it was drawn from the horse in order to prevent

<sup>a</sup> For explanation of occasional irregularities, see p. 63.



clotting and the clear serum subsequently pipetted off. The citrate of soda was afterwards removed by dialysis (Gibson's method) and the plasma proved just as toxic to treated guinea pigs as other horse serum. We also have the following experimental data upon this point:

G. P. No. 4925. Six c. c. normal horse serum (roan) containing 1 per cent citrate soda injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Cutter 1465).]

G. P. No. 4918. Injected as above. Dead in 5 minutes.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Stearns 16 H).]

G. P. (control). The above guinea pigs were controlled by injecting 6 c. c. of the same serum containing 1 per cent citrate of soda into the peritoneal cavity of a normal pig. The only effects of this inoculation were symptoms of irritation which soon passed away.

#### INFLUENCE OF ANTISEPTICS, ETC.

As much of this work was done with horse serum preserved by means of chloroform and trikresol (0.4 per cent) we made a control experiment, using horse serum as fresh as possible without the addition of any substance. The following experiment eliminates these factors as having any influence upon the toxicity of the serum:

G. P. No. 125. Three c. c. normal horse serum (roan) freshly drawn, centrifuged and injected into the peritoneal cavity as soon as the serum separated. Dead in 45 minutes.

[Previous treatment: 49 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Stearns 1351).]

#### EFFECT OF OLD HORSE SERUM UPON SUSCEPTIBLE GUINEA PIGS.

At one time in our work it appeared to us that the toxic properties of horse serum gradually diminished in toxicity with the age of the serum. It was, however, soon proved by the following experiments that very old serums may be quite as poisonous as those freshly drawn.

G. P. No. 4496. Four c. c. antitoxic horse serum (Natl. V. 2) injected into the peritoneal cavity. The serum was at least *one and a half years old* when injected. Dead in 30 minutes.

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit of horse serum (Standard B27).]

G. P. No. 4921. Six c. c. antitoxic horse serum *over 3 years old* (P. D. & Co. 0495 prepared Dec. 15, 1902) inoculated into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Cutter 1465).]

G. P. No. 4922. Same injection. No symptoms.<sup>a</sup>

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Cutter 1465).]

G. P. No. 4899. Same injection. Dead in 6 minutes.

[Previous treatment: 48 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{10}$  c. c. antitoxic horse serum (Alex. 192).]

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<sup>a</sup> Explanation of occasional irregularities, p. 63.

The following guinea pigs were inoculated with horse serum eight and one-half years old:

G. P. No. 258. Six c. c. antiamarillic serum injected into the peritoneal cavity. Dead in 40 min.

[Previous treatment: 24 days prior, inoculated subcutaneously with  $\frac{1}{10}$  c. c. normal horse serum (roan) heated to 60° C. for two hours.]

G. P. No. 256. Same injection. Symptoms, recovered.

[Previous treatment: 20 days prior, inoculated subcutaneously with  $\frac{1}{10}$  c. c. normal horse serum (roan) heated to 60° C. for two hours.]

From the above experiments we see that blood serum over eight and one-half years old is as poisonous to sensitized pigs as fresh serum.

#### EFFECT OF X-RAYS UPON THE TOXIC PRINCIPLE.

Normal horse serum (roan) was exposed to the action of X rays, 40 amperes, 4 inches from the tube for forty minutes, then injected into a susceptible guinea pig. It was found as poisonous as horse serum not so treated.

G. P. No. 6A. Six c.c. above serum injected into peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 33 days prior, inoculated subcutaneously with 0.293 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B28).]



## Part V.

### THE INFLUENCE OF TIME.

#### TIME NECESSARY TO RENDER A GUINEA PIG SUSCEPTIBLE.

We know from our preliminary work that some time is necessary after the first injection of horse serum before a guinea pig is rendered susceptible to the toxic action of a second injection. The following series demonstrates that this time is about ten days:

- G. P. No. 4464. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: *5 days* prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]
- G. P. No. 4485. Six c. c. same serum and injection as g. p. 4484. No symptoms.  
[Previous treatment: *6 days* prior, as g. p. 4484.]
- G. P. No. 4486. Six c. c. same serum and injection as g. p. 4484. No symptoms.  
[Previous treatment: *7 days* prior, as g. p. 4484.]
- G. P. No. 4533. Six c. c. same serum and injection as g. p. 4484. No symptoms.  
[Previous treatment: *8 days* prior, as g. p. 4484.]
- G. P. No. 4530. Six c. c. same serum and injection as g. p. 4484. Symptoms; recovered.  
[Previous treatment: *10 days* prior, as g. p. 4484.]
- G. P. No. 4532. Six c. c. same serum and injection as g. p. 4484. Symptoms; recovered.  
[Previous treatment: *11 days* prior, as g. p. 4484.]
- G. P. No. 4488. Six c. c. same serum and injection as g. p. 4484. Dead in 10 minutes.  
[Previous treatment: *12 days* prior, as g. p. 4484.]
- G. P. No. 4525. Six c. c. same serum and injection as g. p. 4484. Dead in 45 minutes.  
[Previous treatment: *12 days* prior, as g. p. 4484.]
- G. P. No. 4487. Six c. c. same serum and injection as g. p. 4484. Dead in 60 minutes.  
[Previous treatment: *13 days* prior, as g. p. 4484.]
- G. P. No. 4531. Six c. c. same serum and injection as g. p. 4484. Dead in 45 minutes.  
[Previous treatment: *13 days* prior, as g. p. 4484.]
- G. P. No. 4476. Six c. c. same serum and injection as g. p. 4484. Dead in 10 minutes.  
[Previous treatment: *14 days* prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{10}$  c. c. antitoxic serum (Natl. IX. 18).]
- G. P. No. 4490. Six c. c. same serum and injection as g. p. 4484. Dead in 46 minutes.  
[Previous treatment: *14 days* prior, as g. p. 4484.]
- G. P. No. 4529. Six c. c. same serum and injection as g. p. 4484. Dead in 47 minutes.  
[Previous treatment: *15 days* prior, as g. p. 4484.]

#### 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 160 days elapsed between the first treatment and the second

injection. How much longer this action may continue will be ascertained and reported upon at a subsequent time.

G. P. No. 4507. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Dead in 20 minutes.

[Previous treatment: 60 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4A. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 22 minutes.

[Previous treatment: 84 days prior, inoculated subcutaneously with 0.293 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 3827. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 33 minutes.

[Previous treatment: 160 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. horse serum (Jordan No. 201).]

## Part VI.

### DOSAGE AS A FACTOR.

#### MINIMAL AMOUNT OF HORSE SERUM NECESSARY TO RENDER A GUINEA PIG SUSCEPTIBLE.

A large part of our work was done upon guinea pigs which had been used to measure the strength of antitoxic serums. As the strength of diphtheria antitoxin runs between 250 and 1,000 units to the c. c. most of our guinea pigs were therefore rendered susceptible by doses of horse serum varying from  $\frac{1}{100}$  to  $\frac{1}{1000}$  c. c. It now becomes interesting to know how small a quantity of horse serum injected into a healthy guinea pig would render it susceptible to the toxic action of horse serum.

G. P. No. 217. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with  $\frac{1}{100}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 219. Do.

G. P. No. 216. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with  $\frac{1}{100}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 222. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with  $\frac{1}{1000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 223. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 130 minutes.

[Previous treatment: 45 days prior, inoculated subcutaneously with  $\frac{1}{1000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 220. Do. Dead in 68 minutes.

G. P. No. 225. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with  $\frac{1}{1000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 224. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with  $\frac{1}{1000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 227. Do.

G. P. No. 229. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with  $\frac{1}{1000000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 230. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with  $\frac{1}{1000000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 228. Do.

G. P. No. 232. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 24 days prior, inoculated subcutaneously with  $\frac{1}{1000000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 235. Do.

G. P. No. 234. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with  $\frac{1}{1000000}$  c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 233. Do. Symptoms; recovered.

It is plain from the above series that guinea pigs may be sensitized with very small amounts of horse serum. Guinea pig No. 233 received only  $\frac{1}{1000000}$  of a c. c. at the first injection and this quantity was sufficient to render it susceptible to a subsequent injection of horse serum.

#### MINIMAL AMOUNT OF HORSE SERUM NECESSARY TO POISON A SUSCEPTIBLE GUINEA PIG.

From the following series of guinea pigs it will be seen that the toxic principle must be present in exceedingly small quantities of horse serum.

G. P. No. 4348. Four c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 30 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{400}$  antitoxic horse serum (PD 08021).]

G. P. No. 4099. Three c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{100}$  c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 3954. Three c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 57 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{300}$  c. c. antitoxic horse serum (Hubbert 1).]

G. P. No. 4364. Three c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Dead in 45 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{300}$  c. c. antitoxic horse serum (PD 07173).]

G. P. No. 4350. Two c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{300}$  c. c. antitoxic horse serum (PD 08021).]

G. P. No. 4352. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{4}\frac{1}{8}$  c. c. antitoxic horse serum (PD 08021).]

G. P. No. 4101. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{2}\frac{1}{8}$  c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 4511. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4512. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Dead in 81 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4623. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}\frac{1}{8}$  c. c. antitoxic horse serum (Alex. 192).]

G. P. No. 4514. Two-tenths c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4520. Two-tenths c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 65 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 130. One-tenth c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Dead in 55 minutes.

[Previous treatment: 45 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}\frac{1}{8}$  c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 151. One-tenth c. c. antitoxic horse serum (Natl. IX. 19) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}\frac{1}{8}$  c. c. antitoxic horse serum (Stearns).]

G. P. No. 136. Do. Symptoms; recovered.

G. P. No. 144. Do. No symptoms.

G. P. No. 165. One hundredth c. c. antitoxic horse serum (Natl. IX. 17) inoculated subcutaneously. No symptoms.

3 hrs. 45 min. later, 0.1 c. c. Do. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}\frac{1}{8}$  c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 148. One-hundredth c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

3 hrs. 45 min. later, 0.1 c. c. subcutaneously. Symptoms; recovered.

It will be seen from the above series that one-tenth of a cubic centimeter of horse serum into the peritoneal cavity of a susceptible guinea pig is sufficient to cause the death of the animal, whereas the same quantity subcutaneously causes symptoms.



THE INFLUENCE OF LARGE QUANTITIES IN RENDERING GUINEA PIGS  
SUSCEPTIBLE.

The guinea pigs in the following series were first used as control to show that the injection of large quantities of horse serum into the peritoneal cavity produces no effect. They were subsequently injected with horse serum again in large quantities into the peritoneal cavity to determine their susceptibility:

G. P. No. 296. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 14 days prior, 6 c. c. donkey serum injected into the peritoneal cavity.]

G. P. No. 297. Do.

G. P. No. 298. Same injection. No symptoms.

[Previous treatment: 14 days prior, 6 c. c. normal horse serum (Sp.) injected into the peritoneal cavity.]

G. P. No. 299. Do. Symptoms; recovered.

G. P. No. 300. Same injection. Symptoms; recovered.

[Previous treatment: 22 days prior, 6 c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity.]

G. P. No. 301. Do.

G. P. No. 302. Same injection. Symptoms; recovered.

[Previous treatment: 14 days prior 6 c. c. antitoxic horse serum (Natl. V. 14) injected into the peritoneal cavity.]

G. P. No. 303. Do. Symptoms (?)

G. P. No. 304. Same injection. Dead in 65 minutes.

[Previous treatment: 14 days prior 6 c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity.]

G. P. No. 305. Do. Dead in 95 minutes.

G. P. No. 306. Same injection. Symptoms; recovered.

[Previous treatment: 14 days prior, 3.5 c. c. antitoxic horse serum (NYBH 10B Gibson) injected into the peritoneal cavity.]

G. P. No. 307. Same injection. Dead in 48 minutes.

[Previous treatment: 14 days prior, 2 c. c. antitoxic horse serum (NYBH 13C Gibson) injected into the peritoneal cavity.]

G. P. No. 308. Same injection. Dead in 43 minutes.

[Previous treatment: 14 days prior, 3 c. c. antitoxic horse serum (Alex. A211) injected into the peritoneal cavity.]

G. P. No. 309. Same injection. Symptoms; recovered.

[Previous treatment: 14 days prior, 6 c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity.]

G. P. No. 310. Do. Symptoms; recovered.

It is plain, therefore, that guinea pigs may be rendered susceptible by the injection of large amounts of horse serum into the peritoneal cavity. We have seen, in another part of this bulletin, that these animals may also be rendered susceptible by the injection of large amounts into the subcutaneous tissue.

We believe, however, that guinea pigs are rendered more susceptible by the injection of the toxine-antitoxin mixture than by the horse serum alone.

## Part VII.

### SENSITIZING SUBSTANCE.

We already know that guinea pigs may be sensitized with either normal or antitoxic horse serum. It is of course both interesting and important to know whether the substance contained in horse serum which renders the guinea pigs sensitive is the same as the substance which exerts its poisonous action upon the second injection.

#### GUINEA PIGS MAY BE SENSITIZED WITH PRECIPITATED AND DIALYZED SERUM.

The following examples selected from our work demonstrate this point:

G. P. No. 4972. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 22 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{8}$  c. c. antitoxic horse serum (NYBH 13C, precipitated and dialyzed in accordance with Gibson's method).]

G. P. No. 4973. Do.

G. P. No. 4955. Six c. c. same serum. Dead in 27 minutes.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{8}$  c. c. antitoxic horse serum (NYBH 10B).]

G. P. No. 4957. Same injection. Symptoms; recovered.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{8}$  c. c. antitoxic horse serum (NYBH 10B).]

#### DRYING DOES NOT INJURE THE SENSITIZING SUBSTANCE.

A number of guinea pigs which were used by us in this work were sensitized with the antitoxic serum preserved under special conditions in this laboratory as the official standard in accordance with the law of July 1, 1902. This serum was reduced to dryness under special conditions and must be redissolved before using. Compare page 39.

The following are selected as experimental data proving that drying does not injure the sensitizing substance:

G. P. No. 6B. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 27 minutes.

[Previous treatment: 86 days prior inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B25).]

G. P. No. 4488. Same injection. Dead in 10 minutes.

[Previous treatment: 12 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

SMALL QUANTITIES OF HORSE SERUM MAY RENDER GUINEA PIGS MORE SUSCEPTIBLE THAN LARGE QUANTITIES.

Before we learned that the antitoxin contained in the blood serum of the horse plays no rôle in rendering guinea pigs susceptible to subsequent inoculations of horse serum we planned and carried out an extensive series of experiments to determine just what relation the susceptibility of guinea pigs bears to the antitoxic potency of the horse serum injected. One series of guinea pigs was injected subcutaneously with one unit of an antitoxic horse serum containing 250 units to the c. c. This series, therefore, received  $\frac{1}{250}$  c. c. of horse serum. The second series was treated subcutaneously with 250 units—that is, the guinea pigs received 1 c. c. of the horse serum. The third series of guinea pigs was treated with 1,000 units—that is, each guinea pig received 4 c. c. of the antitoxic horse serum. At varying intervals following these injections the pigs were given a second injection of horse serum in order to determine their susceptibility.

The following guinea pigs received  $\frac{1}{250}$  c. c. antitoxic horse serum containing 1 unit:

- G. P. No. 18. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity.  
 [Previous treatment: 1 day prior, inoculated subcutaneously with 1 unit, viz,  $\frac{1}{250}$  c. c., horse serum (Natl. IX. 18).] No symptoms.
- G. P. No. 17. Same injection as g. p. 18. No symptoms.  
 [Previous treatment: 4 days prior, as g. p. 18.]
- G. P. No. 11. Same injection as g. p. 18. Dead in 35 minutes.  
 [Previous treatment: 17 days prior, as g. p. 18.]
- G. P. No. 16. Same injection as g. p. 18. Symptoms; recovered.  
 [Previous treatment: 18 days prior, as g. p. 18.]
- G. P. No. 15. Same injection as g. p. 18. Dead in 18 minutes.  
 [Previous treatment: 24 days prior, as g. p. 18.]
- G. P. No. 19. Same injection as g. p. 18. Symptoms; recovered.  
 [Previous treatment: 32 days prior, as g. p. 18.]
- G. P. No. 12. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into peritoneal cavity. Dead in 44 minutes.  
 [Previous treatment: 47 days prior, as g. p. 18.]
- G. P. No. 10. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. No symptoms.  
 [Previous treatment: 47 days prior, as g. p. 18.]
- G. P. No. 13. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.  
 [Previous treatment: 68 days prior, as g. p. 18.]

The following guinea pigs received 1 c. c. antitoxic horse serum containing 250 units:

- G. P. No. 29. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.  
 [Previous treatment: 1 day prior, inoculated subcutaneously with 250 units, viz, 1 c. c., horse serum (Natl. IX. 18).]

- G. P. No. 27. Same injection as g. p. 29. No symptoms.  
[Previous treatment 4 days prior, as g. p. 29.]
- G. P. No. 28. Same injection as g. p. 29. Symptoms; recovered.  
[Previous treatment 1 day prior, as g. p. 29.]
- G. P. No. 26. Same injection as g. p. 29. Symptoms; recovered.  
[Previous treatment: 18 days prior, as g. p. 29.]
- G. P. No. 25. Same injection as g. p. 29. Symptoms; recovered.  
[Previous treatment 24 days prior, as g. p. 29.]
- G. P. No. 23. Same injection as g. p. 29. Symptoms; recovered.  
[Previous treatment: 32 days prior, as g. p. 29.]
- G. P. No. 24. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Dead in 45 minutes.  
[Previous treatment: 47 days prior, as g. p. 29.]
- G. P. No. 22. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment 47 days prior, as g. p. 29.]
- G. P. No. 21. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 44 minutes.  
[Previous treatment: 67 days prior, as g. p. 29.]
- G. P. No. 20. Four c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 83 days prior, as g. p. 29.]

The following guinea pigs received 4 c. c. of antitoxic horse serum containing 1,000 units:

- G. P. No. 38. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: 1 day prior, inoculated subcutaneously with 1,000 units, viz, 4 c. c., antitoxic horse serum (Natl. IX. 18).]
- G. P. No. 35. Same injection as g. p. 38. No symptoms.  
[Previous treatment 4 days prior, as g. p. 38.]
- G. P. No. 36. Same injection as g. p. 38. Symptoms; recovered.  
[Previous treatment 17 days prior, as g. p. 38.]
- G. P. No. 32. Same injection as g. p. 38. Symptoms; recovered.  
[Previous treatment 18 days prior, as g. p. 38.]
- G. P. No. 34. Same injection as g. p. 38. Symptoms; recovered.  
[Previous treatment 24 days prior, as g. p. 38.]
- G. P. No. 37. Same injection as g. p. 38. No symptoms.  
[Previous treatment: 32 days prior, as g. p. 38.]
- G. P. No. 39. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: 47 days prior, as g. p. 38.]
- G. P. No. 31. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 47 days prior, as g. p. 38.]
- G. P. No. 33. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 46 minutes.  
[Previous treatment: 68 days prior, as g. p. 38.]
- G. P. No. 30. Four c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 83 days prior, as g. p. 38.]

We see from the above series that it required 17 days (or less) for the guinea pigs to become susceptible.

We learn from these three series that guinea pigs receiving 4 c. c. of antitoxic horse serum are apparently not quite so susceptible as those receiving the smaller amounts ( $\frac{1}{3}$  c. c. and 1 c. c.); also, that those which received 1 c. c. are apparently not quite so susceptible as those which received only  $\frac{1}{3}$  c. c. of the horse serum. From our work we know that it is the quantity of horse serum, and not the number of units contained in the serum, which plays the important rôle. If this relation of the amount of serum is true, we presume that animals receiving large quantities of horse serum for the first injection are less susceptible because they are rendered slightly immune, just as animals may be immunized against this action by daily repeated inoculations. When large quantities are given at the first injection, the horse serum is probably absorbed very slowly and acts somewhat in the same manner.

It would also seem from the above that guinea pigs are not rendered quite as susceptible by first injections of horse serum as by injections of the toxine-antitoxin mixture.

#### THE SENSITIZING SUBSTANCE IS NOT FREE IN THE BLOOD SERUM.

The following work seems to indicate that the substance which renders the guinea pigs susceptible to a second injection of horse serum is not free in the blood serum of the guinea pig in the same sense that the free receptors are in diphtheria antitoxic serum:

G. P. No. 298 (normal). Eight c. c. of a mixture containing 4 c. c. serum of susceptible guinea pigs and 4 c. c. normal horse serum, injected into the peritoneal cavity; the serums were mixed and injected at once. No symptoms.

G. P. No. 299. Ten c. c. above mixture injected into the peritoneal cavity. No symptoms.

G. P. No. 297. Five c. c. above mixture injected into the peritoneal cavity. No symptoms.

From the fact that the sensitizing substance does not appear to be free in the blood of the guinea pig and from the further fact that we were unable to transfer the immunity we might infer that this reaction may not reside so much in the blood serum as in the cells of the body.

#### THE EFFECT OF HEAT UPON THE SENSITIZING SUBSTANCE.

The following experiments plainly prove that the sensitizing substance is very resistant to heat, for guinea pigs previously treated with small quantities of horse serum heated to 60° C. for six hours subsequently proved highly susceptible:

G. P. No. 265. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 38 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with  $\frac{1}{3}$  c. c. normal horse serum (roan) heated to 60° C. for 6 hours.]

G. P. No. 266. Do. Dead in 20 minutes.

G. P. No. 267. Do. Dead in 25 minutes.

## Part VIII.

### ACTION OF HORSE SERUM UPON MAN AND OTHER ANIMALS.

#### MAN.

It may be that man can not be sensitized in the same way that we have shown is the case with guinea pigs. We made no human experiments, but have experimental data done by others which has a direct bearing on this question.

Pirquet and Schick injected children with antitoxic horse serum at intervals.

It has been customary to immunize numbers of children when exposed to diphtheria with antitoxic horse serum at intervals of from three to four weeks.

We have no doubt that there are many such instances on record and, so far as we know, this practice has never caused death.

Repeated injections of horse serum into man is not an infrequent occurrence. Patients suffering with diphtheria are often given injections of antitoxic serum at short and frequent intervals. It is also not rare for persons to have several attacks of diphtheria at long intervals and to be treated each time with antidiphtheric serum.

Certain serums, for example, the antitubercle serum of Maragliano, or the antirheumatic serum of Menzer are habitually used by giving injections at intervals of days or weeks.

In all of these cases of frequent and repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to this work. Pirquet and Schick in their work on Serumkrankheit give the following instances in which children received two injections of horse serum at intervals of from sixteen to forty-two days between the first and second injections.

*Leopold H.*—October 3, 1902. 100 c. c. scarlet-fever serum (Moser). Eight days following this injection symptoms of the serum disease appeared and lasted several weeks.

December 2, 1902. Fifty days after the first injection patient received 2 c. c. antidiphtheric serum under skin of arm.

In fifteen minutes following this second injection stormy symptoms set in. The boy began to cry and complained of nausea. Edema of the lip set in and soon spread over the whole face. In several hours general urticaria.

*Heinrich K.*—Three years old. June 13, 1902. 100 c. c. scarlet-fever serum. On the eighth day following the injection, symptoms of the serum disease appeared which lasted until the twenty-sixth day.

July 7. Thirty-four days following the first injection, 1 c. c. antidiphtheric serum injected into the left arm. The same afternoon urticaria and swelling of the lips. The next morning the arm was highly edematous.

*Alexandrine K.* Nine years old. May 28, 1902. 180 c. c. normal horse serum. Eleven days after the injection severe symptoms appeared, which lasted until June 17.

Sixteen days following the first injection, given 1 c. c. diphtheric antitoxic serum in the right forearm. Next morning, the hand swollen, very painful, and smartly to such an extent that the infection was considered of an erysipelatosus nature. All redness and swelling disappeared in a few days.

*Elizabeth K.* Six and one-half years old. May 3, 1902. Given 180 c. c. scarlet-fever serum. May 15, ten days following, sudden rise of temperature, 39.4° C. Swelling of the lymph nodes. No exanthem.

Nineteen days after the first injection 50 c. c. scarlet-fever serum, following which occurred severe and painful edema of the skin of the abdomen, which spread to the labia and thighs, and disappeared in about a week.

*Franz Z.* June 6, 1903. 1 c. c. antidiphtheric serum. June 16 had urticaria, about nine days after the injection. August 2, eight weeks after first injection again given 5 c. c. antidiphtheric serum. Shortly following, urticaria and swelling of the face.

*Frieda Z.* June 10, 1903. 1 c. c. antidiphtheric serum. June 26, sixteen days following, the same injection repeated and acted in all respects similar to the above case, Franz Z., her brother. On August 2 she was again given 5 c. c. antidiphtheric serum. One hour later her face became red and swollen. The lids became so edematous that she could not open her right eye. In two hours there was general urticaria. Temperature, 38.6° C. Moderate swelling, locally.

*Elli M.* Four months old. First injection June 20, 1904. 12 c. c. antidiphtheric serum. Ten days later sudden rise of temperature 39° C. and urticaria. The fever lasted two days. Eruption later became measles-like and remained several days.

Nineteen days after the first injection 5 c. c. antidiphtheric serum. One hour after this severe general urticaria. From the site of injection, swelling of the skin of the abdomen. In the afternoon the temperature rose to 39.5° C.; frequent vomiting.

*Leopoldine K.* December 1, 1903. 5 c. c. horse serum. Twenty-seven days following the first injection, patient received 1 c. c. antidiphtheric serum. One hour following this last injection, definite swelling of the right hand, which later spread to the entire arm.

All these eight cases show this in common, that after the first injection of horse serum, the symptoms of the serum disease appear after the normal period of incubation, namely, between the eighth to thirteenth day. But when the same individuals are again injected with horse serum after intervals of sixteen to forty-two days, there reappears at once, or at least within twenty-four hours, symptoms of the serum disease.

Von Pirquet and Schick further give a list of 60 children who were injected with antitoxic horse serum at intervals from six days to seven and a half years between the first and second injections. They found that when the second injection was given from fourteen days to four months after the first injection they obtained, with great regularity, what they termed "the immediate reaction," but

When the interval between the first and second injection is over four months they obtained little or no immediate reaction, but what they termed "an accelerated reaction," for the fever, urticaria, and other symptoms of the serum disease appeared on the fifth, sixth, seventh, or eighth day. It will be remembered that the normal period of incubation for the symptoms of the serum disease to appear after the first injection is between the eighth and thirteenth day. Von Pirquet and Schick lay special stress upon the phenomena of the "immediate" and "accelerated" reactions following the second injection.

We might also conclude despite the suggestion contained in Part X of our work upon sensitizing guinea pigs by feeding them with horse serum or horse meat that children may not be sensitized to the toxic action in horse serum by eating horse meat from the fact that horse meat is a favorite article of diet in certain European countries, and there is nothing on record to show that the injection of horse serum in those countries is fraught with more danger than where this practice does not obtain. We must, however, remember that our work has shown that guinea pigs are sensitized with exceedingly minute quantities of the strange proteid, and that repeated injections cause an immunity; and it is possible that the same action may be true of feeding.

Man reacts to the first injection of horse serum after a period of incubation of eight to thirteen days. Guinea pigs show practically no reaction following the first injection. Both react to a second injection. The reactions in man and the guinea pig, however, differ both in severity and kind. The relation, therefore, that our observations upon the guinea pig may have in its application to man must await further study. Of course, the fact that other animals besides man and guinea pigs react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action.

We have tested monkeys, rabbits, mice, dogs, cats, rats, chickens, and pigeons to determine whether any of these animals may be sensitized to the action of horse serum. Thus far we have obtained a response in the dogs, rabbits, and cats. This work is still in progress and will be reported at a future date.

Von Pirquet and Schick also found that the first injection into rabbits caused no clinical effect, but that subsequent subcutaneous injections caused immediate reaction in the production of local edema which extended even to gangrene. Second injections, when introduced intravenously, produced symptoms of collapse and even death.



Arthus also found that the injection of horse serum into rabbits caused no symptoms, whether the horse serum was injected subcutaneously or intravenously, but when he injected the serum every six days subcutaneously he obtained, after the fourth injection, edema and local reaction which continued to gangrene.

A rabbit which von Pirquet and Schick had previously treated with 8 injections was then given 2 c. c. of horse serum into the ear vein. The rabbit reacted to this in a minute and, after characteristic symptoms similar to those we see in guinea pigs, died in about four minutes.

Arthus obtained similar results with repeated injections of sterilized milk.

## Part IX.

### • IMMUNITY.

#### ACTIVE IMMUNITY.

#### GUINEA PIGS MAY BE IMMUNIZED AGAINST THE TOXIC SUBSTANCE IN HORSE SERUM.

Guinea pigs may be immunized against this toxic substance, following the lines of active immunity produced by repeated inoculations of bacterial toxins. For instance, a guinea pig that has received an injection daily for ten days of small quantities (1 c. c.) of horse serum develops a high degree of immunity to subsequent injections of horse serum. Guinea pigs that have received more than ten inoculations on ten successive days develop a still higher degree of immunity.

G. P. No. 109. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 15 to 27 days prior, 10 subcutaneous inoculations, 1 c. c. each, of antitoxic horse serum (Natl. IX. 19).]

G. P. No. 111. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 36 to 48 days prior; 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

G. P. No. 107. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 15 to 27 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

G. P. No. 106. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 36 to 48 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

G. P. No. 108. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Prior treatment: 37 to 50 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

6 days later, 1 c. c. normal horse serum (roan) inoculated subcutaneously on each of 12 successive days. No symptoms.

6 days later, 6 c. c. normal horse serum (roan) into peritoneal cavity. No symptoms.

G. P. No. 110. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 to 50 days prior, 10 subcutaneous inoculations, 1 c. c. each, antitoxic horse serum (Natl. IX. 19).]

6 days later, 1 c. c. normal horse serum (roan) inoculated subcutaneously on each of 12 successive days. No symptoms.

6 days later, 6 c. c. normal horse serum (roan) into peritoneal cavity. No symptoms.

We noticed that the subcutaneous injection of horse serum in guinea pigs sometimes occasions a local reaction, as indicated by edema at the site of inoculation, but the above guinea pigs Nos. 108 and 110, which received a large number of injections, seemed to react less each time.

We give below a series of 11 guinea pigs injected with large quantities of horse serum at irregular intervals. It is plain from these experiments that a rather high degree of immunity may be induced by repeated injections.

G. P. No. 4101. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{2}$  c. c. antitoxic horse serum (Alex. 190).]

8 days later, 1 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms, less severe.

16 days later, 6 c. c. into peritoneal cavity. No symptoms.

6 days later, 6 c. c. into peritoneal cavity. Symptoms.

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

G. P. No. 4530. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 10 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7 + 1 unit antitoxic horse serum (Standard B27).]

15 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms.

2 days later, 6 c. c. into peritoneal cavity. Restless.

6 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

9 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

33 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

21 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 4532. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 11 days prior, inoculated subcutaneously with 0.19 c. c. toxine. No. 7 + 1 unit antitoxic horse serum (Standard B27).]

14 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

8 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. Symptoms; recovered.

20 days later, 6 c. c. into peritoneal cavity. Symptoms; recovered.

G. P. No. 4432. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 7 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]

22 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

15 days later, 6 c. c. into peritoneal cavity. Symptoms.

- 33 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.
- 21 days later, 6 c. c. into peritoneal cavity. No symptoms.
- G. P. No. 4426. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: 8 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]
- 27 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms (?).
- 9 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 34 days later, 6 c. c. into peritoneal cavity. Symptoms.
- 20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) in peritoneal cavity. No symptoms.
- G. P. No. 4593 Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: 3 days prior, inoculated subcutaneously with 0.001 c. c. toxine No. 7.]
- 18 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms.
- 8 days later, 6 c. c. into peritoneal cavity. Symptoms.
- 9 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 34 days later, 6 c. c. into peritoneal cavity. Symptoms.
- 20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.
- G. P. No. 4377. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.  
[Previous treatment: 21 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{3}$  c. c. antitoxic horse serum (Natl. V. 7).]
- Same day, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms, less severe.
- 10 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 2 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 15 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 34 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.
- G. P. No. 4. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms, recovered.  
[Previous treatment: 27 days prior, inoculated subcutaneously with 6 c. c. antitoxic horse serum (Alex. 189).]
- 3 days later, 6 cc. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.
- 19 days later, 6 c. c. into peritoneal cavity. Symptoms.
- 6 days later, 6 c. c. into peritoneal cavity. No symptoms.
- 9 days later, 6 c. c. into peritoneal cavity. No symptoms.
- G. P. No. 4534. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.  
[Previous treatment: 4 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]
- 10 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.
- 18 days later, 6 c. c. into peritoneal cavity. Symptoms (?).
- 9 days later, 6 c. c. into peritoneal cavity. No symptoms.

33 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

21 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 2. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. No symptoms.

[Previous treatment: None.]

3 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

19 days later, 6 c. c. into peritoneal cavity. Symptoms.

6 days later, 6 c. c. into peritoneal cavity. No symptoms.

43 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. Symptoms.

20 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 3. Ten c. c. normal horse serum (roan) injected into the peritoneal cavity. No symptoms.

[Previous treatment: None.]

2 days later, 6 c. c. same serum into peritoneal cavity. No symptoms.

3 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

10 days later, 6 c. c. into peritoneal cavity. No symptoms.

#### PASSIVE IMMUNITY.

#### EFFECT OF NORMAL GUINEA-PIG BLOOD AND ORGANS UPON THE TOXICITY OF HORSE SERUM.

As a normal untreated guinea pig is entirely unsusceptible to the toxic substances found in horse serum, it naturally occurred to us to try to ascertain whether the blood serum or the juices from the various organs of such a normal guinea pig had a restraining, neutralizing, or immunizing action when mixed with horse serum and the mixture inoculated into susceptible guinea pigs.

Normal G. P. A large, full-grown female guinea pig was chloroformed, then exsanguinated.

Various organs, mentioned below, were minced and ground separately in a mortar with 10 c. c. of antitoxic horse serum (National VIII. 17). The mixtures were allowed to stand at room temperature 3 hours, then strained through cheese cloth, and injected into the peritoneal cavity of susceptible guinea pigs, as follows:

G. P. No. 4365. Six c. c. of a mixture containing equal parts of antitoxic horse serum and normal guinea-pig serum injected into the peritoneal cavity. Death in 40 minutes.

[Previous treatment: 31 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (PD&Co. No. 07173).]

G. P. No. 4463. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig liver injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{2}$  c. c. antitoxic serum (Natl. VIII. 17).]

G. P. No. 4393. Seven c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig liver injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.2 c. c. toxine No. 59+1 unit antitoxic serum (Standard B27).]

- G. P. No. 4470. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Nat. VIII. 17) and normal guinea-pig spleen injected into the peritoneal cavity. No symptoms. For occasional irregularities see below.  
 [Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (Nat. IX. 17).]  
 6 c. c. antitoxic horse serum (Nat. VIII. 17) injected into peritoneal cavity of same pig one day later. Symptoms, recovered.
- G. P. No. 4475. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Nat. VIII. 17) and normal guinea-pig ovary injected into the peritoneal cavity. Death in 45 minutes.  
 [Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (Nat. IX. 18).]
- G. P. No. 4466. Six c. c. of mixture containing equal parts of antitoxic horse serum (Nat. VIII. 17) and normal guinea-pig kidney injected into the peritoneal cavity. Death in 45 minutes.  
 [Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (Nat. VIII. 17).]
- G. P. No. 4378. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Nat. VIII. 17) and normal guinea-pig suprarenal injected into the peritoneal cavity. Death in 10 minutes.  
 [Previous treatment: 31 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (Nat. V. 10).]
- G. P. No. 4464. Six c. c. of mixture containing equal parts of antitoxic horse serum (Nat. VIII. 17) and normal guinea-pig thyroid injected into the peritoneal cavity. Symptoms, recovered.  
 [Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (Nat. VIII. 17).]
- G. P. No. 4469. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Nat. VIII. 17) and normal guinea-pig brain injected into the peritoneal cavity. Symptoms, recovered.  
 [Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{3}$  c. c. antitoxic serum (Nat. IX. 17).]

We may conclude from the above that neither the blood serum nor the organs of a normal guinea pig have any neutralizing action upon the toxicity of horse serum when injected into a sensitized guinea pig.

Guinea pig 4470, which received an injection of horse serum plus the spleen pulp of a normal guinea pig, showed no symptoms. We therefore repeated this experiment as follows:

- G. P. No. 4493. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Nat. IX. 19) and normal guinea-pig spleen injected into the peritoneal cavity. Symptoms, recovered.  
 [Previous treatment: 27 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7 + 1 unit horse serum (Standard B27).]

We believe that occasional irregularities like this which have exceptionally occurred in this work may be accounted for by the fact that sometimes when the stomach and intestines are full and distended the inoculating needle enters the intestinal tract instead of the peritoneal cavity. While we suspected this possibility, it was proved to our satisfaction one day; after injecting a guinea pig, the intestinal

contents flowed back into the barrel of the syringe, owing to accidental withdrawal of the piston. This particular guinea pig showed no symptoms as a result of the injection; whereas we knew it to be susceptible, because a subsequent operation, done a few hours later — caused characteristic symptoms.

For intraperitoneal inoculations the needle should be short and blunt to avoid this accident and the injection should be given over the region where the coils of small intestine lie close to the abdominal walls.

NEUTRALIZING EFFECT OF THE BLOOD AND ORGANS OF AN IMMUNIZED GUINEA PIG UPON THE TOXICITY OF HORSE SERUM.

We found that repeated injections of horse serum into a guinea pig produces a certain degree of immunity to the subsequent action of horse serum, and it naturally occurred to us to try whether such guinea pigs contained in their body juices an immunizing substance in sufficient quantity to neutralize the poisonous substance found in horse serum.

G. P. No. 109 (immunized). This guinea pig had received 10 subcutaneous inoculations of 1 c. c. each of antitoxic horse serum (Natl. IX. 19) on 10 successive days from Feb. 8 to 20; 15 days later, viz, March 7, it was inoculated with 6 c. c. antitoxic horse serum (Natl. IX. 19) into the peritoneal cavity. No symptoms.

Twenty-one days later, viz, March 28, it was again tested by injecting 6 c. c. of antitoxic horse serum (Natl. VIII. 18) into the peritoneal cavity. No symptoms.

As this guinea pig showed a high degree of resistance to the toxic action of horse serum it was killed two days later, March 30, with chloroform. About 15 c. c. of the blood was taken from the heart and defibrinated, the various organs were removed and placed in separate mortars and ground up with 10 c. c. of antitoxic horse serum (Natl. VIII. 18). This mixture of horse blood with organ juices of the guinea pig was allowed to stand about 3 hours at room temperature and strained through cheese cloth. It was then inoculated into the guinea pig.

G. P. No. 166. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *suprarenal* of g. p. 109 injected into the peritoneal cavity. Dead in 9 minutes.

[Previous treatment: 49 days prior, inoculated with 0.22 c. c. toxine No. 7 +  $\frac{1}{3}$  c. c. horse serum (Stearns No. 1351).]

G. P. No. 158. Six c. c. same serum as g. p. 166 mixed with whole (defibrinated) *blood* of g. p. 109 injected into peritoneal cavity. Dead in 15 minutes.

[Previous treatment: As g. p. 166.]

G. P. No. 126. Six c. c. same serum as g. p. 166 mixed with *thyroid* of g. p. 109 injected into peritoneal cavity. Dead in 16 minutes.

[Previous treatment: As g. p. 166.]

G. P. No. 132. Six c. c. same serum as g. p. 166 mixed with *kidney* of g. p. 109 injected into peritoneal cavity. Symptoms, recovered.

[Previous treatment: As g. p. 166.]

G. P. No. 164. Six c. c. same serum as g. p. 166 mixed with *spleen* of g. p. 109 injected into peritoneal cavity. Symptoms, recovered.

[Previous treatment: As g. p. 166.]

P. No. 129. Six c. c. same serum as g. p. 166 mixed with *liver* of g. p. 109 injected into peritoneal cavity. No symptoms.

[Previous treatment: As g. p. 166.]

2 hr. 45 min. later. *To test susceptibility.* 6 c. c. same serum as g. p. 166 injected into peritoneal cavity. Dead in 30 minutes.

G. P. No. 147. Six c. c. same serum as g. p. 166 mixed with *brain* of g. p. 109 injected into peritoneal cavity. No symptoms.

[Previous treatment: As g. p. 166.]

1 hr. 20 min. later. *To test susceptibility.* 6 c. c. same serum as g. p. 166 injected into peritoneal cavity. Dead in 35 minutes.

G. P. No. 139 (control). Six c. c. same serum as g. p. 166 injected alone into peritoneal cavity. Dead in 15 minutes.

[Previous treatment: As g. p. 166.]

Owing to the fact that guinea pigs 129 and 147 showed no symptoms, the experiment was repeated, using organs from a similarly treated pig as follows:

G. P. No. 108 (immunized). This guinea pig had received 10 subcutaneous inoculations of 1 c. c. each of normal horse serum (Sam) on 10 successive days from Feb. 8 to 20; 37 days later, viz, March 29, it was inoculated with 6 c. c. antitoxic horse serum (Natl. VIII. 18) into the peritoneal cavity. Symptoms, recovered.

Beginning six days later, viz, April 4, and continuing 12 days, it was given a daily subcutaneous inoculation of 1 c. c. normal horse serum (roan). No symptoms.

Six days after the last inoculation the pig was given 6 c. c. normal horse serum (roan) into the peritoneal cavity. No symptoms.

This guinea pig, having showed a strong resistance to the toxic action of horse serum, was killed with chloroform and the brain, part of the spinal cord, and liver were minced and ground separately in a mortar with 20 c. c. antitoxic horse serum (Natl. VIII. 18). The mixture was allowed to stand about 3 hours at room temperature and strained through cheese cloth and then inoculated.

G. P. No. 4998. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *liver* of g. p. 108 injected into the peritoneal cavity. Dead in 95 minutes.

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{8}\frac{1}{8}$  c. c. antitoxic horse serum (Wellcome 474A).]

G. P. No. 5044. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *brain* and portions of the *spinal cord* of g. p. 108 injected into the peritoneal cavity. Dead in 85 minutes.

[Previous treatment: 33 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 +  $\frac{1}{8}\frac{1}{8}$  c. c. antitoxic horse serum (NYBH 13C).]

G. P. No. 252. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *brain* and portion of *spinal cord* of g. p. 108 injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 53 days prior, inoculated subcutaneously with  $\frac{1}{8}\frac{1}{8}$  c. c. normal horse serum (roan) heated to 60° C. for 30 min.]

G. P. No. 251. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *liver* of g. p. 108 injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 53 days prior, inoculated subcutaneously with  $\frac{1}{8}\frac{1}{8}$  c. c. normal horse serum (roan) heated to 60° C. for 30 min.]



As a further proof that the blood serum of an immunized guinea pig can not transfer this immunity, we added such serum from several guinea pigs immunized by repeated injections with normal horse serum; but when this mixture is injected into susceptible pigs they react in a characteristic manner. The experimental data upon this point will be seen in Part IV, page 37, upon the relation of precipitin to the toxic action.

It therefore appears that the "immune bodies," if such exist against the toxic action of horse serum, are not free in the blood and body juices, contrary to the case in diphtheria.

## Part X.

### FEEDING EXPERIMENTS.

#### GUINEA PIGS MAY BE SENSITIZED BY FEEDING HORSE SERUM.

Our work up to this point seemed to carry us very far away from an explanation of the cause of sudden death in man following the injection of horse serum. A thorough study of the literature upon this subject discloses the fact that almost all the unfortunate cases of this kind have been due to the first injection of horse serum. If, therefore, man may be sensitized to a toxic principle in horse serum, it must be by some other means than a previous injection of horse serum. We have seen that von Pirquet and Schick, in their studies on serum disease, have actually injected children at intervals of several weeks without any such effect as we have found upon guinea pigs. Further, that on numerous occasions children have been given successive injections of diphtheria antitoxin at intervals of three or four weeks for purposes of immunization.

In casting about for another explanation it occurred to us that probably this action may take place through eating horse meat. We therefore carried on a number of feeding experiments upon guinea pigs. We first fed guinea pigs with normal horse serum by means of a stomach tube, with the following results:

- G. P. No. 116. One feeding of 5 c. c. normal horse serum (Jane) into stomach.  
27 days later inoculated with 6 c. c. antitoxic horse serum (Natl. IX. 19) into the peritoneal cavity. No symptoms.
- G. P. No. 114. Four daily feedings, each of 5 c. c., same serum.  
27 days after first feeding and 23 after last, same inoculation as g. p. 116. No symptoms.
- G. P. No. 112. Five daily feedings, 5 c. c. each, same serum.  
27 days after first feeding and 22 after last, same inoculation as g. p. 116. No symptoms.
- G. P. No. 113. Six daily feedings, 5 c. c. each, same serum.  
27 days after first feeding and 21 after last, same inoculation as g. p. 116. No symptoms.
- G. P. No. 115. Ten daily feedings, 5 c. c. each, same serum.  
27 days after first feeding and 15 after last, same inoculation as g. p. 116. Symptoms, recovered.

Only one of these five pigs showed definite and characteristic symptoms following the intrastomachal administration of the horse serum. This pig, No. 115, which was sensitive to the subsequent injections

of horse serum, had received ten feedings of 5 c. c. each of normal horse serum daily. In view of the fact, however, that exceedingly small quantities (1/1000 c. c.) of horse serum is sometimes sufficient to sensitize a guinea pig, it seemed to us that feeding animals with a stomach tube might cause slight abrasions, resulting in absorption, so that pigs fed in this manner might really be sensitized through minute wounds of the mucous membrane.

We therefore used another series of pigs and fed the animals without using artificial means. As guinea pigs will not drink horse serum, we found it necessary to dry the serum, reduce it to powder, and then mix it with ground carrots. In this way they took the serum unknowingly with their food. By this means definite amounts of the horse serum may be given and the quantity given to each animal accurately controlled. Half a gram of the dried normal horse serum was thus fed to each pig, which was afterwards tested for susceptibility, as follows:

- G. P. No. 4551. Fed 0.5 gm. dried normal horse serum daily from March 15 to 28. Injected March 28 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX. 19), 13 days from first and 0 days from last feeding. Symptoms; recovered.
- G. P. No. 245. Fed 0.5 gm. dried normal horse serum daily from March 15 to 28. Injected March 28 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX. 18), 13 days from first and 0 days from last feeding. No symptoms.
- G. P. No. 4581. Fed 0.5 gm. dried normal horse serum daily from March 15 to 29. Injected April 5 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 7 days from last feeding. Symptoms; recovered.
- G. P. No. 4540. Fed 0.5 gm. dried normal horse serum daily from March 15 to 31. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 6 days from last feeding. Symptoms; recovered.
- G. P. No. 4546. Do.
- G. P. No. 4575. Fed 0.5 gm. dried normal horse serum daily from March 15 to April 6. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 0 days from last feeding. Symptoms; recovered.
- G. P. No. 172. Fed 0.5 gm. dried normal horse serum daily from March 15 to April 5. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 1 day from last feeding. Symptoms; recovered.
- G. P. No. 173. Do.
- G. P. No. 276. Fed 0.5 gm. dried normal horse serum daily from March 15 to April 9. Injected April 9 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 25 days from first and 0 days from last feeding. Dead in 55 min.

There is no doubt from the above experiments that guinea pigs may be sensitized by feeding to them horse serum. Of the nine pigs so fed in the above series eight reacted to subsequent injections of horse serum, one of these died.

These remarkable results are not surprising when we remember that Uhlenhuth <sup>a</sup> showed the possibility of the intrastomachal immu-

<sup>a</sup> Uhlenhuth, —: Neuer Beitrag zum spezifischen Nachweis von Eiereiweiss auf biologischem Wege. Deut. med. Woch., Bd. 26, 1900, p. 734-735.

nization in working on antiproteid immunization. He fed rabbits daily for a number of weeks with egg albumin by means of a stomach tube. The rabbits' serum was examined every eight days. After twenty-four days it developed the power to precipitate egg albumin.

Metalnikoff<sup>a</sup> reports that he attained immunization by feeding one animal with the blood of another. He fed horse's blood to white rats. After three or four feedings no agglutination or hemolytic action of the rats' blood upon the horse's corpuscles was noted. In one week the action appeared feeble and increased to decided strength after one or two months.

#### GUINEA PIGS MAY BE SENSITIZED BY FEEDING HORSE MEAT.

After demonstrating the possibility of rendering guinea pigs susceptible by feeding them with horse serum, we next fed a series with horse meat. The following series was fed with indefinite amounts of horse meat, both fresh and dried ground up with carrots, as guinea pigs do not like fresh horse meat and must be very hungry in order to eat it.

G. P. No. 242. Fed 9 days on horse meat from Feb. 15. Injected March 9 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX, 17), 24 days after first and 15 days after last feeding. Symptoms; recovered.

G. P. No. 237. Fed 9 days on horse meat from Feb. 15. Injected March 10 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX, 17), 25 days after first and 16 days after last feeding. Symptoms; recovered.

G. P. No. 238. Do.

G. P. No. 239. Do.

G. P. No. 240. Do.

G. P. No. 244. Do.

G. P. No. 241. Fed 9 days on horse meat from Feb. 15. Injected April 5 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 49 days after first and 40 days after last feeding. Symptoms?; recovered.

G. P. No. 243. Fed 9 days on horse meat from Feb. 15. Injected March 13 into peritoneal cavity with 10 c. c. aqueous extract dry horse meat, 26 days after first and 17 days after last feeding. No symptoms.

As the above series was fed with indefinite amounts, another series of guinea pigs was placed upon definite weighed amounts of horse meat. Four grams of the dried ground horse meat were mixed with fresh carrots and fed to the pigs daily. Almost all the pigs placed upon this diet did poorly, their coats became shaggy and the animals lost weight.

G. P. No. 246. Fed 4 gm. dried horse meat daily from March 15 to 23. Injected March 23 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 13 days after first and 0 day after last feeding. Symptoms?; recovered.

G. P. No. 4562. Do.

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<sup>a</sup> Metalnikoff, —: Ueber hamolytisches Serum durch Blutfütterung. Centbl. f. Bakt., v. 29, 1901, p. 531-533.

- G. P. No. 4544. Fed 4 gm. dried horse meat daily from March 15 to 29. Injected April 5 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 7 days after last feeding. Symptoms; recovered.
- G. P. No. 4543. Fed 4 gm. dried horse meat daily from March 15 to April 6. Injected April 6 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 0 days after last feeding. No symptoms.
- G. P. No. 4563. Do. Symptoms?; recovered.
- G. P. No. 4371. Do. No symptoms.
- G. P. No. 270. Do. Symptoms; recovered.
- G. P. No. 4549. Do. No symptoms.
- G. P. No. 4538. Fed 4 gm. dried horse meat daily from March 15 to 31. Injected April 6 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 6 days after last feeding. Symptoms; recovered.
- G. P. No. 174. Do. Dead in 20 minutes.
- G. P. No. 275. Fed 4 gm. dried horse meat daily from March 15 to April 9. Injected April 9 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 25 days after first and 0 days after last feeding. No symptoms.
- G. P. No. 4557. Do.
- G. P. No. 4574. Do. Symptoms?; recovered.
- G. P. No. 4569. Do. No symptoms.
- G. P. No. 4566. Do. Symptoms; recovered.

It is perfectly plain from the above that guinea pigs may be sensitized by feeding them horse meat as well as horse serum.

#### MAY GUINEA PIGS BE SENSITIZED BY FEEDING BEEF?

Another series of experiments was then conducted by feeding guinea pigs with beef. The meat was dried, ground, and mixed in definite weighed amounts with ground carrots.

- G. P. No. 247. Fed 4 gm. dried beef daily from March 15 to 28. Injected March 28 into peritoneal cavity with 5 c. c. antitoxic horse serum (Natl. VIII, 18), 13 days after first and 0 day after last feeding. Symptoms?; recovered.
- G. P. No. 269. Fed 4 gm. dried beef daily from March 15 to 29. Injected April 5 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 7 days after last feeding. Symptoms?; recovered.
- G. P. No. 171. Fed 4 gm. dried beef daily from March 15 to April 6. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 0 day after last feeding. No symptoms.

Five guinea pigs were placed in this series, but two of them died, apparently as a result of the diet.

We conclude, as a result of these feeding experiments, that guinea pigs may be highly sensitized to the toxic action of horse serum by previous feeding with horse serum or horseflesh. So far as conclusions may be drawn from the few experiments done upon feeding guinea pigs with beef, we feel justified in stating that guinea pigs may also be rendered slightly susceptible in this way. Whether pigs fed with beef would be more susceptible subsequently to the

**n**jection of cattle serum than horse serum is reserved for further study.

The fact that guinea pigs may be rendered susceptible by the feeding of strange proteid matter opens an interesting question as to whether sensitized guinea pigs may also be poisoned by feeding with the same serum. If man can be sensitized in a similar way by the eating of certain proteid substances it may throw light upon those interesting and obscure cases in which the eating of fish and other sea food, etc., by certain individuals habitually causes sudden and sometimes serious symptoms.



## Part XI.

### HEREDITARY TRANSMISSION OF THE SUSCEPTIBILITY IN GUINEA PIGS.

So far as may be judged from the following data on this subject the susceptibility to the toxic effects of horse serum may be transmitted from the mother guinea pig to her young:

#### FAMILY NO. 1.

**Mother g. p. No. 105.** One c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 55 days prior, inoculated subcutaneously with  $\frac{1}{10}$  c. c. normal horse serum (Jane).]

The progeny of the above guinea pig, when about five days old, was tested as follows:

**G. P. No. 105A.** One c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

**G. P. No. 105B.** One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

#### FAMILY NO. 2.

**Mother g. p. No. 66.** Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 120 minutes.

[Previous treatment: 85 days prior, 6 c. c. normal horse serum (Sam) injected into the peritoneal cavity.]

The progeny of the above guinea pig, when about a week old, was tested as follows:

**G. P. No. 66A.** One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

**G. P. No. 66B.** Do.

#### FAMILY NO. 3.

**Mother g. p. No. 103.** Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 71 days prior,  $\frac{1}{10}$  c. c. normal horse serum (Jane) inoculated subcutaneously; 33 days later 6 c. c. antitoxic horse serum (Natl. IX, 17) injected into the peritoneal cavity. Symptoms.]

The progeny of the above guinea pig, when about ten days old, was tested as follows:

**G. P. No. 103A.** One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

#### FAMILY NO. 4.

**Mother g. p. No. 4463.** Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms (i).

[Previous treatment: 99 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 +  $\frac{1}{10}$  c. c. antitoxic horse serum (Natl. VIII, 17). 26 days later, 6 c. c. antitoxic horse serum (Natl. VIII, 17) + liver pulp of normal guinea pig injected into the peritoneal cavity. Symptoms.]



The progeny of the above guinea pig, when about ten days old, was tested as follows:

G. P. No. 4463A. One c. c. normal horse serum (roan) injected into the peritoneal cavity.  
Symptoms.

G. P. No. 4463B. Do.

FAMILY NO. 5.

Mother g. p. No. 14. Six c. c. normal horse serum (roan) injected into the peritoneal cavity  
Symptoms; recovered.

[Previous treatment: 94 days prior, inoculated subcutaneously with  $\frac{1}{50}$  c. c. antitoxic horse serum (Natl. IX, 18.)]

The progeny of the above guinea pig, when five days old, was tested as follows:

G.P. No. 14A. One c. c. normal horse serum (roan) injected into the peritoneal cavity  
Dead in 30 minutes.

G. P. 14B. Do.

G. P. No. 14C. Do. Dead in 35 minutes.

It is plain from the above that young guinea pigs born of a susceptible mother are themselves susceptible, often more so than the mother herself.

To control the effect of horse serum upon such young guinea pigs the following data are given:

(All controls born of normal parents.)

Control g. p. No. 01. One c. c. normal horse serum (roan) inoculated subcutaneously when 5 days old. No symptoms.

Control g. p. No. 02. Two c. c. same serum when 1 week old. No symptoms.

Control g. p. No. 03. Same injection when 6 days old. No symptoms.

Control g. p. No. 04. Same injection when 4 days old. No symptoms.

These four controls were selected from four different litters.

These results upon the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea pigs may throw light upon the well-known inherited tendency to tuberculosis in children born of a tuberculous parent.

There are certain analogies between the action of tuberculosis and horse serum. Both produce a hypersensitiveness and also a certain degree of immunity. Now that we have proved that this hypersensitiveness or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea pigs, may it not throw light upon the fact that tuberculosis "runs in families?" While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young this is, as far as we know, the first recorded instance in which hypersensitiveness, or anaphylaxis, has been experimentally shown to be hereditarily transmitted from a mother to her young.

## Part XII.

### TOXIC ACTION OF OTHER ALBUMINOUS SUBSTANCES.

As we found that guinea pigs may be so highly sensitized to the toxic action of horse serum and to a certain extent the serum from other animals we made a few experiments to determine whether other albuminous substances possessed the same toxic properties under these conditions. For this purpose we used egg albumin, peptone, skimmed milk, hemoglobin and vegetable proteid.

G. P. No. 4366. Six c. c. of a saturated solution of egg albumin were injected into the peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{4}$  $\frac{1}{8}$  c. c. horse serum (PD & Co. 08022-A)].

Two days later this guinea pig was tested for susceptibility by the inoculation of 6 c. c. antitoxic horse serum (Natl. VIII. 17) into the peritoneal cavity. Dead in 50 minutes.

G. P. No. 4924. Six c. c. of a saturated solution of peptone injected into the peritoneal cavity. No symptoms.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}$  $\frac{1}{8}$  c. c. antitoxic horse serum (Cutter 1540)].

Three days later this guinea pig was tested for susceptibility by the inoculation of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Symptoms; recovered.

G. P. No. 4926. Six c. c. skimmed milk injected into the peritoneal cavity. No symptoms.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{4}$  $\frac{1}{8}$  c. c. antitoxic horse serum (Cutter 1540)].

Three days later tested for susceptibility by the injection of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Dead in 11 minutes.

G. P. No. 4919. Ten c. c. vegetable proteid from oats injected into the peritoneal cavity. No symptoms.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{2}$  $\frac{1}{8}$  c. c. antitoxic horse serum (Stearns 16 H)].

Three days later tested for susceptibility by the injection of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Dead in 25 minutes.

G. P. No. 4925. Six c. c. hemoglobin solution in water from washed normal (roan horse) corpuscles injected into the peritoneal cavity. No symptoms (?)

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+ $\frac{1}{3}$  $\frac{1}{8}$  c. c. antitoxic horse serum (Cutter 1540)].

Three days later tested for susceptibility by the injection of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Symptoms; recovered.

G. P. (normal). Four and a half c. c. solution of hemoglobin (watery solution of washed red blood corpuscles of roan horse) injected into peritoneal cavity. No symptoms.

G. P. No. 4989. Ten c. c. same injection. Symptoms (?)

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.22 c. c. to ~~ine~~  
No. 7+~~z~~~~0~~ c. c. antitoxic horse serum (Alex. A202)].

To control the two preceding pigs a solution of hemoglobin, obtained by dissolving red blood corpuscles from a normal horse (roan), was injected into the peritoneal cavity of a normal guinea pig, with negative results.

Whether guinea pigs treated with these albuminous substances are rendered susceptible to subsequent injections of the same albumin is now being studied.

### Part XIII.

#### A REVIEW OF THE LITERATURE RELATING TO OUR WORK.

We feel it incumbent upon us to compare our results with the work of others and, therefore, have introduced this discussion upon the toxic action of serums in general, especially in relation to the production of immunity and hypersensitiveness.

NOTE.—In this historical development we have drawn largely upon the splendid review of the literature as given by Uhlenhuth in "Zur Kenntniss der giftigen Eigenschaften des Blutserums" (*Zeit. f. Hyg.*, v. 26, 1897), and C. Frh. von Pirquet and B. Schick in "Die Serunkrankheit" (K. K. Universitäts-Kinder-Klinik, Wien, 1905).

Transfusion was formerly used as a therapeutic measure. The first reference to this practice is June 15, 1667, when von Denis transfused lamb's blood (cited in Landois's article on transfusion, *Eulenburg's Realenzyklopädie*, 3. Auflage). This practice gradually fell into disuse but was again revived about the beginning of the last century, when transfusion was used for acute anemia and poisonings.

The intravenous injection of lamb's blood was soon shown to be associated with great danger. High fever in half an hour, emboli, hemorrhages, hemoglobinuria, etc., were often noticed. The causes of these serious symptoms, as well as the scientific basis of our knowledge upon the effects of transfusion, were first made clear by the work of Landois and Ponfick in the years 1873 and 1875. These authors showed that the blood of alien species caused solution of the corpuscles when brought into the circulation. Naunyn and Francken showed that the injection of dissolved hemoglobin into the circulation caused coagulation. Great numbers of the white corpuscles were so acted upon by the dissolved hemoglobin that the fibrin-forming elements were thus set free, resulting in the formation of clots within the vessels. When this coagulation is extensive, death may result from asphyxia.

Dallera <sup>a</sup> in 1874 reported that urticarial eruptions may follow the transfusion of blood.

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<sup>a</sup> Dallera: Considerazioni e casi clinici di transfusione del sangue. *Il Morgagni*, 1874, 7.

Neudörfer<sup>a</sup> refers to the fact that urticaria frequently follows transfusion.

Landois<sup>b</sup> also refers to this complication.

In consequence of the severe injury which often resulted from the transfusion of lamb's blood the practice was entirely discontinued. Recently, however, Dominici<sup>c</sup> has again taken up the subject.

In England at one time the transfusion of milk was practiced, but proved dangerous and without benefit, as stated by Montard, Martin, and Richet. (Injections intraveineuses de lait et de sucre, Soc. de Biol., 1879.)

Latterly the transfusion of lamb's blood has given place to physiological salt solution and also to the transfusion of defibrinated human blood.

In the year 1894 the use of diphtheria antitoxin introduced the widespread practice of injecting horse serum. This practice differed from the previous work with transfusion in that the injections are generally not given intravenously, but subcutaneously, and the quantity of the alien albuminous substance is relatively small. According to von Pirquet and Schick this accounts partly for the fact that in the majority of these injections no injurious consequences followed. Luebinski (Über eine Nachwirkung des Antitoxins bei Behandlung des Diphtherie, Deut. med. Woch., 1894) was the first to call attention to a case of exanthematous eruption following the injection of antitoxin serum. Soon afterwards Schütz,<sup>d</sup> Cnyrim,<sup>e</sup> Asch,<sup>f</sup> Rembold,<sup>g</sup> and Trey mann<sup>h</sup> reported urticarial eruptions following the prophylactic injection of antitoxic horse serum. Following these came a great mass of evidence which made it clear that following the injection of antidiphtheric serum these sequelæ were comparatively harmless.

In relation to the cause of these symptoms it was generally held that the serum contained some toxic substance, probably a globulin. This explanation led manufacturers to the commendable practice of starving their horses about twenty-four hours prior to bleeding, in order to eliminate as far as practicable the freshly assimilated products of digestion.

<sup>a</sup> Neudörfer: Beiträge zur Bluttransfusion. Zeitschr. f. Chirurgie, Bd. 6.

<sup>b</sup> Landois: Die Transfusion des Blutes, Leipzig, 1875. —: Transfusion. Eulenburg's Realencyclopädie, 3. Aufl., 1900.

<sup>c</sup> Dominici: Transfusion. Wiener med. Wochenschr., 1895.

<sup>d</sup> Schütz: Discussionsbemerkung zu Piorkowski's Vorträge. Berl. med. Gesellsch., 1904.

<sup>e</sup> Cnyrim: Zwei Fälle von Erkrankungen nach Anwendung des Diphtherieserums. Deut. med. Woch., 1894, p. 898.

<sup>f</sup> Asch: Zur Casuistik der Heilserumexantheme. Berl. klin. Woch., 1894.

<sup>g</sup> Rembold: Zwei Fälle von Erkrankung nach Anwendung des Heilserums. Deut. med. Woch., 1894, p. 963.

<sup>h</sup> Trey mann, Otto: Ein Fall von acuter hämorrhagischer Nephritis nach Anwendung des Behring'schen Diphtherieheilserums. Deut. med. Woch., 1894, p. 952.

Heubner <sup>a</sup> and von Bókay <sup>b</sup> expressed the opinion that these manifestations were due to other properties than the antitoxin in the serum.

Johannessen <sup>c</sup> believed that the agent in horse serum which produced these symptoms was due to the introduction into the body of an alien serum, for he produced the same effects by injecting normal horse serum. In 22 cases fever resulted, and skin eruptions in 12.

Sévestre <sup>d</sup> referred the exanthems which appear in eight to ten days back to the serum which had been injected. However, he thought that the measles-like eruption and fever was the expression of a streptococcus infection. Netter, however, in the discussion, believed these symptoms to be caused by the serum.

Almost all authors express the opinion that the symptoms caused by the injection of serum are of minor consequence. The fact that from time to time serious symptoms and even death followed the injection of diphtheria antitoxic serum does not alter the general impression that as a rule such injections are harmless. *The benefits far outweigh the danger.*

Gottstein, <sup>e</sup> in addition to the Langerhans case, collected 8 deaths following the injection of serum in those having diphtheria and 4 in those not sick with diphtheria.

We have collected from the literature 19 cases of such unfortunate results, and know personally of several more which have not been reported. Von Pirquet and Schick express the opinion that a critical examination of these cases may be otherwise explained, and authors generally have not permitted this rare accident to counterbalance the great benefit possible from the use of diphtheria antitoxic serum.

It seems to have been definitely shown that the skin eruptions and other symptoms following the injection of horse serum follow in direct proportion to the amount of serum injected. This has resulted in endeavors to concentrate the serum as much as possible.

<sup>a</sup> Heubner: Praktische Winke zur Behandlung der Diphtherie mit Heilserum. Deut. med. Woch., 1894, p. 701. — Klinische Studien über die Behandlung der Diphtherie mit dem Behring'schen Heilserum. Leipzig, 1895. — Über die Anwendung des Heilserums bei der Diphtherie. Jahrb. f. Kdheilk., Bd. 38, p. 231.

<sup>b</sup> Von Bókay: Die Heilserumbehandlung gegen Diphtherie im Budapeste Stefanie-Kinderspital. H. f. Kdh., Bd. 44, 1897. — Meine Erfolge mit Behring's Diphtherieheilserum. Deut. med. Woch., 1895, p. 233. — Meine Erfahrungen mit dem Moser'schen polyvalenten Scharlach-Streptokokkenserum. Deut. med. Woch., 1904.

<sup>c</sup> Johannessen: Über Injektionen mit antidiphtherischen Serum und reinem Pferdeserum. Deut. med. Woch., 1895. — Über Immunisierung bei Diphtherie. Deut. med. Woch., 1895.

<sup>d</sup> Sévestre: Des accidents causés par le streptococque à la suite des injections de sérum de Roux. Bull. de la Soc. méd. des hôpitéaux, July 19, 1895, Jan. 31, 1896. La méd. moderne, 1896.

<sup>e</sup> Gottstein: Über Todesfälle, welche bei der Anwendung des Diphtherieheilserums beobachtet worden sind. Therap. Monatsh., 1896.

Bujwid <sup>a</sup> believed that these manifestations were due largely to fresh horse serum, and favors the use of old seasoned antitoxin for therapeutic purposes.

Hamburger and Moro <sup>b</sup> have shown that the injection of alien serums into man result in the formation of precipitins.

Hamburger, <sup>c</sup> in a further work, drew a distinction between alien albuminous substances introduced through the digestive tract and by other channels.

Upon the general toxic properties of blood we quote the following instances, taken largely from Uhlenhuth: <sup>d</sup>

Stern, <sup>e</sup> 1893, found that the blood from a case of erysipelas in amounts of 0.5 to 1 c. c. killed white mice, while normal human blood required 3 c. c. or more to kill the animals.

Rummo, <sup>f</sup> 1891, showed that blood serum from cases of typhoid fever, malaria, pneumonia, and eclampsia had a high grade of toxicity for rabbits when injected intravenously.

Albu, <sup>g</sup> 1897, confirm Rummo's experiments, and found an increase of toxicity in persons suffering from pneumonia, severe chronic bronchitis, epilepsy, uræmia, and puerperal sepsis.

Upon the toxicity of the blood serum of man in health and disease further work has been done by Charrin, <sup>h</sup> Leclainche and Rémond, <sup>i</sup> Chambrelent and Tarnier, <sup>j</sup> Ludwig and Savor, <sup>k</sup> Guinard and Dumarest, <sup>l</sup>

<sup>a</sup> Bujwid, O.: Kann des Antidiphtherieserum schädlich sein? Polnisch, ref. Virchow's Jahrb., 1897, II, p. 659.

<sup>b</sup> Hamburger, F., and Moro: Über die biologischen nachweisbaren Veränderungen des menschlichen Blutes nach Seruminjektion. Wiener klin. Woch., 1903.

<sup>c</sup> Hamburger, F.: Zur Frage der Immunisierung gegen Eiweiss. Wiener klin. Woch., 1902. —: Arzeigenheit und Assimilation. Wien, 1903.

<sup>d</sup> Uhlenhuth: Zur Kenntniss der giftigen Eigenschaften des Blutserums. Zeit. f. Hyg., vol. 26, 1897, p. 384.

<sup>e</sup> Stern: Ueber einige Beziehungen zwischen menschlichen Blutserum und pathogenen Bakterien. (XII Congress für innere Medicin, Wiesbaden, 1893): Deut. med. Woch., 1893.

<sup>f</sup> Rummo: Ueber die Giftigkeit des Blutserums bei Menschen und Thieren im normalen Zustande und bei Infectiouskrankheiten. Wiener med. Woch., 1891.

<sup>g</sup> Albu: Untersuchungen über die Toxicität normaler u. pathol. Serumflüssigkeit. Virchow's Archiv., bd. 140, 1897.

<sup>h</sup> Charrin: Compt. rend. soc. biol., 1890.

<sup>i</sup> Leclainche and Rémond: Compt. rend. soc. biol., 1890, p. 1037.

<sup>j</sup> Ludwig and Savor: Monatschrift für Geburtshulfe, bd. 1, 1895.

<sup>k</sup> Tarnier and Chambrelent: Note relative à la recherche de la toxicité du sérum sanguin dans deux cas d'eclampsie puerpérale. Soc. de biolog., 1892.

<sup>l</sup> Guinard and Dumarest: Note sur la détermination de la toxicité du sérum sanguin: Techniques et résultats. Compt. rend., 1897, No. 15. Variations de la toxicité du sérum sanguin dans certaines infections expérimentales. Loc. cit., No. 18. A propos de la détermination physiol. et clinique de la toxicité du sérum humain. Loc. cit., No. 18.

Bar and Rénon,<sup>a</sup> Picolini and Conti,<sup>b</sup> Mairet and Bosc.<sup>c</sup> All of these authors determined the toxicity of the blood serum in accordance with Rummo's method of intravenous injections into rabbits.

The poisonous properties of the blood serum from healthy men, according to the work of these authors, varies considerably, as may be judged from the following summary:

Rummo found 10 c. c. as the fatal dose for 1 kilogram of rabbit.

Lugwig and Savor, 8 to 9 c. c.

Chambrelet and Tarnier, 10 c. c.

Albu, 9.5 to 11 c. c.

Mairet and Bosc, 12.5 to 18.0 c. c.

Guinard and Dumarest, 17 c. c.

Leclainche and Rémond, 23 c. c.

Charrin, 27 c. c.

It will therefore be seen that the fatal dose of normal human serum, according to these different investigations, varies between 8 c. c. and 27 c. c. per kilogram of rabbit.

Uhlenhuth found that the intravenous injection of from 7 to 10 c. c. of normal blood serum per kilogram of rabbit regularly caused death within one-half hour.

The blood serum of other animals was injected intravenously into rabbits and showed considerable differences in their toxicity. The quantity necessary to kill in proportion of 1 kilogram of rabbit was:

	c.c.
Sheep serum .....	11
Hog serum .....	12
Cattle serum .....	6

Horse serum on the other hand, when injected in such large quantities as 60 c. c. and more per 1 kilogram of rabbit, not only did not kill but caused no reaction at all.

In 1888 Mosso<sup>d</sup> discovered that eel serum in doses as small as 0.02 c. c. per 1 kilogram of rabbit caused death.

Uhlenhuth, finding intravenous injections into rabbits unsatisfactory, used subcutaneous inoculations into guinea pigs. He found that the injection of small quantities (0.5 c. c.) of human,

<sup>a</sup> Bar and Rénon: De la toxicité du sang et de l'urine chez une femme atteinte de troubles gravido-cardiaques. Compt. rend. soc. biol., 1894.

<sup>b</sup> Picolini and Conti: Toxicité du sérum sanguin et de l'urine dans la pneumonie.

<sup>c</sup> Mairet and Bosc: Recherches sur les causes de la toxicité du sérum du sang. Compt. rend., v. 119, p. 292. Toxicité du sérum du sang de l'homme sain. Comp. rend., 1897. \*Des effets de la chaleur sur la toxicité du sérum. Compt. rend., 1894.

<sup>d</sup> Mosso: Die giftige Wirkung des Serums der Muraeniden. Arch. f. exper. pathol. u. pharmakol., bd. 25, p. 111.



The hastening power of the reaction must be regarded as an advantage that the organism has won for itself through the occurrence of the first disease.

In applying this view to disease von Pirquet and Schick find that an organism in the stage of the free antibodies is an expression of the immediate power of reaction or hypersensitiveness. This stage has only a limited duration. The free antibodies disappear; however, the individual remains immune. The fact of this immunity consists, however, no longer in the power of immediate reaction against the cause of the infection, but in a hastened power of reproduction of antibodies. An organism has the power to combat a newly introduced infection and localize it, as is well shown in the case of vaccination. Von Pirquet and Schick believe that this property does not depend so much upon free antibodies in the fluids as upon a property acquired by the cells through the first disease, in which we see an expression of cellular immunity.

In Wassermann's<sup>a</sup> observation that the adaptation of the tissues to the existence of microorganisms which were naturally pathogenic without any apparent reaction and production of antibodies we see an instance of immunity through insusceptibility.

A series of diseases—for example, smallpox, measles, varicella, rötthlen—cause after one attack the individual to remain more or less protected throughout the rest of his life. They have the common clinical characteristic that, after a long and definite period of incubation, the disease runs a course in a definite and comparatively short time. If the organism does not die as a result of the disease, then it has won a complete victory over the cause of infection; the same is no longer able to produce harm in such an organism.

According to von Pirquet and Schick the fact that the clinical immunity against this group of diseases, for which we may take vaccinia as a type, does not consist of an acquired nonsusceptibility against the cause of the infection, but the power of an accelerated reaction. They believe they have shown from their work that they have reawakened interest in the serum disease and that this syndrome is not only of interest clinically but, from the standpoint of general pathology, is of the greatest importance.

Our explanation of the cause of sudden death following the second injection of horse serum does not differ essentially from the theory (which has just come to our notice as our manuscript is being edited for press) published by von Pirquet and Schick, relative to the reaction of the serum disease in man, although these similar deductions were reached independently and from different premises.

<sup>a</sup>Wassermann, A.: *Über natürliche und künstliche Immunität*. *Zeit. f. Hyg.*, *bd. 37*, p. 173. — *Wesen der Infection*, in Kollé und Wassermann. *Handb. der pathogen. Mikroorganismen*, 1903.

We believe that the substance which sensitizes the animal is identical with that which later poisons it, absurd as that may seem at first glance. However, the first substance must cause a reaction in the organism resulting in the production of "antibodies" and it is these antibodies combining with a substance in the horse serum that produce the toxic action. We have found that small quantities of serum produce, after a definite period of incubation, a condition of anaphylaxis. Large quantities probably produce a lesser grade of hypersusceptibility. Multiple or repeated injections produce immunity. We therefore possess in horse serum a substance capable of causing both anaphylaxis and prophylaxis.



## SUMMARY AND CONCLUSIONS.

Normal horse serum, when injected into the peritoneal cavity of a normal guinea pig, produces no symptoms. When injected subcutaneously there may result at most a slight local reaction consisting of swelling and edema, which gradually disappears.

Antitoxic horse serum is equally harmless for normal guinea pigs.

Horse serum is, however, poisonous to a guinea pig which has previously been injected with horse serum. The "period of incubation" or time necessary to elapse between the first and second injection is about ten days. Under these circumstances, horse serum is poisonous whether injected subcutaneously or into the peritoneal cavity.

The first injection of horse serum renders the guinea pig susceptible.

The symptoms caused by the injection of horse serum into a susceptible guinea pig are respiratory embarrassment, paralysis, and convulsions, followed by death. The symptoms come on usually within ten minutes after the injection, and when death results it usually occurs within one hour, frequently in less than thirty minutes, and sometimes within a few minutes.

The poisonous principle in horse serum appears to act upon the respiratory centers. The heart continues to beat long after respiration ceases.

The toxic action of horse serum bears no relation to diphtheria. The poison is not *toxone*. Guinea pigs can not be rendered susceptible by previous infections with the *B. diphtheriæ* or by previous injections with diphtheria toxine.

It seems from our work, however, that guinea pigs first injected with a mixture of diphtheria toxine plus horse serum are more sensitive to subsequent injections of horse serum than are guinea pigs sensitized with a first injection of horse serum alone.

DIPHThERIA ANTITOXIN PLAYS NO PART IN THIS POISONOUS ACTION AND IN ITSELF IS HARMLESS.

As soon as we realized that the toxic principle in horse serum exerts its action in quantities so minute as to place it almost in the category of the ferments and, further, when we concluded from our work that this toxic principle is doubtless one of those highly organized and complex proteid substances belonging to the "haptin group" in the sense used by Ehrlich, we recognized how futile it would be with present methods to attempt to isolate this substance.

Nevertheless we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eliminating or neutralizing this toxic principle in horse serum is at once evident.

It is probable that when the strange proteid is introduced into the guinea pig it causes a reaction resulting in a production of "antibodies," so that when a second injection of horse serum is given there is probably either a union or a reaction between the antibodies and a substance in the horse serum which produces the poisonous effect.

This poisonous principle is quantitatively specific; that is, guinea pigs treated with horse serum are rendered somewhat susceptible to the subsequent injection of the serum of another animal. Guinea pigs treated with the serum of another animal are slightly sensitive to the toxic action of horse serum.

Guinea pigs treated with the serums of various animals and subsequently injected, are much more susceptible to homologous serums than to heterologous serums.

This poisonous action has no relation to hemolysis. Our work proves that blood serum may contain an acute poison entirely independent of any hemolytic action. Normal horse serum has no lytic power upon the red corpuscles of the normal guinea pig.

This poisonous action has no relation to the specific albuminous precipitins.

The poisonous principle in horse serum is not affected by a temperature of 60° C. for 6 hours, but it is destroyed at 100° C. for 15 minutes.

The poisonous principle is filterable through porcelain, is not injured by drying, and can not be separated by precipitation with ammonium sulphate and subsequent dialysis.

The following chemical substances do not oxidize, neutralize, or precipitate the poisonous principle in horse serum: Butyric acid, permanganate of potash, citrate of soda, alcohol, succinic peroxide acid (alphozone), hydrogen dioxide, and ammonium sulphate. The presence of chloroform or trikresol (0.4 per cent) does not interfere with this poisonous action.

Serums eight years old are as toxic as those freshly separated.

Exposure to X-rays does not affect the poisonous action of horse serum.

It requires about 10 days after the first injection of horse serum for a guinea pig to show susceptibility to a second injection. A guinea pig remains susceptible a very long time, at least 160 days.

As small a quantity as  $\frac{1}{1,000,000}$  c. c. of horse serum was sufficient in one instance to render a guinea pig susceptible. Quantities

varying from  $\frac{1}{10}$  to  $\frac{1}{100}$  c. c. almost invariably render guinea pigs highly susceptible when given in the toxine-antitoxine mixture.

One-tenth c. c. of horse serum injected into the peritoneal cavity of a susceptible guinea pig is sufficient to cause death. The same quantity inoculated subcutaneously may cause serious symptoms.

There is some evidence to show that the sensitizing substance in horse serum is the same as the poisonous substance. The sensitizing substance is not affected by precipitation with ammonium sulphate and dialysis.

Guinea pigs may be sensitized with horse serum that has been dried and redissolved.

The sensitizing substance is not affected by a temperature of 60° C. for 6 hours.

It is probable that small quantities of horse serum render a guinea pig more susceptible than do large quantities. If this be true, it is due, perhaps, to the fact that large quantities, owing to slow absorption or prolonged reaction, partly immunize the guinea pig at the same time that it is being sensitized.

The sensitizing substance apparently is not free in the blood serum of guinea pigs.

An active immunity against this toxic principle may readily be established by repeated injections of horse serum, at short intervals, into a guinea pig. Although guinea pigs may be immunized actively in this manner we have not yet succeeded in transferring this immunity in the blood serum or body juices to another guinea pig. It therefore appears that the immune bodies, if such exist against the toxic action of horse serum, are not free in the blood and body juices contrary to the case in diphtheria.

Guinea pigs may be sensitized to the toxic action of horse serum by feeding them with horse serum or horse meat.

The fact that guinea pigs may be rendered susceptible by the feeding of strange proteid matter opens an interesting question as to whether sensitive guinea pigs may also be poisoned by feeding with the same serum given after a proper interval of time. If man can be sensitized in a similar way by the eating of certain proteid substances may not this throw light upon those interesting and obscure cases in which the eating of fish, sea food, and other articles of diet habitually cause sudden and sometimes serious symptoms?

The susceptibility to the toxic action of horse serum is transmitted hereditarily from the mother guinea pig to her young.

These results upon the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea pigs **in** throw light upon the well-known hereditary tendency to tuberculosis in children born of a tuberculous parent. There are **certain** analogies between the action of tuberculosis and horse serum.

may produce a hypersusceptibility and also a certain degree of immunity. Now that we have proved that this hypersusceptibility or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea pigs, may it not throw light upon the fact that tuberculosis "runs in families?"

Demonstrations of the hereditary transmission of acquired characters are comparatively rare in biology. While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young, yet, as far as we know, this is the first recorded instance in which hypersensitiveness, or anaphylaxis, has been experimentally shown to be transmitted from a mother to her young.

Other albuminous substances, such as skimmed milk, peptone, hemoglobin, egg albumin, and vegetable proteids possess no poisonous action upon guinea pigs sensitized with horse serum. Whether guinea pigs are rendered susceptible to a subsequent injection with the same albuminous matter with which they have been sensitized will be reported in a later paper.

We believe that the substance which sensitizes the animal is identical with that which later poisons it. However, the substance must first cause a reaction in the organism resulting in a production of antibodies. We have found that small quantities of horse serum produce, after a definite period of incubation, a condition of anaphylaxis; multiple or repeated injections produce immunity. We therefore possess in horse serum a substance capable of causing both anaphylaxis and prophylaxis.

It may be that man can not be sensitized in the same way that we have shown to be the case with guinea pigs. Children have, in a number of instances, been injected with antidiphtheric horse serum at short and long intervals without, so far as we are aware, causing death. Certain serums, for example, the antitubercle serum of Maragliano and the antirheumatic serum of Menzer, are habitually used by giving injections at intervals of days or weeks. In all such cases of frequent and repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to our work. Von Pirquet and Schick have shown that a second injection of horse serum into children causes an "immediate" or an "accelerated" reaction. Both the immediate and the accelerated reaction in children are characterized by symptoms of "the serum disease."

We might conclude that children may not be sensitized to the toxic action of horse serum by eating horse meat, for horse meat is a favorite article of diet in certain European countries and there is nothing upon record to show that the injection of horse serum in those countries is fraught with more danger than where this diet is not used. It should,

however, be borne in mind that our work has shown that guinea pigs may be sensitized with exceedingly minute quantities of a strange proteid, and that repeated injections cause an immunity, and it does not seem impossible that the same action may be true of food.

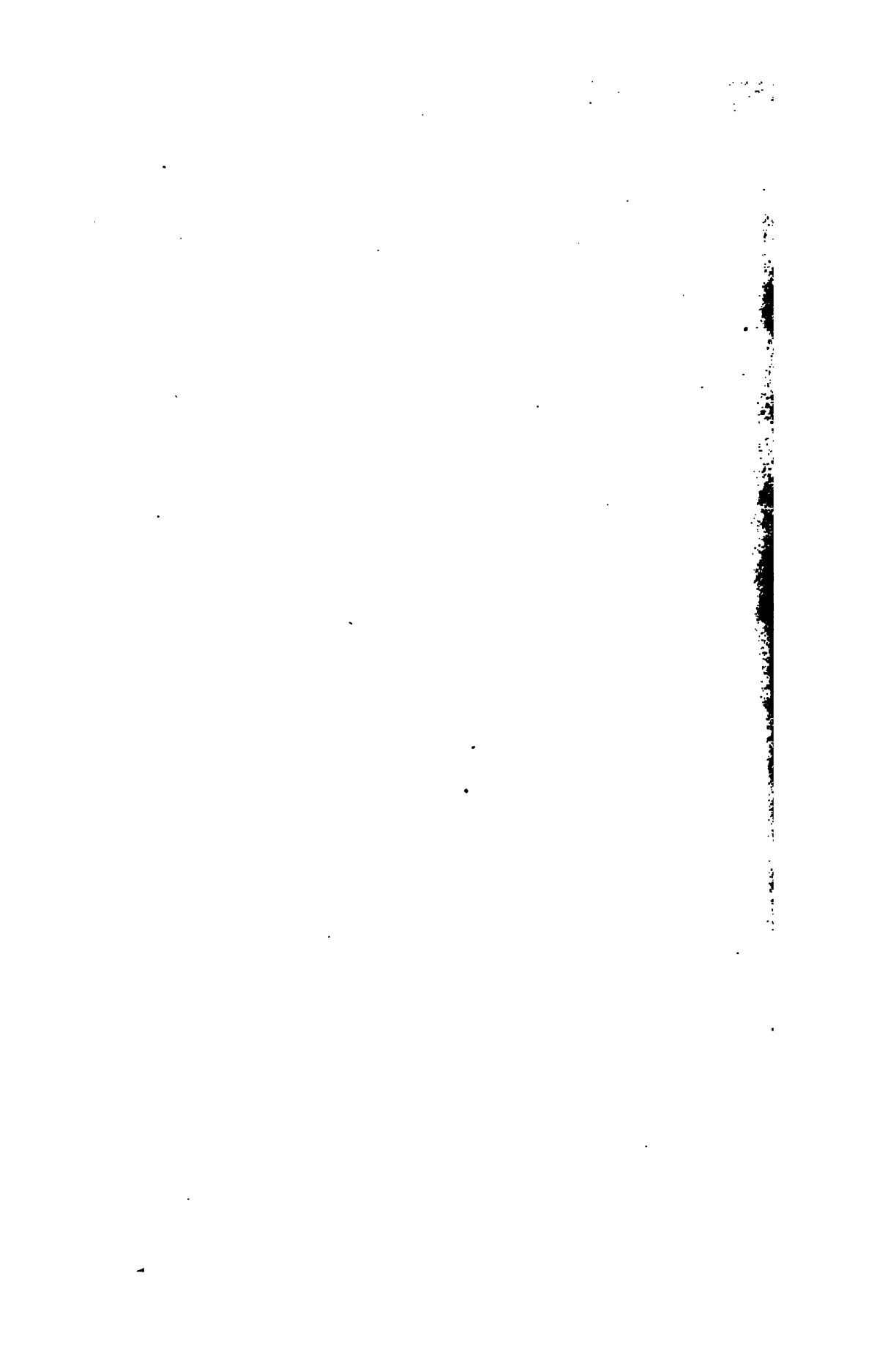
Man reacts to the first injection of horse serum after a period of eight to thirteen days; guinea pigs show no reaction as a result of the first injection; both man and guinea pigs react to a second injection. The reactions in man and the guinea pig differ, however, both in severity and in kind. The relation, therefore, that our observations upon the guinea pig may have in their application to man must await further study.

The fact that other animals beside man and the guinea pig react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action.

We believe that our results make it probable that man may be rendered sensitive to the injection of a strange proteid, as is the case with the guinea pig and other animals, and that this explanation must be considered as well as the *status lymphaticus*, which has heretofore been assigned as the cause of sudden death following the injection of horse serum.

[POST SCRIPTUM.—After the galley proof of this article had left our hands an article by R. Otto entitled "Das Theobald Smithsche Phänomen der Serum-Ueberempfindlichkeit," reprinted from Leuthold-Gedenkschrift, band 1, first came to our notice. His paper deals with some of the problems we have studied and his results are in harmony with many of our conclusions.]





(Continued from second page of cover.)

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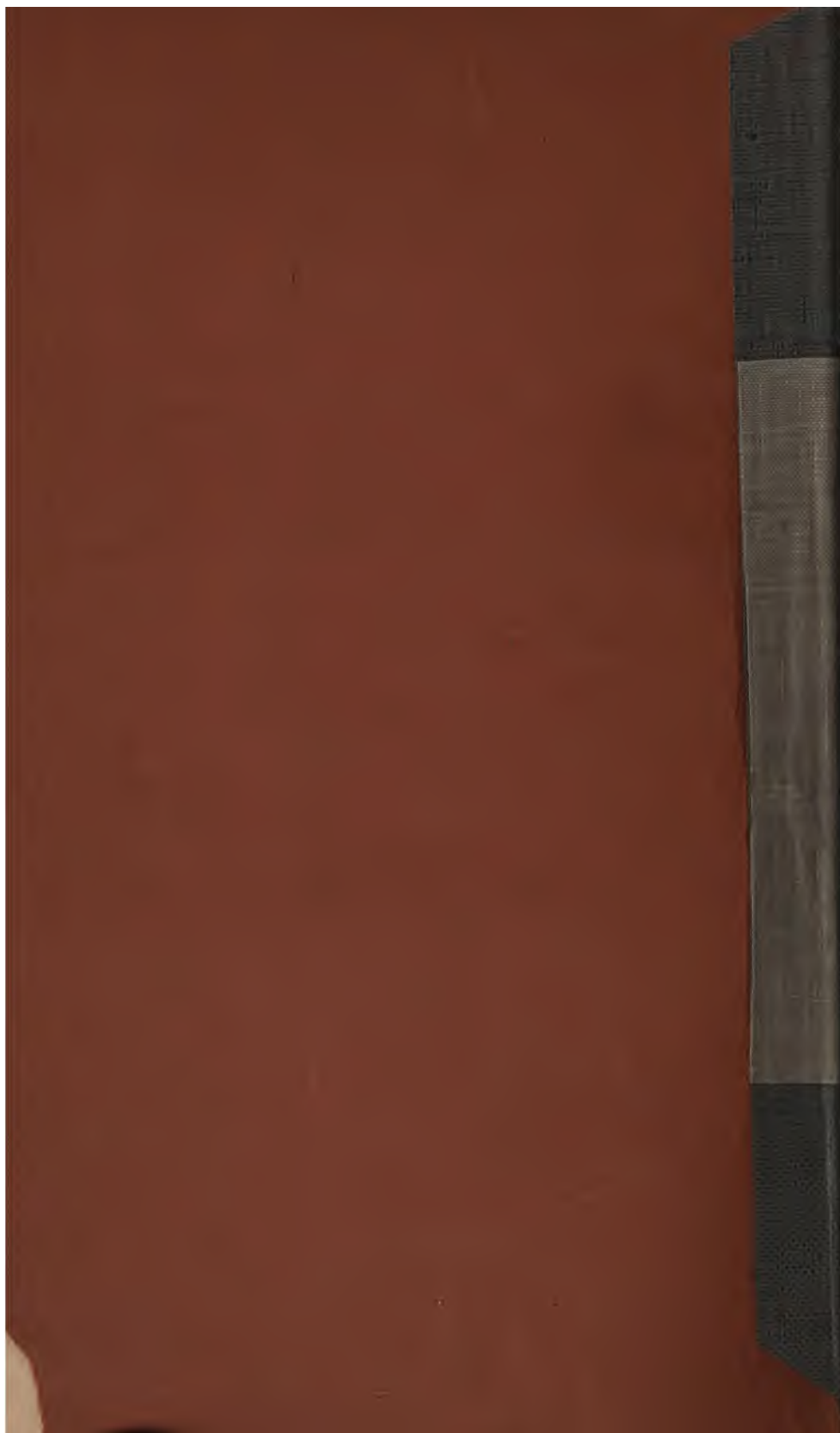
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