

# ANAPHYLATOXIN AND ANAPHYLAXIS

## VII. PEPTONE ANAPHYLATOXIN

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### SYNOPSIS

#### INTRODUCTION

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EFFECT ON BLOOD COAGULATION

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SYMPTOMS; THE LETHAL DOSE; IMMUNITY

EFFECT ON BLOOD COAGULATION

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RAT SERUM; IN GUINEA-PIG SERUM

#### THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN RATS; IN

GUINEA-PIGS; IN RABBITS

#### SUMMARY

The observations made in Ludwig's laboratory by Schmidt-Mülheim,<sup>1</sup> and by Fano,<sup>2</sup> that an injection of digestion products, that is, peptone, caused an intoxication characterized by a noncoagulable blood, served as the starting point of a long series of investigations. The early work was necessarily made with mixtures consisting of various hydrolytic products, and in view of the inherent difficulties it is not surprising, therefore, to find that very little was learned regarding the nature of the substance concerned, and much less regarding its mode of action. Even as late as 1900, Pick and Spiro<sup>3</sup> resorted to the conception of a hypothetical substance or substances which they designated as "peptozyme," a term which represented no advance over the "peptotoxin" of Brieger.<sup>4</sup> Although Witte's peptone, which consists largely of albumoses, was used frequently in such work, it was by no means certain that the action was due to these bodies. The studies of Chittenden, Mendel, and Henderson,<sup>5</sup> of Underhill,<sup>6</sup> and of

<sup>1</sup> Arch. f. Anat. u. Physiol., 1880, p. 33.

<sup>2</sup> Ibid., 1881, p. 277. Arch. ital. de biol., 1882, 2, p. 146.

<sup>3</sup> Ztschr. f. physiol. Chem., 1900, 31, p. 272.

<sup>4</sup> Ueber Ptomaine, 1885, 1, p. 17; Deutsch. med. Wehnschr., 1891, 17, pp. 821, 917.

<sup>5</sup> Am. Jour. Physiol., 1899, 2, p. 142.

<sup>6</sup> Ibid., 1903, 9, p. 345. Underhill and Hendrix, Jour. Biol. Chem., 1915, 22, p. 443.

others, however, served to show that certain proteoses were responsible for the observed effects.

The question of peptone-intoxication acquired a new interest with the discovery of anaphylaxis, for the reason that the symptoms and findings were essentially those of anaphylactic shock. The work of Vaughan<sup>7</sup> directed attention to the protein-cleavage products as the probable poison in anaphylaxis, and about the same time, DeWaele<sup>8</sup> pointed out the striking analogy between the two phenomena. Somewhat later, Biedl and Kraus<sup>9</sup> emphasized the similarity of the two forms of poisoning in dogs, while Hirschfelder<sup>10</sup> showed that the injection of Witte's peptone into guinea-pigs reproduced the picture of anaphylaxis. These facts obviously did not prove that anaphylactic shock was due to intoxication with peptone, but they at least served to show that the two had something in common.

If it was difficult to explain anaphylactic shock, it was equally difficult to account for the markedly poisonous property of proteoses. It was easy enough to assume that peptone was poisonous per se, or that on further cleavage it gave rise to the real poison. On the other hand it was conceivable that the proteose, strictly speaking, was not toxic but that it induced poison-production in the plasma of the animal. This view, rendered highly probable by the work on trypanosome and agar anaphylatoxin (Parts I to IV), was subjected to the test of experiment with the result that anaphylatoxin-production was shown to occur. This poison is formed in vitro when the peptone and serum are mixed; it is also produced in vivo when the peptone is injected into animals. Peptone, agar, and cellular matter (trypanosomes, bacteria, etc.) have, therefore, a common mode of action.

#### INJECTION OF PEPTONE INTO GUINEA-PIGS

At the outset, it was desirable to ascertain the effects of Witte's peptone on guinea-pigs, notwithstanding the fact that a number of investigators had concerned themselves with this subject.

Thus, Persano<sup>11</sup> found that the injection of more than 0.6 gm. per kilo of a commercial preparation caused rapid death, while DeWaele<sup>8</sup> obtained some effect by intraperitoneal injections of Witte's peptone, as did also Pfeiffer and Mita.<sup>12</sup> Hirschfelder<sup>10</sup> like Persano found that the intravenous injection of this

<sup>7</sup> Jour. Infect. Dis., 1907, 4, p. 504.

<sup>8</sup> Bull. de l'Acad. roy. de med. de Belgique, 1907, 21, p. 715.

<sup>9</sup> Wien. klin. Wchnschr., 1909, 22, p. 363. Ztschr. f. Immunitätsf., 1910, 7, p. 222.

<sup>10</sup> Jour. Exper. Med., 1910, 12, p. 586.

<sup>11</sup> Arch. ital. de biol., 1902, 37, p. 409.

product caused acute death and in addition, he noted the similarity to that of anaphylaxis; the dose employed by him was 0.98 to 2.8 gm. per kilo. Biedl and Kraus,<sup>13</sup> using a dose of 1 to 1.2 gm. per kilo, obtained similar results.

Kumagai<sup>14</sup> established 1.5 c.c. of 10% Witte's peptone as a single lethal dose per 200 gm. (0.75 gm. per kilo), while Ritz<sup>15</sup> employing a 12% solution found the lowest dose to be 1.8 c.c. (0.87 to 0.93 gm. per kilo). It will be shown presently that an average lethal dose, one which kills about 50% of the test animals, may be as low as 0.6 c.c. of the 10% solution (0.3 gm. per kilo), an amount which is appreciably less than hitherto reported.

For the preliminary tests given in Table 70, a 10% solution of Witte's peptone was used; this was prepared by dissolving 10 gm. in 100 c.c. of salt solution (0.85%) at 60 C. The resulting suspension can be used as such, but for most of the work it was autoclaved at 110 C. for 15 minutes and then either filtered or centrifugated.

In Exper. A, the cloudy suspension, obtained by warming at 60 C., was used; for Exper. B, this suspension was centrifugated at 3000 revolutions for 4 minutes; for Exper. C, it was centrifugated at 8000 revolutions for 45 minutes; for Exper. D, it was heated to 110 C. for 15 minutes and filtered. The tests with these four solutions were made on the same day, while the others were made at different times. In Exper. E, the solution was autoclaved at 110 C. and then centrifugated at 8000 r. p. m. for 40 minutes. For Exper. F, the dry peptone was sterilized at 150 C. for 1 hour, then dissolved in sterile salt solution at 60 C. In Exper. G, the peptone was dissolved in distilled water at 60 C., and used as such, unfiltered, for Test 17, while for the two preceding tests it was first heated to 110 C. and filtered.

The symptoms and findings in guinea-pigs injected with peptone were the same as those produced by anaphylatoxin, by agar, or in specific anaphylactic shock. In addition to the usual effects—dyspnea, spasms, convulsions, and urination—a marked exophthalmos was noted. This symptom has seemingly been overlooked by previous observers. It is a striking feature when large guinea-pigs are used, and especially is it in evidence in rabbits injected with peptone. If recovery occurs, it disappears in a few minutes. The necropsy findings were those of typical anaphylactic shock—maximal distention of lungs, heart beating, absence of clot, blood fluid, and low blood pressure, as indicated by the difficulty of drawing blood from the heart for transfusion and other tests.

<sup>12</sup> Ztschr. f. Immunitätsf., 1909, 4, p. 439.

<sup>13</sup> Zentralbl. f. Physiol., 1910, 24, p. 258. Zentralbl. f. Bakteriöl., R., 1910, 47, Beiheft, p. 35.

<sup>14</sup> Ztschr. f. Immunitätsf., 1913, 17, pp. 626, 636.

<sup>15</sup> Ibid., 1911, 12, p. 654.

*The Lethal Dose.*—An inspection of the table will show that the lowest fatal dose was obtained in Expts. A and F, in which the unfiltered suspension, made at 60 C., was used. Seemingly, short centrifugation at 3000 r. p. m. decreased the toxicity (B); this was lowered still more by prolonged centrifugation at 8000 r. p. m. (C), or by autoclaving and filtering (D). In other words, the clear solution was less toxic than the cloudy suspension, the lethal dose of the former being 1.5 c.c. or more (C, D, E), while that of the latter was 0.75 to 1 c.c. (F, A). These figures, however, do not represent the minimal fatal dose, for it will be shown that smaller amounts may prove fatal.

TABLE 70  
INJECTION OF 10% PEPTONE SOLUTION INTO GUINEA-PIGS

Expt.	Guinea-Pig		Peptone Solution*		Result
	Number	Weight	c.c.	gm. per kilo	
A	1	210	1.5	0.71	3/40". Typical shock
	2	220	1.0	0.45	3/40". " "
	3	220	0.5		Very slight
B	4	207	1.5	0.72	3/40". Typical shock
	5	185	1.0		Slight
C	6	215	4.0	1.8	3". Atypical shock
	7	250	2.0	0.8	3". Typical shock
	8	225	1.5		Very slight
D	9	210	2.0	0.95	3/10". Typical shock
	10	212	1.5		Severe
E	11	198	1.5	0.75	4/25". Typical shock
	12	170	"	0.88	6/10". " "
F	13	195	0.75	0.38	6". Atypical shock
	14	198	0.5		Slight
G	15	185	4.0	2.1	2/20". Typical shock
	16	235	2.5	1.06	3". " "
	17	206	1.5	0.72	3/40". " "

\* Intravenously injected.

Thus, it will be seen from Tables 71 and 72 that 1 c.c. of the clear peptone solution may kill in about one-half of the tests (= 0.5 gm. per kilo), and furthermore (Table 75), even 0.6 c.c. (0.3 gm. per kilo) will give a like result. On the other hand, very exceptionally, recovery has been observed after a rapid injection (5 to 7 seconds) of 1 gm. per kilo. These facts indicate that the guinea-pig shows considerable variation as to its susceptibility to this intoxication. Moreover, it is to be noted that peptone is considerably less toxic than agar, which, as shown in Part VI, may kill in dose of 10 mg. per kilo.

*Variable Resistance.*—From what has just been said it is evident that no two guinea-pigs will necessarily show the same behavior to a given dose of peptone. As was the case with anaphylatoxic serum, individual variation in susceptibility is a striking feature which extends not only to the symptom complex as a whole, but also to the individual features thereof. Table 71, for example, presents the results of a series of tests made at intervals of 15 minutes, the peptone being kept at 37 C. Although the injection time was the same (45 seconds) and the dose employed was but 1 c.c., 4 tests proved fatal, whereas the other 6 showed slight or practically no effect. This variation in response may extend to the individual symptoms or changes. Thus, given the same injection of peptone into a series of guinea-pigs, the speed of coagulation of the blood when transferred to the test tube will vary considerably. Similarly, the drop in blood pressure is subject to much variation.

TABLE 71  
INJECTION OF 10% PEPTONE, INCUBATED AT 37 C., INTO GUINEA-PIGS

Guinea-Pig		Intravenous Injection (c.c.)	Peptone Incuba- tion at 37 C. (hr.)	Result
Number	Weight			
1	208	1.0	—	Very slight
2	192	"	¼	11'35"
3	191	"	½	2'55"
4	198	"	¾	Very slight
5	212	"	1	Slight
6	196	"	1¼	Very slight
7	196	"	1½	3'15"
8	208	"	1¾	2'
9	186	"	2	Fair
10	196	"	2¼	Slight

The lethal dose is 0.49 to 0.52 gm. per kilo of guinea-pig.

*Speed of Injection.*—Table 72 presents another instance of variation, the experiment being similar to that given in Table 45. The animals were tested in pairs, the second one of each pair being injected within 30 seconds of the first, and the entire set of 6 pairs being injected in less than 45 minutes and in the order given. The immediate object of the experiment was to ascertain the effect of the speed of injection, and with that in mind the first 3 pairs were injected slowly, the injection being interrupted and 0.25 c.c. being given every 15 seconds; the total time of the injection was 45 seconds. The second 3 pairs were injected very rapidly, that is, in from 2 to 3 seconds. The same number of acute deaths was obtained in the first as in the second set, but the latter, in addition, developed 2 slow deaths.

The experiment shows that a very rapid injection is somewhat more injurious than one made slowly, a fact which has been recognized, concerning the dog, from the time of Schmidt-Mülheim and Fano. With an injection time of 2 minutes, a dose of 1.5 c.c. of peptone, even if diluted with salt solution or with distilled water, can be given quite safely, but the same dose injected in half that time is usually fatal.

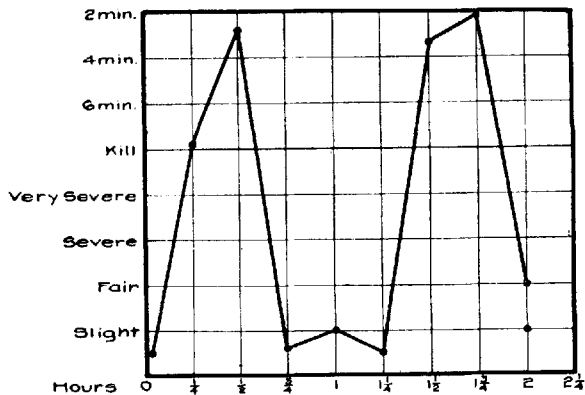


Chart 10. Apparent variation in the toxicity of a 10% solution of Witte's peptone, kept at 37 C. (Table 71).

TABLE 72  
APPARENT VARIATION IN THE TOXICITY OF PEPTONE. INDIVIDUAL RESISTANCE.  
SPEED OF INJECTION \*

Exper.	Guinea-Pig		Peptonet (c.c.)	Result	Exper.	Guinea-Pig		Peptonet (c.c.)	Result
	No.	Weight				No.	Weight		
A	1	187	1.0	2'40"	B	4	200	1.0	10 hr.
	1a	185	"	Slight		4a	200	"	7 "
	2	202	"	Moderate		5	196	"	3'50"
	2a	205	"	2'47"		5a	195	"	Slight
	3	205	"	Slight		6	203	"	3'52"
	3a	204	"	3'17"		6a	204	"	3'40"

\* Nos. 1 to 3a given interrupted slow injection; Nos. 4 to 6a given very rapid injection.  
† Intravenously injected.

*Immunity.*—Another well-known fact regarding peptone is that a certain immunity or tolerance is established by a nonfatal injection. In dogs, this immunity is seen in the behavior of the blood pressure, and of the blood itself which does not become incoagulable as it did after the first injection. In treated guinea-pigs, the immunity is evidenced by the fact that the second injection of an otherwise surely fatal dose

is tolerated with little or no effect. Thus, No. 1a of Table 72 when injected 3 hours later with 1.5 c.c. of a 10% peptone showed some slight respiratory disturbance and urinated, but developed no spasms, and when bled an hour later, the blood coagulated in the tube in 5 minutes.

Numbers 2 and 3 of Table 72 were likewise reinjected with 1.5 c.c. of the peptone, 3 hours after the first injection. Both showed practically no effect and consequently, half an hour later, they were given a third injection of a like amount, with no effect other than depression and some uneasiness. Twenty minutes later, they were given a fourth injection as before; No. 2 showed no effect, and 15 minutes later it was bled from the heart to ascertain the effect on the blood; this coagulated in the tube in 6 minutes. No. 3, however, after the fourth injection, developed slight jerky spasms which kept up for half an hour. An hour and a half after the injection it was very weak and on its side, and clearly was about to die. It was accordingly bled from the heart, and the blood was found to clot in the tube in 3 minutes.

By contrast with the foregoing, No. 5a, which also was reinjected with 1.5 c.c. of the peptone solution, 3 hours after the first treatment, responded with a severe typical shock and died in 6 minutes; the blood drawn from the heart 3 minutes after death clotted in the tube in 2 minutes. This does not mean that the blood was more coagulable than in the preceding instances, for if the coagulation time is reckoned from the moment of death, it would be 5 minutes. This result shows that in some individuals the immunity is either very fugitive or very fragile.

The repeated injection of a sublethal dose of peptone (0.5 c.c.), at intervals of 15 minutes, was also tried, with the object of learning the effect of such injections on the coagulability of the blood. It was rather unexpected to find that 1 guinea-pig died in 8 minutes after the second injection; the blood drawn from the heart 5 minutes after death did not clot in the tube until 7 minutes later, or 12 minutes after death. In another test, the guinea-pig showed no effect from the first and second injections, but died in about 15 minutes after the third; its blood coagulated in the tube in 3 minutes. Apparently, the injection of sublethal doses at short intervals is not tolerated as well as that of larger doses at longer intervals.

The immunity as regards a given dose of peptone (1.5 c.c.) can often be broken down by diluting this dose with 6 to 9 parts of salt

solution or distilled water. Nos. 1, 2, 4, and 5 of Table 78 present instances of this kind. This result, however, is not constant, especially if the first dose had been similarly diluted.

*Antagonistic Action of Salt Solution.*—Ritz,<sup>15</sup> in testing the protective action of salt solution against peptone intoxication obtained somewhat irregular results, which in the main, however, showed that salt could protect. The variable results, without doubt, were due in part to the individual resistance of the animals, and, in part, to the delayed injections. It was desirable to make independent tests of the behavior of salt, especially for comparison with similar experiments with sodium carbonate.

A 10% solution of Witte's peptone (10 gm. + 100 c.c. of 0.85% NaCl) was used for the tests given in Tables 73 and 74. A so-called 30% solution of salt was prepared by dissolving 7.5 gm. of Kahlbaum's sodium chlorid in 25 c.c. of distilled water. The injections were made, as usual, into the jugular vein. As Ritz and others have noted, the injection of the concentrated salt solution must be made slowly to avoid a fatal shock. A careful watch of the respiration of the guinea-pig during the injection is desirable. The irritant action of the salt is usually seen in the production of tremors involving the abdominal muscles. The time required for the injection of 1 c.c. of the salt solution in the following tests ranged from  $1\frac{1}{2}$  to  $1\frac{3}{4}$  minutes. Immediately after the injection of the salt, the syringe was disconnected from the needle, which remained in the vein; a syringe with peptone was then attached and its contents injected, the time for the latter injection being 5 to 8 seconds. The total time from the beginning to the end of this double injection was  $1\frac{3}{4}$  to 2 minutes.

From Exper. A, Table 73, it will be seen that 1 c.c. of the salt solution protected perfectly, in 3 of 4 tests, against 1.5 c.c. of the peptone solution, a dose which is almost invariably fatal. This amount of salt, however, did not protect against 2 c.c. of the peptone solution (Exper. B), corresponding in this respect to the like tests with sodium carbonate (Table 74, Exper. B). In the work with the latter, however, it was found that the alkali did protect against this amount of peptone, provided it was injected immediately before and after the peptone. Similar tests with salt (Exper. C) failed to give protection. The procedure followed in Exper. C was first to inject 0.5 c.c. of the salt solution, then the peptone which was followed, at once, by another 0.5 c.c. of salt. The total time from the beginning to the end of these injections (Nos. 9 and 10) was  $1\frac{3}{4}$  and  $2\frac{1}{4}$  minutes, respectively.

TABLE 73  
PROTECTIVE ACTION OF SALT AGAINST PEPTONE

Exper.	Guinea-Pig		c.c. 30% NaCl	c.c. 10% Peptone	Result
	Number	Weight			
Control	1	193	—	1.5	4/15". Typical shock
	2	182	—	"	13/20"
A	3	185	1.0	1.5	Practically nil
	4	190	"	"	5". Typical shock
	5	193	"	"	Like No. 3
	6	206	"	"	" " "
B	7	186	1.0	2.0	2 hr. 6 min.
	8	191	"	"	5/25". Typical shock
C	9	191	1.0*	2.0	5 hr.
	10	190	"	"	3/45"

\* Half before and half after peptone injection.

*Antagonistic Action of Sodium Carbonate.*—It was pointed out in Part III that Friedberger and Moreschi had observed that anaphylatoxin was destroyed by treatment with normal NaOH, and it was shown, further, that a triple alkalized serum did not give rise to the poison on subsequent treatment with agar (Table 36). Other tests, as yet not described, have demonstrated that anaphylatoxin is destroyed on contact with sodium carbonate, and these facts suggested that a similar reaction could take place in vivo, and that protection could thus be obtained against peptone and even against specific anaphylactic shock. The experiments given in Table 74 show that sodium carbonate actually does protect against peptone intoxication.

The method of procedure was the same as that given above in connection with the tests with salt. A normal solution of sodium carbonate was employed, and, as this was tolerated much better than the more concentrated salt solution, the injection time was reduced to 1 minute. The injection time for the peptone ranged from 8 to 20 seconds, and the total time from the beginning to the end of the double injection was  $1\frac{1}{4}$  to  $1\frac{3}{4}$  minutes.

It will be seen from Exper. A that the alkali protected against an otherwise fatal dose of peptone, the result corresponding to that of the like test, Exper. A of Table 73 where concentrated salt solution was used. The same amount of alkali, given in a single dose, did not protect against 2 c.c. of the peptone (Exper. B), but given in 2 injections, 0.5 c.c. before and 0.5 c.c. immediately after the peptone, it did protect (Exper. C). In the last test, No. 9, the alkali was given in 2 injec-

tions, each of 1 c.c., with the result that death occurred. An excess of alkali (2 c.c. or more) injected alone, can produce an acute death. Autopsy will disclose findings like those of anaphylatoxic poisoning.

On comparing the results given in Tables 73 and 74, it will be noted that normal  $\text{Na}_2\text{CO}_3$  (5.3%), in about one-sixth the concentration of the salt, is as active and even more so than the latter. In view of the fact that peptone intoxication is due to the in-vivo production of anaphylatoxin, it is significant that the action of the latter can be completely neutralized or destroyed by the administration of a small amount of alkali; the presence of the latter may even prevent the disturbance which leads to poison-production. While this is not the place to consider the fundamental conception regarding anaphylatoxin-production, it may be well to state the belief that the clinical application of this principle is of far-reaching importance; applied in 3 very severe cases (salvarsan poisoning, Landry's ascending paralysis, and multiple exudative erythema) it has yielded already surprising results.

TABLE 74  
PROTECTIVE ACTION OF NORMAL SODIUM CARBONATE AGAINST PEPTONE

Exper.	Guinea-Pig		c.c. Normal $\text{Na}_2\text{CO}_3$	c.c. 10% Peptone	Result
	Number	Weight			
Control	1	192	—	1.5	7'20"
	2	192	—	"	5'40"
A	3	190	1.0	1.5	Practically nil
	4	200	"	"	" "
B	5	200	1.0	2.0	3'30"
	6	198	"	"	3'55"
C	7	185	1.0*	2.0	Practically nil
	8	184	"	"	" "
	9	185	2.0*	"	5'10".

\* Half before and half after peptone injection.

*Antagonistic Action of Normal Serum.*—It is well known that the addition of normal serum to an organ extract renders the latter less toxic. A similar observation concerning kaolin anaphylatoxin was made by Mutermilch,<sup>10</sup> who found that the toxic property disappeared on treatment with either fresh serum or extracts of organs of guinea-pigs. His interpretation of a limited number of experiments may be questioned in view of the fact that guinea-pigs vary considerably in resistance (Table 45). Be that as it may, the belief that serum is antag-

<sup>10</sup> Ann. de l'Inst. Pasteur, 1913, 27, p. 90.

onistic to organ extracts is generally held, and it would be in order to expect a like behavior concerning peptone. In an extensive series of tests, having for their object the demonstration of anaphylatoxin-production in vitro, it was repeatedly observed that a serum-peptone mixture could be relatively inert, though the amount of peptone present, were it given by itself, would yield a large percentage of deaths. Thus, it will be shown that serum mixtures containing 0.5 or 0.3 c.c. of 20% peptone are quite nontoxic, though, as demonstrated in Tables 71 and 72, a dose of 1 c.c. of a 10% solution of peptone was fatal in about one-half of the tests; the control tests, Nos. 6 to 7a of Table 75, gave a similar result with 0.3 c.c. of a 20% solution of peptone.

TABLE 75

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. PROTECTIVE ACTION OF GUINEA-PIG SERUM AGAINST PEPTONE. THE DOSE REPRESENTS 3 C.C. SERUM AND 0.3 C.C. OF 20% PEPTONE

Exper. and Ratio	Guinea-Pig		Mixture			Result
	No.	Weight	Incubation at 0 C. (min.)	Incubation at 38 C. (min.)	Intra- venous Injection	
Control A 1:50	1	184	2	—	3.3	Nil
	1a	186	"	—	"	"
	2	190	6	—	"	"
	2a	191	"	—	"	Very slight
B 1:50	3	193	9	10	3.3	7'43"
	3a	198	"	"	"	Very slight
	4	205	"	15	"	" "
	4a	200	"	"	"	Moderate
	5	196	"	25	"	Very slight
	5a	197	"	"	"	45'
Control C	6	205	—	—	3.3*	4'20"
	6a	200	—	—	"	Slight
	7	190	—	—	"	Very slight
	7a	190	—	—	"	5'50"

\* The control C received a mixture of 3 c.c. of salt solution + 0.3 c.c. of 20% peptone.

For the experiments given in Tables 75 and 76, made on the same day, a 20% solution of Witte's peptone was prepared by adding 20 gm. of the latter to 100 c.c. of 0.85% salt solution; the mixture was heated on the water bath at 100 C. for 15 minutes and then filtered. For the tests given in Table 75, a single pool of 30 c.c. of fresh guinea-pig serum was used. To this serum, previously iced, 3 c.c. of the peptone

solution were added, and the mixture, after being swung for half a minute, was placed in cracked ice. The injections were made in pairs, the first being within 2 minutes and the second within 6 minutes after preparing the mixture; these first 4 tests served as preliminary controls. When injections were made in pairs, the second animal received its dose within half a minute of the first.

After Tests 1 to 2a had been made and the mixture kept at 0 C. for 9 minutes, it was transferred to the Roux water bath at 38 C., and further duplicate tests were made after 10, 15, and 25 minutes, with

TABLE 76  
ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. PROTECTIVE ACTION OF GUINEA-PIG SERUM AGAINST PEPTONE. THE DOSE REPRESENTS 3 C.C. OF SERUM AND 0.5 C.C. OF 20% PEPTONE

Exper. and Ratio	Guinea-Pig		Mixture			Result
	No.	Weight	Incubation at 0 C. (min.)	Incubation at 38 C. (min.)	Intra- venous Injection	
A 1:30	1	200	16	—	3.5	Very severe shock
	2	197	18	—	"	Slight
	3	201	20	—	"	Very slight
	4	195	23	—	"	"
B 1:30	5	200	—	10	3.5	Very slight
	5a	200	—	"	"	Severe
	6	200	—	20	"	Very slight
	6a	200	—	"	"	"
	7	210	—	30	"	"
	7a	208	—	"	"	"
	8	190	—	50	"	"
	8a	195	—	"	"	"

For control tests as to the action of corresponding amount of peptone, see Tables 71 and 72.

the expectation of finding an increased toxicity due to anaphylatoxin-production. The first test (No. 3) gave the desired result, but the companion test (No. 3a) and the subsequent trials were not as favorable. The apparent flare-up in toxicity might be ascribed as due to a very susceptible guinea-pig (No. 3); it is conceivable, however, that the anaphylatoxin-production was started, then checked and destroyed by the excess of the serum, and more especially by its alkaline content. Whether this be true or not, the striking fact remains that of 10 tests only 1 acute and 1 subacute death were obtained, whereas of the 4 control tests (Nos. 6 to 7a), which received the same amount of peptone diluted with salt solution, two acutely fatal shocks resulted.

It would appear from the series of tests just given that the toxic action of peptone is counteracted by normal guinea-pig serum. This conclusion is fully corroborated by the two series of tests given in Table 76 which were made with another pooled serum (50 c.c.) used about 3 hours after bleeding. The same peptone solution was used as that of the preceding series of tests (Table 75).

In Exper. A, which was of a preliminary character, the mixture consisting of 2 c.c. of the 20% peptone and 12 c.c. of the pooled serum was placed in cracked ice and tests (Nos. 1 to 4) were made at intervals as indicated; these tests clearly show that the toxicity of the peptone, in the dose employed, namely, 0.5 c.c. 20% solution, was decreased by admixture with serum at 0 C. This dose of peptone is the equivalent of 1 c.c. of 10% solution, which, as shown in Tables 71 and 72, is fatal in about one-half of the tests. This result was confirmed by the series of tests in Exper. B, where a mixture of 5.5 c.c. of the 20% peptone and 33 c.c. of the same pooled serum was placed at once at 38 C., the tests in this case being made in pairs. The entire series of tests (Nos. 1 to 8a), it may be added, were made within 70 minutes. It will be noted that in Exper. B there is practically no evidence of toxicity, though the dose of peptone present in the serum mixture, were it given by itself, would have produced fatal results in one-half the tests. The injection time in all of these tests, it may be added, was but 5 to 10 seconds.

These experiments clearly show that the peptone toxicity is suppressed, regardless of the question as to whether such toxicity is due directly to the peptone, or to anaphylatoxin induced by its presence. As mentioned, it is possible that this antagonistic action of the serum is, in part at least, due to the alkali content of the serum. Given a pool of serum with a relatively high alkali content, actual or potential, it is to be expected that it would be less easily toxified by peptone; or, in other words, it would show a greater antagonistic action than one having a relatively lower alkali content. Or, stated in another way, a serum having a low primary toxicity may be expected to show a greater neutralizing power concerning peptone than one possessing a high primary toxicity.

The neutralizing action of serum, as shown toward peptone, may furnish a basis for the rather empirical practice of injecting serum, or of transfusing either normal blood or that of alkalinized persons.

*Coagulation of Blood in Guinea-Pigs Injected with Peptone.*—The

fact that various substances when injected intravenously into dogs render the blood noncoagulable is well established. The first observation of this kind was made by Albertoni,<sup>17</sup> who found that the injection of hydrochloric acid and pepsin, or of pancreatin, produced this effect. Schmidt-Mülheim followed with the discovery of the action of peptone, and this was promptly confirmed by Albertoni and by Fano. A little later, Salvioli<sup>18</sup> observed a similar effect in dogs when injected with diastatic enzymes of plant or animal origin, including whole saliva; he noted, however, that twice the dose given dogs had no effect in the treatment of rabbits or guinea-pigs, either upon the coagulability or upon blood pressure. Persano,<sup>11</sup> as mentioned heretofore, found that the injection of a peptone caused rapid death in guinea-pigs and that such injection had no anticoagulant effect; in fasting guinea-pigs, he found that a nonfatal dose gave a retard of 3 to 5 minutes, provided the blood was drawn from 2 to 5 minutes after the injection. Neither Hirschfelder, Biedl and Kraus, Ritz, or Kumagai made any special observations as to the state of the blood other than that, after death, the blood in the heart was fluid.

As mentioned heretofore, the heart of guinea-pigs which die from peptone injection is free from clot and the blood is fluid when the examination is made a few minutes after death. While no special effort was made to determine how long this fluidity would persist, the records show a score of observations in which the blood within the heart was perfectly fluid for 6 to 19 minutes after death. How much longer the heart would have remained free from clot cannot be stated. The fact remains that a peptone shock leaves the blood in the heart in a less coagulable state.

In view of the fact that the dog's blood after peptone injection remains perfectly fluid in the test tube, it seemed as if under proper conditions a similar result should be obtainable with the guinea-pig. This was all the more to be expected since retarded coagulation, and even complete incoagulability had been obtained with the blood of guinea-pigs injected with anaphylatoxin (Part V), and the latter condition had also been successfully procured in specific anaphylactic shock (Part IX). Considerable effort was therefore made to secure like results with guinea-pigs after peptone injections, but the outcome, tho fair, was by no means entirely satisfactory, since delayed coagulation but not strictly incoagulable blood was obtained. Perhaps this is

<sup>17</sup> *Centralbl. f. d. med. Wissensch.*, 1878, 16, p. 641; 1879, 17, p. 596; 1880, 18, p. 577.

<sup>18</sup> *Ibid.*, 1885, 23, p. 913.

to be expected in the light of results to be presented which indicate that, concerning the guinea-pig and rabbit, the peptone is a relatively weak, disturbing agent.

In order to test the coagulability of the blood after peptone injection, the heart was exposed either before, or just at death, or at a definite time after death, and the blood was quickly drawn up into a plain heart pipet (Fig. 1), the tip of which was then sealed with wax. Obviously, the glass rod, ordinarily present in the pipet, was omitted. Observations were made every minute and the time of beginning and that of completed coagulation were noted. The tables indicate the injection time as well as the time which elapsed from the end of the injection to the end of the bleeding operation. The actual time required for drawing the blood varied usually from 5 to 10 seconds, but when drawn after death, on account of the fall in blood pressure, this time was somewhat increased. The amount of blood thus drawn varied from 2 to 5 c.c. The index of death was the last nasal twitch.

The alteration in coagulability of guinea-pig blood is subject to individual peculiarities of the species, and is not entirely conditioned by the amount of the peptone, or by the speed of injection, or by the time of withdrawal. This change in the blood, like that of pressure and respiration, etc., is influenced by factors inherent or developed within a given individual. It is well known that dogs show similar variations, and this will also be found to be the case with rabbits. It may be added that Camus<sup>19</sup> found that injections of milk into dogs likewise gave extreme variations as to coagulability. As a rule, dogs respond readily to peptone injection with an incoagulable blood, whereas guinea-pigs, it will be seen, offer great resistance to this change.

A series of tests, made under varying conditions, are presented in Table 77. In Tests 2 to 5, inclusive, in which, according to plan, the heart blood was drawn exactly 3 minutes after the injection of the peptone, there were 2 marked delays in coagulation (Nos. 2 and 4), while 2 coagulated in about normal time. The blood of No. 2 remained perfectly fluid in the test tube for 15 minutes after withdrawal, but when reexamined 10 minutes later it had coagulated. The heart of this animal examined 16 minutes after the injection was found to be free from clot.

The first 12 tests given in Table 77 were made on one day. The blood was drawn at a definite time, as just stated, or at once after

<sup>19</sup> *Compt. rend. Soc. de biol.*, 1900, 52, p. 787.

death, or at a fixed time thereafter, or before death as soon as the eye reflex had been lost. With the two exceptions noted, little effect was seen in regard to coagulation. The dose of peptone, which was the same in all twelve, represented a usually fatal amount. It was possible that a greater disturbance could be set up with a larger dose, and accordingly, on the following day, Tests 13 to 19, were made. In these the blood was drawn at or before death; of the 7 tests, 3 showed some delay, especially No. 15. It is possible that a better result would have been obtained with these increased doses had the blood been drawn 1 or 2 minutes after death. As the results stand, a moderate increase in the amount of peptone had no marked effect.

More striking changes, however, were obtained when a 30% solution of peptone was injected in doses corresponding to 3, 6, and 9 gm. per kilo (Nos. 20, 21, 22). These relatively large doses of peptone produced the most marked effect on the coagulation. Thus, in No. 22, the blood showed no sign of clotting until 22 minutes; at 35 minutes the clot was soft and floating and this incomplete clot persisted for 24 hours. In Test 21 the clot showed exactly the same behavior. Test 20, it will be seen, likewise showed a marked retardation, complete coagulation not taking place until 40 minutes.

It is evident from the results given in Tables 77 and 78 that, in so far as coagulation is concerned, the guinea-pig offers an extreme resistance to the action of peptone; this behavior is in striking contrast to that observed in dogs, where according to Thompson,<sup>20</sup> even as small an amount as 0.02 gm. per kilo may cause incoagulability. In guinea-pig 22, 450 times this amount of peptone produced but an incomplete coagulation. It is quite possible that if a larger number of tests, similar to Nos. 21 and 22 had been made, some would have shown complete incoagulability. Be that as it may, the fact remains that while peptone produces in guinea-pigs an acute fatal shock more readily than in dogs, the reverse is true in regard to the blood behavior. In the guinea-pig, peptone does not readily induce sufficient disturbance to give rise to much poison, or to a marked change in the blood. As pointed out in connection with the question of lethal dose, peptone is at best only one-thirtieth as toxic as an agar suspension.

It may be of interest to note that if a second portion of blood is drawn from the heart, it appears to coagulate more rapidly than the first. Indeed such blood may clot within 1 minute after removal. If,

<sup>20</sup> Jour. Physiol., 1896, 20, p. 455.

however, the time which has elapsed from the end of the injection to this second withdrawal is added to this coagulation time it will be found to correspond to that of the first portion. Thus, in Test 15, in which the coagulation time of the first portion was 16 minutes, a second portion drawn 16 minutes after the injection clotted in 2 minutes (+++). Again, in Test 17, in which coagulation occurred in 9 minutes, a second portion drawn 9 minutes after the injection clotted

TABLE 77

EFFECT OF PEPTONE ON COAGULATION OF BLOOD IN GUINEA-PIGS. Nos. 20 to 22 RECEIVED A 30% SOLUTION; ALL OTHERS A 10% SOLUTION OF WITTE'S PEPTONE

Exper.	Guinea-Pig		Intra-venous Injection (c.c.)	Injection Time (sec.)	Interval from End of Injection to End of Bleeding	Time of Death	Clot Begins (+) (min.)*	Clot Complete (+++)(min.)
	No.	Weight						
A	1	192	1.5	4	2'33"	3'33"	(3)	4
	2	207	"	5	3'		(15)	25
	3	199	"	"	3' 5"		2	3
	4	204	"	"	3'		12	14
	5	211	"	"	3'	4'30"	2	3
	6	205	"	"	4'		(1)	2
	7	190	"	3	4' 7"	6'	2	3
	8	185	"	5	4' 5"	5'15"	(2)	3
	9	215	"	4	4'11"	5'	(8)	4
	10	209	"	5	5'	3'45"	(2)	3
	11	210	"	3	5'32"	3'40"	2	3
	12	197	"	5	3'20"	3'15"	3	6
B	13	195	2	5	2'40"	4'	(3)	4
	14	202	"	"	2'35"	2' 5"	5	6
	15	212	"	"	2'10"	3'15"	14	16
	16	195	3	10	1'50"	2'40"	(1)	2
	17	201	"	"	2'55"	3'20"	6	9+++
	18	193	"	5	2'50"	2'35"	(1)	2
	19	202	"	3	3' 5"	3'	(2)	3
C	20	197	2	8	3'37"	2'10"	7	40
	21	197	4	10	3'28"	2' 3"	18	40+++
	22	195	6	"	4'	1'30"	22	35+++

\* The figure in parenthesis indicates that no coagulation had taken place at that time. The last nasal twitch, indicating death, was observed at times after the blood was drawn. In Nos. 21 and 22 the coagulation remained incomplete for 24 hours.

in 1 minute (+++). It is evident, therefore, that while the blood which remains in the heart is perfectly fluid, it is nevertheless undergoing a change parallel to that in the first tube, and that withdrawal of such blood, with the added disturbance due to glass contact, suddenly forces the reaction to completion.

In the tests given in Table 77, the peptone solution was injected in the original concentration. In view of the fact that an injection of

diluted agar (Table 61) gave an incoagulable blood, it seemed that possibly similar results could be obtained, if the action of the peptone was assisted, as it were, by the addition of distilled water. With this object in view, a series of tests were made in which the peptone was diluted with 4 to 9 parts of salt solution or with distilled water. The results are given in Table 78.

TABLE 78  
EFFECT OF DILUTED PEPTONE (10%) ON COAGULATION OF BLOOD IN GUINEA-PIGS. DILUTIONS  
MADE WITH DISTILLED WATER, EXCEPT IN NOS. 1 TO 3, IN WHICH  
SALT SOLUTION WAS USED

Exper.	Guinea-Pig		Peptone	Injection Time (sec.)	Interval from End of Injection to End of Bleeding	Time of Death*	Clot Begins (+) (min.)	Clot Complete (++++) (min.)
	No.	Weight	Intravenous Injection (c.c.)					
A	1	183	15 (1.5 + 13.5)	80	4'40"	3'	6	7
	2	175	15 ( " + " )	70	8'45"	6'50"	12	14
	3	175	15 ( " + " )	63	16'30"	14'35"	(3)	4
B	4	185	10 (1 + 9)	60	3'25"	1'50"	7	8
	5	180	10 ( " + " )	55	4'10"	2'30"	(4)	5
	6	203	10 ( " + " )	60	4'	2'40"	7	10
	7	210	10 ( " + " )	"	4'55"	3'20"	(2)	3
	8	200	10 ( " + " )	"	15'40"	12'15"	(1)	2
	9	199	10 ( " + " )	55	13'15"	11'10"	3	4
C	10	205	10 (1 + 9)	55	2'45"		(2)	3
	11	187	10 (1.5 + 8.5)	"	2'15"		(1)	2
	12	204	10 ( " + " )	"	3'30"		(15)	20+
	13	204	10 ( " + " )	"	4' 2"		3	4
	14	204	10 ( " + " )	60	3' 2"		5	6
	15	205	10 ( " + " )	90	13'50"	13'	(15)	28+ + +
D	16	213	10 (2 + 8)	55	2'50"	2'40"	2	3
	17	200	10 ( " + " )	60	3'30"	2' 5"	3	5+ + +
	18	217	10 ( " + " )	55	3'55"	2'35"	2	3
	19	210	15 (2 + 13)	65	3'45"	2'55"	(2)	3

\* The time of death is the interval from end of injection to the last nasal twitch. In No. 15, the blood remained incompletely coagulated and soft for 45 minutes, after which it was not examined. In No. 12, the blood had a slight clot (+) and remained so for 2 hours.

An examination of this table will show that while the majority of tests show no marked retardation, a few gave evidence of delayed coagulation. One can safely designate as a delay any test in which the first sign of coagulation does not appear until 5 minutes or later. On this basis 7 of the 19 tests showed delay. The most marked reactions were noted in Tests 2, 12, and 15. In No. 12, there was no evidence of coagulation for 15 minutes; reexamined at 20 minutes it appeared

to have a slight deposit of fibrin on the glass, but there was no appreciable increase for 2 hours (+). This was the nearest approach to an incoagulable blood that was obtained. The heart of No. 12, when opened 19 minutes after the injection, showed no sign of clot. In No. 15, in which death was subacute, the blood was also perfectly fluid up to 15 minutes; reexamined at 28 minutes it had a soft floating clot (+++), which remained thus during the next 20 minutes, after which no further observations were made.

Incidentally, it may be mentioned that while 1 c.c. of the 10% peptone is fatal in about half of the tests (Tables 71 and 72), when this amount of peptone is diluted with 10 parts of distilled water, it appears to be more fatal (Nos. 4 to 9). It has already been pointed out that an injection of peptone which would confer immunity or tolerance to subsequent reinjection of 1.5 c.c. of the solution, does not protect, as a rule, against 1 c.c. which has been diluted with 9 parts of water. Exceptions, however, are to be found, and a previous dose of diluted peptone may protect against a second dose of 1.5 c.c. likewise diluted.

Another attempt at producing a more marked noncoagulation consisted in giving a preliminary injection of N/5 HCl followed at once by 4 c.c. of a 20% peptone. With 0.65 c.c. of the acid, coagulation occurred in 4 minutes; with 1.4 c.c. of the acid, the first sign (+) was obtained at 8 minutes and the coagulation was complete at 10 minutes. With 2 c.c. of acid, the blood coagulated in 5 minutes. It may be added that with an injection time of 1 minute, even 7 c.c. of the acid could be given without any effect; 10 c.c. caused death in about 12 minutes; and 15 c.c. resulted in death in 65 seconds, with dyspnea and convulsions. Blood drawn from the heart, in this case, 3 minutes after death remained entirely free from clot for 33 minutes, after which no further examination was made.

A few attempts were made to favor the action of the peptone by adding 0.1 c.c. normal sodium carbonate to 0.5 or 1 c.c. of the 10% peptone. In these tests, the blood coagulated in from 3 to 5 minutes. A larger dose of alkali may have given a better result, but it is to be remembered that an excess of alkali may counteract the toxic effect of peptone (Table 74).

## INJECTION OF PEPTONE INTO WHITE RATS

Apparently, the rat has not been used hitherto in work with peptone. The white mouse has been tested by Ritz and Sachs,<sup>21</sup> who found that peptone was toxic for this species while anaphylatoxin produced no severe effects and never death; since mice may die of specific anaphylactic shock they concluded that evidently more poison was produced in-vivo than was injected with the anaphylatoxic serum.

It was particularly desirable to ascertain the effects of injections of Witte's peptone into white rats, in view of the results which had been obtained with agar (Table 64). It was somewhat surprising to find that such injections produced severe intoxication and even death, more readily than was the case with agar, but a consideration of the dosage employed soon showed that peptone, in reality, was the more feeble of the two. The symptoms and findings were those of anaphylactic poisoning and, as will be shown, they are due not to peptone directly, but to changes induced within the rat.

For the tests given in Table 79, a 10% solution of the peptone in 0.85% salt solution was used. This was sterilized at 110 C. and then

TABLE 79  
INJECTION OF 10% PEPTONE SOLUTION INTO WHITE RATS

Exper.	Rat		Peptone Solution*		Result
	No.	Weight	c.c.	gm. per kilo	
A	1	120	2.0	1.6	At once on side; dyspnea
	2	105	"	1.9	Dyspnea; depressed
	3	145	2.5	1.7	Moderate dyspnea
	4	135	3.0	2.2	" " ; slight spasm
	5	110	"	2.7	4'30". Typical shock and autopsy
	6	150	5.0	2.3	5'. " " " "
B	7	148	3.0	2.0	At first nil, then severe shock
	8	200	4.0	2.0	2'31". Typical shock and autopsy
	9	145	3.7	2.5	28'. Typical shock

\* Intravenously injected.

centrifugated. The injections were made intravenously and as a rule into the femoral vein, which is incomparably better adapted for the purpose than that of the tail. The injection time was not noted in the early tests, but in those made later it ranged from 10 to 30 seconds. The two groups of tests were made on different days and with different peptone solutions.

<sup>21</sup> Centralbl. f. Bakteriöl., R, 1911, 50, Beiheft, p. 45.

The results obtained were very striking. Thus, while 1.5 c.c. of the peptone solution appeared to give little or no effect, a dose of 2 to 2.5 c.c. developed more or less dyspnea from which the rat soon recovered. A dose of 3 c.c. may be taken as the average lethal amount, since it proved fatal in 1 of 3 trials. Individual variation in the resistance of the rat occurs, the same as in other animals. In the fatal cases, after a slight delay, dyspnea rapidly developed together with severe spasms and convulsions; the animal was soon thrown and acute death resulted in 3 of the tests; in No. 9, the first shock caused a near-kill, but the respiration gradually returned; complete recovery, however, did not take place and a subacute death occurred.

The autopsy findings were perfectly typical of anaphylactic shock — maximal distention of lungs, heart beating, absence of clot, and blood perfectly fluid. The heart blood of No. 5, 5 minutes after death, was transferred to a small test tube and examined every minute; it began to clot 7 minutes later and was solid at 8 minutes, or 13 minutes after death. In the case of No. 8, the blood was drawn 10 minutes after death; 5 minutes later it was still fluid, but it had set (++++ ) at 6 minutes, or 16 minutes after death. In another test, in which the rat (155 gm.) received an injection of 3 c.c. and was used for a transfusion experiment, the blood drawn 10 minutes after death showed the first indication of clot, 11 minutes later, and gave a complete clot at 12 minutes, or 22 minutes after death (Table 92, No. 3). These results clearly show that considerable retardation in clotting time occurs as the result of peptone injection into rats. Considering the number of tests made, they appear to be better than those given by the guinea-pig (Table 77).

*The Lethal Dose.*—Knowing the relative resistance of the rat to anaphylatoxin and to agar, it was to be expected that peptone would be less poisonous than it was for the guinea-pig. It will be seen from Table 79 that the least lethal dose per kilo of rat is 2 gm.; an amount which is about 7 times larger than that of the guinea-pig (0.3 gm. per kilo) (Table 75). It is clear, therefore, that the rat shows a greater resistance than does the guinea-pig to peptone, agar, anaphylatoxin, and specific shock.

Of further interest is the fact that peptone is much less toxic than agar. Thus, on reference to Table 64 it will be seen that the least lethal dose of agar per kilo of rat was 0.027 gm., whereas that of peptone, as shown, was 2 gm., a ratio of 1:74. In other words, in regard

to the rat, agar when in the proper state of division may be 74 times more toxic than Witte's peptone. A similar comparison of the two substances in regard to the guinea-pig shows that agar is at least 30 times more toxic than peptone (0.009 gm. vs. 0.3 gm. per kilo).

#### INJECTION OF PEPTONE INTO RABBITS

The rabbit has served for a number of peptone studies, largely because of its peculiar behavior to this substance. Fano noted that the rabbit, unlike the dog, did not respond to peptone injections, either with a drop in blood pressure or by a change in the coagulability of the blood. The amount used by him, however, was but 0.3 gm. per kilo, which is altogether too small for the rabbits. Albertoni (1880), a year before, had found that peptone was inactive in the treatment of the rabbit and sheep. These and other observations, based on insufficient data, led to the general belief that the rabbit was wholly refractory, or naturally immune to peptone. Incidentally, it may be well to recall that the rabbit is also looked upon as being relatively immune to specific anaphylactic shock.

The rabbit, however, is not immune either to peptone or to the anaphylactic shock. Grosjean<sup>22</sup> was the first to show that rabbits did give some reaction with a propeptone (proteose) and a peptone which he prepared. He found that the injection of 0.3 gm. or less per kilo of his propeptone had no effect on clotting; the injection of 1.7 gm. per kilo likewise failed to show any effect on the coagulation, but in this experiment the blood was not drawn until 50 minutes after the injection. Had it been drawn shortly after injection, the result, without doubt, would have been different. This dose, however, caused a very rapid fall in blood pressure, which soon returned to the normal. The peptone in like dose gave a slight drop, followed by a rise in pressure which then gradually fell below normal. After the injection of this peptone, the blood drawn 80 minutes later coagulated normally, whereas, when drawn 15 minutes after the injection, it began to coagulate in 13 minutes, and the coagulation was complete in 45 and 60 minutes (2 tubes from 1 rabbit).

The work of Grosjean was extended a few years later by Gley,<sup>23</sup> who tested a propeptone prepared according to the method of the former. This product in dose of 1.5 gm. per kilo was always rapidly

<sup>22</sup> *Mémoires couronnés et autres mémoires, l'Acad. roy. de Belgique*, 1892, 46, Mémoir 2, pp. 24, 25, 28. *Arch. de Biol.*, 1892, 12, p. 381.

<sup>23</sup> *Compt. rend. de la Soc. de biol.*, 1896, 48, p. 658.

fatal, a result, it may be added, not obtained by Grosjean. With the blood of 6 of 7 animals, thus tested, Gley obtained an appreciable decrease in coagulability. In 1 instance, the coagulation was retarded for 20 minutes, in 1, for 30 minutes, in 3 others, for more than an hour, and in 1, the blood remained fluid for 2 hours and then formed a soft clot. The extreme activity of his product almost raises the question whether all the ammonium sulfate used in the process had been removed. The animals injected showed a rapid fall in blood pressure, violent tetanic convulsions, the respiration stopped at once as did also the heart.

It may be added that Gley was unable to obtain incoagulable blood with Witte's peptone, and in a later paper<sup>24</sup> he apparently contradicted himself by stating that propeptone (presumably meaning Witte's peptone) exerted no influence upon the coagulability of blood of the rabbit. On the other hand, Nolf<sup>25</sup> using Witte's peptone, was able to get a fall in blood pressure, but while 0.03 gm. per kilo was active in the dog, even 1 gm. per kilo was not always active in the rabbit. Similarly, the rabbit is resistant to injections of milk, snail's blood (Camus), strawberry extract (Gley), crawfish, eel serum, and a host of other extracts, all of which in the dog produce a typical drop in blood pressure and an incoagulable blood.

Pozerski<sup>26</sup> found that while the injection of Witte's peptone in treated rabbits had no influence upon the coagulation of the blood, it caused a disappearance of complement and hence of hemolysis in regard to sheep corpuscles. He, therefore, concluded that there was no relationship between the phenomena of incoagulability and loss of complement. This view, however, is not strictly justified inasmuch as change in coagulation time, loss of complement, and production of toxicity are to be considered as expressions of one and the same disturbance involving the highly labile constituents of the blood plasma. The rabbit is not strictly refractory to the anticoagulant action of peptone as he supposed it to be.

Brieger<sup>4</sup> found that rabbits of about 1 kilo weight when given subcutaneously from 0.5 to 1 gm. of peptotoxin, developed posterior paralysis, became somnolent, and died after some hours. Witte's peptone was tolerated in dose of 20 gm., while a peptone which he prepared by peptic digestion of fibrin was quickly fatal when given subcutaneously in dose of 2 gm.

<sup>24</sup> Cinquantenaire de la Soc. de biol., 1899, p. 707.

<sup>25</sup> Arch. internat. de physiol., 1906, 3, p. 218.

<sup>26</sup> Compt. rend. Soc. de biol., 1913, 74, p. 577.

Fatal results in rabbits following the intravenous injection of Witte's peptone are mentioned by Gley.<sup>23</sup> Hirschfelder noted collapse of the lungs in 2 rabbits killed with peptone, but failed to give any further data. Kumagai,<sup>14</sup> likewise obtained 2 fatal results following the injection of 3.08 and 4.2 gm. per kilo; death was almost immediate, with dyspnea and collapsed lungs. This appears to be the extent of available data regarding the action of peptone on rabbits. It was necessary for the purpose of this study to confirm and extend these observations. It will be shown that Witte's peptone does produce severe and even fatal effects in rabbits, among which drop in blood pressure and decreased coagulability are easily noted. In addition it will be shown that the rabbit may respond with a perfectly typical anaphylactic shock and findings, in which respect it falls in line with the rat and the guinea-pig.<sup>27</sup>

For the tests given in Table 80, the 10 and 20% solutions of Witte's peptone were prepared in the usual way by dissolving the peptone in salt solution in a water bath at 100 C., and then filtering through paper. In the case of the 30% solution, filtration was found to be very slow and was abandoned in favor of centrifugation at 8000 r.p.m. Even at this speed, the fluid will not be clarified if the temperature is that of the room, the viscosity of the liquid being sufficient to hold up some of the very fine material. This difficulty was overcome by centrifugating the hot solution. The peptone solution employed in all of the injections was perfectly clear. On account of the large bulk of solution to be injected, and especially because of the viscosity, it was found desirable to warm up the solution to 40 C. before use. Obviously, the best result can be expected if the peptone solution is in a condition to mix freely with the blood.

The ordinary syringe was used for the injection of all but Nos. 16 and 17. Since the largest syringe had a capacity of but 20 c.c., it was often necessary to make use of 2 or 3; after the first was discharged, the syringe was separated from the needle, which remained in the jugular, and the next one was attached and the contents injected. In this way, considerable speed was possible, especially if the needle was of fair caliber. In the last 2 tests this method was supplanted by a more convenient procedure in which a 50 c.c. cylinder was equipped with an outflow tube carrying the needle, and an inflow tube attached

<sup>27</sup> In a very recent publication (*Jour. Biol. Chem.*, 1917, 29, p. 129), Kuriyama states that in a few cases 0.5 to 1 gm. of Witte's peptone per kilo of body weight killed the rabbits immediately or in a short time. Prostration, weak heart action, and convulsions were noted.

to a compression bulb. The difference in the injection time of Nos. 16 and 17 was due to the use of different needles, that for the former being short and wide, while that for the latter was very long and fine.

TABLE 80  
INJECTION OF PEPTONE INTO RABBITS

Exper.	Rabbit		Peptone		Injection Time (sec.)	Result
	No.	Weight	Intra-venous Injection (c.c.)	gm. per kilo		
A 10% Peptone	1	1,400	14	1	25	Nil
	2	1,220	24.4	2	50	Practically nil
	3	1,235	37.1	3	70	Severe
	4	1,085	32.5	3	70	2/45". Typical shock and autopsy
	5	1,970	59	3	100	Nil
B 20% Peptone	6	1,300	19.5	3	50	Practically nil
	7	1,075	16.1	3	35	Severe
	8	1,525	30.5	4	60	Nil
	9	1,800	45	5	130	Slight
C 30% Peptone	10	1,360	22.6	5	20	7". Typical shock and autopsy
	11	1,050	17.4	"	12	7" 8". " " " "
	12	1,550	35.7	"	27	3". " " " "
D 30% Peptone	13	1,350	22.4	5	45	Slight
	14	1,060	17.6	"	15	"
	15	1,510	24.9	"	30	Moderate
	16	2,075	34.5	"	15	3". Atypical
	17	1,930	32	"	120	Very slight

No. 17 was reinjected 26 minutes later with a like dose and died in 3/20"; the toxicity of the blood is given in Table 95 (Nos. 7 to 9).

The rabbits used for these tests were all new animals. An inspection of the table will show at once that there is a great variation in the resistance of the rabbits to peptone. Of the 17 tests, but 5 proved fatal; and of these, 4 were perfectly typical anaphylactic shocks with characteristic autopsy findings, namely, maximal distention of lungs, heart beating strongly, low blood pressure, the heart free of clot and the blood of normal fluidity. The small intestine in addition to increased peristalsis, showed most intense transverse contractions which fairly wrinkled the entire gut. The lungs were very light pink in color and free from petechiae. The time of death was reckoned from the end of the injection.

The symptoms developed after a slight incubation period of  $\frac{3}{4}$  to 1 minute; the respiration became shallow, dyspnea set in, followed by

spasms and convulsions, the animal being thrown; in one instance (No. 11) there was a frothy discharge from the nose. The difficulty in respiration is usually shown by a wheezing or gurgling sound. After the acute effects pass off, the animal becomes more or less somnolent, a condition first observed in dogs by Fano. A very striking symptom, hitherto unobserved, was an exophthalmos which in the fatal cases came on rapidly and was most marked at death; if recovery occurs, this condition disappears in a few minutes. In No. 16, in which, as stated, the injection was made very rapidly, the exophthalmos was extreme; the pupil, at first wide, became constricted, and the iris appeared quite blanched.\*

*The Lethal Dose.*—The lowest amount of peptone which was fatal was 3 gm. per kilo (No. 4). It is rather striking to find that of 9 tests, in which 5 gm. per kilo were given, only 4 proved acutely fatal. This fact clearly shows that the rabbit, like the guinea-pig, is subject to individual variations. This is quite independent of the speed of injection, as will be seen on inspection of the injection times in Exper. D, Table 80, in which the same peptone solution was used and the tests made one after another. It is very likely that the slight effect produced in No. 17 was due to the long injection period. On the other hand, it is also probably true that very rapid injections may cause a speedy death with lungs in collapse, as was the case in No. 16. A moderate speed of about half a minute with thorough intermingling of the peptone and blood is perhaps the condition to be desired.

On comparison of Tables 79 and 80, it will be seen that the rabbit is more resistant to peptone than the rat. Thus, while the least fatal dose for the rat was found to be 2 gm. per kilo, that for the rabbit was 3 gm. per kilo. Compared with the guinea-pig, the least fatal dose for which is 0.3 gm., the rabbit has at least a ten-fold resistance. It is noteworthy that in regard to agar, peptone, anaphylatoxin, and specific anaphylaxis, the rabbit is considerably more resistant than the guinea-pig.

With reference to agar, it should be pointed out that apparently the rabbit is more susceptible than the rat. It is to be noted, however, that only 2 very young rabbits were tested with agar, and it is quite likely that a different result would have been obtained had animals of 1 to 1½ kilos been used, as in the case of the work with peptone.

\* It should be mentioned that Camus and Gley (Arch. internat. de pharmacodynamie, 1898, 5, p. 272) observed, at times, a very marked exophthalmos in rabbits injected with celandine serum (0.3 c.c.). The other toxic effects of this serum, which have been compared to those of peptone by Delezenne, are without doubt due to the production of anaphylatoxin.

The following tabulation of the least fatal dose in grams per kilo may be of interest:

	Agar	Peptone	Ratio
Guinea-pig .....	0.009	0.3	1:33
Rabbit .....	0.016	3.0	1:188
Rat .....	0.027	2.0	1:74

*Immunity.*—Like the dog, the rabbit, as well as the guinea-pig, shows an increased resistance to peptone after recovery from the effects of the first injection. If the second injection is given too soon after the first it is likely to cause death, just as in the case of guinea-pigs injected with 0.5 c.c. doses, at intervals of 15 minutes. By way of illustration, it may be stated that Rabbit 17 of Table 80 was reinjected 26 minutes later with the same dose, the injection time being 40 seconds. It died with typical shock in 3 minutes, 20 seconds, but on autopsy the lungs were in collapse, though the heart was beating and the blood was fluid. Transfusion of this blood showed that it was highly toxic (Table 95, B). Again, Rabbit 3 was reinjected 3 hours later, the dose being 4 gm. per kilo, and the injection time, 55 seconds; it was at once limp, showed exophthalmos, and died in 1 minute, 35 seconds after the end of the injection. The autopsy findings were the same as those of No. 17; the blood condition of both will be referred to later.

By contrast, Table 81 presents the results of multiple injections into 2 rabbits of the same series, the second injection being given on the following day. At 20 minutes and at 3½ hours after the third injection of Rabbit 1, blood was drawn from the heart and transferred to a small test tube; the first sign of coagulation was noted at 5 and 3 minutes, respectively, and both samples clotted firmly in 8 minutes. In brief, the blood coagulated in nearly normal time. It was probable that even the interval of 20 minutes was too long and hence after the fourth dosage blood was drawn within 3 minutes after the injection; this remained perfectly fluid for 30 minutes and when reexamined at 40 minutes it showed a slight clot (+) or floater which did not increase during the next 40 minutes. In other words, though the animal showed no symptoms following the fourth injection, its blood was practically incoagulable. The fifth injection was followed by dyspnea, spasms, convulsions, exophthalmos, and death; the autopsy at 2 minutes after death showed collapsed lungs, heart, beating and free of clot, and blood, fluid. The blood transferred to a tube gave no evidence of clotting for 10 minutes, but was solid at 13 minutes.

In Rabbit 6, there was practically no effect observed until after the fifth injection when a transitory exophthalmos and slight respiratory distress developed. After the sixth injection, the eye protrusion came on rapidly, but receded in about 12 minutes; the next injection was followed by spasms and a severe exophthalmos, which, however, again passed off in 10 minutes. This condition recurred after the eighth injection, while the ninth resulted in death. The usual dyspnea, spasms, violent convulsions, and eye protrusion were noted and at autopsy, made within 2 minutes after death, the lungs were collapsed, but the heart was vigorously beating and the blood was fluid. A portion transferred at once to a test tube remained perfectly limpid for 18 minutes; at 19 minutes, the first sign of clot appeared, but even in 30 minutes,

TABLE 81  
MULTIPLE INJECTIONS OF PEPTONE INTO RABBITS. IMMUNITY

Rabbit	Number of Injections	Interval	Injection Time (sec.)	gm. per kilo	Result
1 (Table 80)	1	—	25	1	Nil
	2	24 hrs.	20	3	"
	3	22'20"	10	3	"
	4	3 hrs. 50'	40	4	"
	5	22'15"	55	4	1'5". Typical shock
6 (Table 80)	1	—	50	3	Very slight
	2	21 hrs.	25	"	Nil
	3	20'	30	"	"
	4	4 hrs. 25'	35	4	"
	5	8'30"	50	"	Slight
	6	8'55"	55	"	"
	7	17'55"	40	4	Fair
	8	22'20"	40	"	"
	9	6'10"	50	5	2'. Typical shock

coagulation was not complete, the clot being soft and rolling. A second portion of blood, drawn 9 minutes after the first and while the heart still showed a slight beat, gave the first sign of clot in 11 minutes, and a soft sliding clot like that of the first tube, in 22 minutes. By adding to these the interval between the two portions, it will be seen that the two portions behave alike, as has been pointed out in connection with the coagulation of the blood of guinea-pigs.

*Coagulation of Blood in Rabbits Injected with Peptone.*—Under the preceding heading, several instances have been mentioned going to show that the coagulation changes in rabbit blood may be greatly retarded. It should be added that the blood of Rabbit 3, mentioned

heretofore, was perfectly fluid in the tube for 15 minutes and reexamined at 22 minutes, had a beginning small clot (+), which gradually increased till at 48 minutes it was complete except that it was not firm. Another portion of blood, drawn from the heart 10 minutes after the first lot, began to thicken after 6 minutes, but the process did not go beyond that of a soft clot after which resolution took place. Some of this blood was centrifugated and the clear plasma did not commence to clot for an hour; at the end of 2 hours the clotting was complete (+++++) but soft. This plasma, it may be added, was acutely fatal in dose of 1 c.c.

Rabbit 17 referred to under "Immunity" gave a blood, 4 minutes after death, which showed no sign of coagulation for 35 minutes; at 40 minutes, it developed a very slight clot (+) and this persisted for nearly 2 hours, when it formed a small mass (++) which remained in that condition for 24 hours.

Of especial interest, is the behavior of the blood in those tests in which death occurred after a single injection of peptone. Thus, in No. 4, Table 80, the blood drawn 3 minutes after death showed a small clot (+) at the end of a minute, but this did not increase during the next half hour; in fact it became less so that in an hour and a half it was scarcely to be seen. This resolution or contraction of a very partial clot was repeatedly encountered; it corresponds to the fibrinolysis of Dastre. Another portion of blood, drawn 10 minutes after death, behaved in exactly the same manner. The blood therefore could be said to be practically incoagulable.

In the case of Rabbit 10, Table 80, the blood drawn less than 3 minutes after death remained entirely free of clot for 1 hour; reexamined an hour later it was found to be fully clotted, and showed no retraction for 4 hours.

Rabbit 11 was bled from the heart at 2 minutes after death; the blood gave a small soft clot (+) at 9 minutes, and this did not increase, but after 20 minutes, it began to shrink and the blood appeared to be perfectly fluid and remained so.

In Test 12, the blood was drawn at once after death, and in 11 minutes showed a small clot (+) which gradually increased, so that at 50 minutes it was +++, a soft sliding mass. A second portion drawn 11 minutes after the first gave in 1 minute a small clot (+), corresponding in time to that of the first portion; this clot did not increase, but instead behaved like the others, showing in about 40 minutes a resolution or fibrinolysis.

In Rabbit 16, in which the blood was removed from the heart within 3 minutes after death, no clot was in evidence for 20 minutes; at 22 minutes a very slight clot appeared, which increased a trifle (++) and remained thus, a very soft mass, for more than 2 hours. This result may well be compared with that obtained in No. 13, in which the injection produced but a slight general effect; the heart was exposed and blood drawn 10 minutes after the injection, remained perfectly fluid for 12 minutes, but became solid in 14 minutes (see Table 95, A).

The results of blood examinations of 10 rabbits which received 1 or more injections of peptone have been herein presented. It was deemed important to show, notwithstanding conflicting statements to the contrary, that the rabbit responded to injections of Witte's peptone in essentially the same manner as the dog, the guinea-pig and the rat. There can be no question from the evidence submitted as to this point. The amount of peptone, it is true, must be considerable in order to bring about the disturbance in the rabbit, but when this is done, the result is a typical anaphylactic shock in which the cardinal symptoms, dyspnea, spasms, convulsions, drop in blood pressure, incoagulable blood, and lung distention are to be found.

#### THE IN-VITRO PRODUCTION OF ANAPHYLATOXIN BY PEPTONE

In order to establish the mode of action of peptone, it was not sufficient to show that when injected it gave rise to symptoms and changes similar to, if not identical with, those produced by anaphylatoxin. Such demonstration could be but the first link in the chain of evidence necessary, and it was imperative to show that peptone like agar could make anaphylatoxin by contact with normal serum, and as a further and final proof, it was essential to demonstrate that such a poison was formed in an animal when injected with peptone.

Previous work had shown that the best reagent for anaphylatoxin-production was rat serum, while that of the guinea-pig, usually employed hitherto, was much less satisfactory, and that of the rabbit still less. Hence, in the solution of the problem which presented itself, rat serum was made the first choice and the results met all expectations. A large number of experiments were likewise made with guinea-pig serum, but the outcome, for reasons which will be duly considered, was not as satisfactory. Inasmuch as the rabbit serum was the least promising, no tests, as yet, have been made with it.

An essential condition for this work is a large pool of fresh serum sufficient for a series of tests, and also for all needed controls. This is necessary because of the fact that the normal serum of a given species is a variable. A further condition which was learned by long experience is the need of safe-guarding against the individual variability of the test animal. This can be done to some extent by injecting the animals in pairs, but even then it is possible that chance has brought together either two susceptible or two resistant animals. It is only from a large series of tests, controlled in every possible way, that trustworthy conclusions can be drawn.

*Rat Serum.*—Early in the study on anaphylatoxin, some attempts were made to toxify rat serum with peptone so that 1 c.c. of the serum would be fatal. In view of the fact that an unheated unfiltered solution of peptone was more toxic than one that was perfectly clear (Table 70), the first trials were made with such a suspension, and the results are given in Table 82.

The peptone solution was made by adding 10 gm. of Witte's peptone to 100 c.c. of salt solution; the mixture was then heated, with stirring at 60 C. The cloudy, unfiltered solution was used direct. For Exper. A, 3 c.c. of the peptone were added to 6 c.c. of fresh rat serum, and this mixture was tested at once, after incubation at 37 C., as shown in the Table. Expers. B and C were carried out in like manner, the mixtures being 1 + 4 and 1 + 8, respectively. The injection dose in all cases represented 1 c.c. of serum. An inspection of Table 82 will show at a glance that apparently the peptone does render the serum poisonous. It may, however, be objected to Exper. A that the amount of peptone injected (0.5 c.c.) was too near the lethal dose when given by itself, and that, therefore, the results might be due to the direct action of the peptone. This objection, however, does not hold for Expers. B and C. The serum by itself, in the dose employed, is harmless.

The effects observed after the injection of the incubated mixtures of serum and peptone were the same as with sera, which had been treated with trypanosomes or with agar; they were those of anaphylatoxin, that is, dyspnea, spasms, and convulsions. When death was not acute there was a drop in temperature which in No. 9, in 2½ hours, reached 27 C.; at autopsy the usual picture was obtained, namely, maximal lung distention, heart beating and free of clot. The rat serum, toxified with peptone, frequently produces a marked rigidity of the muscles of the abdomen and extremities not seen when the agar anaphylatoxin is used.

TABLE 82  
ACTION OF CLOUDY UNHEATED PEPTONE SOLUTION (10%) ON NORMAL RAT SERUM

Exper.	Ratio	Guinea-Pig		Mixture		Result
		No.	Weight	Incuba- tion at 37 C. (min.)	Intra- venous Injection (c.c.)	
A	1:20	1	220	—	1.5	Practically nil
		2	200	7½	"	Severe
		3	195	15	"	2/40". Typical shock
		4	205	30	"	2/55". " "
		5	195	60	"	3/20". " "
		6	190	120	"	6'. " "
B	1:40	7	180	—	1.25	Practically nil
		8	220	7½	"	Severe
		9	185	15	"	6½ hr.
		10	180	30	"	Slight
		11	215	60	"	Practically nil
		12	225	120	"	" "
C	1:80	13	202	7½	1.12	Nil
		14	180	15	"	Very slight
		15	208	30	"	3/45". Typical shock
		16	230	120	"	Nil

Different pools of serum were used for these experiments.

It should be pointed out that the mixtures when tested at once, as soon as made, gave little or no effect, but that the toxicity developed on incubation. In view of the small amount of the serum used as a test dose, it will be seen that even a short incubation of a few minutes gives rise to some poison. Thus, when retested in 7½ minutes, severe shocks were obtained in Experiments A and B, and, in the light of subsequent work, it is likely that had duplicate tests been made, possibly even a fatal result might have developed. The incubation for 15 minutes, in Experiment A did bring about the production of more than an average lethal dose of poison since the 4 consecutive tests made after this time caused typical acute shock and death. On the other hand, in Experiment B, in which the mixture contained but half the peptone of the former, the poison-production was much less since no acute death resulted. The guinea-pig injected with the material after incubation for 15 minutes had an intense, quiet shock; the temperature dropped in 2½ hours to 27 C., and death occurred in 6½ hours. Further, it will be seen that in Experiment C, in which the amount of peptone was still further reduced a typical acute shock and death resulted after incubation for 30 minutes. This outcome, on account of the small amounts, cannot possibly be ascribed either to an initial toxicity of the rat serum,

or to that of the peptone, or to the sum of these two; it leads to the conclusion that the poisonous effect is the result of an interaction of the serum with the peptone, anaphylatoxin being formed.

TABLE 83  
ACTION OF CLEAR HEATED PEPTONE SOLUTION (10%) ON NORMAL RAT SERUM

Exper.	Ratio	Guinea-Pig		Mixture		Result
		No.	Weight	Incuba- tion at 37 C. (min.)	Intra- venous Injection (c.c.)	
A	1:20	1	225	7½	1.5	Practically nil
		2	205	15	"	Moderate
		3	198	30	"	4'. Intense quiet shock
		4	200	60	"	Moderate
B	1:20	5	190	7½	1.5	Practically nil
		6	205	15	"	Moderate
		7	185	30	"	4'. Intense quiet shock
		8	213	60	"	Slight

The two experiments in Table 83 were made on the same day as Exper. A of the preceding set and were in the nature of controls. The same peptone solution was used, but it was first heated to 110 C. for 15 minutes; this gave a clear liquid with a small flaky deposit, which was shaken up and the resulting mixture used in the first experiment (A). The balance was centrifugated at 8000 r.p.m., and the perfectly clear solution was employed in the second experiment (B). It will be seen that the two series are practically alike. The clear peptone solution can toxify the rat serum, but the toxicity of the mixture does not approach that of the mixture having the peptone suspension. The cloudy suspension is more active in vivo and also in vitro. Heating, with the resultant dispersion and complete solution of the peptone, clearly reduces its activity. Agar, it is to be noted, is never in perfect solution, since it can be thrown by centrifugation.

The fact that the clear solution of peptone is less active than the cloudy unfiltered suspension corresponds to the observation of Nathan<sup>28</sup> that inulin toxifies guinea-pig serum when in a suspended state, but does not do so when warmed to 68 C. for 2 minutes. Evidently, the extreme dispersion of inulin, like that of peptone, is unfavorable to the reaction, conceivably, because the charge or energy of the aggregate is more intense, applied locally, than that of the fully dispersed phase.

<sup>28</sup> Ztschr. f. Immunitätsf., 1914, 23, p. 209.

Be that as it may, the fact is that the clear peptone solution (Expers. A and B, Table 83) in the same concentration as that of the cloudy one (Exper. A, Table 82) produces a toxic condition in the serum, less rapidly and to a less degree. The effect after incubation for 15 minutes was but moderate, and, seemingly, incubation for 30 minutes was necessary to produce a fatal dose of poison. Designating as the average, or least fatal dose, that amount of the poison which may kill one-half or less of the test animals, it will be readily understood that the cloudy suspension may produce several multiples of such dose since 4 consecutive tests were fatal, whereas the clear solution can give rise to only a single average lethal dose.

After an interval of 10 months, these tests were repeated in an extended form; the peptone solution was prepared in the same way as for Exper. B of Table 83, except that it was made up with distilled water instead of salt solution. A mixture of 10 c.c. of this peptone and 20 c.c. of rat serum was incubated, and at intervals of 15 minutes, tests were made, the dose being 1.5 c.c. Uniformly mild results were obtained in 9 consecutive tests, made in 2 hours. As the only explanation of this failure seemed to be in the use of distilled water which may not have dissolved as much of the peptone as a salt solution, the experiment was repeated on the following day with a peptone solution prepared in the usual way with salt. The results of this experiment are given in Table 84.

It will be seen from this table that 3 of 12 tests gave acutely fatal shocks. More important was the variability in the results which could not be accounted for by an assumed change in the poison, but rather by a variation in the resistance of the test animals. It indicated that the amount of poison produced was small, a single average lethal dose and not a multiple thereof.

While the foregoing experiments rendered it highly probable that peptone acted on the serum and gave rise to anaphylatoxin, yet the fact that the amount of peptone per dose of serum, as used in most of the tests, was relatively large (0.5 c.c.) made it imperative to meet the objection that the effects observed were due to this amount. Decreasing the quantity of peptone as in Table 82 only partially met the objection. It was desirable to obtain a surely fatal dose, one which would kill all or nearly all of the test animals, in which the amount of peptone would be considered negligible. If a minimal amount of peptone did not produce a surely fatal dose of poison in 1 c.c. of serum, it possibly

would do so were the amount of serum doubled or trebled. Ordinarily, normal rat serum can be injected in dose of about 5 to 6 c.c. without any serious effect, but, on the other hand, it is possible to prepare such serum so that in dose of 3 to 4 c.c. it will be acutely fatal (p. 533). Consequently, to be perfectly safe, it was decided not to increase the amount of serum per dose beyond 2 c.c.

TABLE 84  
ACTION OF CLEAR HEATED PEPTONE SOLUTION (10%) ON NORMAL RAT SERUM. VARIATION  
IN RESISTANCE OF GUINEA-PIGS

Ratio	Guinea-Pig		Mixture		Result
	No.	Weight	Incuba- tion at 37 C. (hrs.)	Intra- venous Injection (c.c.)	
1:20	1	195	$\frac{1}{4}$	1.5	Slight
"	2	183	$\frac{1}{2}$	"	3'15"
"	3	192	$\frac{3}{4}$	"	Moderate
"	4	186	1	"	5'20"
"	5	209	$1\frac{1}{4}$	"	Practically nil
"	6	196	$1\frac{1}{2}$	"	Good shock
"	7	207	$1\frac{3}{4}$	"	Practically nil
"	8	189	2	"	"
"	9	181	$2\frac{1}{4}$	"	Fair shock
"	10	177	$2\frac{1}{2}$	"	Very slight
"	11	208	$2\frac{3}{4}$	"	Slight
"	12	176	3	"	3'45"

The experiments which were made under these conditions did not at once yield the desired result and some idea of the effort expended in this final series of tests may be gathered from the fact that about 125 guinea-pigs were used. Eventually, the chief difficulty was found to lie in the serum. In the production of anaphylatoxin out of rat serum by means of agar, it mattered little whether the serum was used perfectly fresh or after being iced for 24 hours; in the peptone work, however, it did make a great deal of difference in results, according as the serum was fresh or had been iced for from 6 to 9 or 12 hours. Seemingly, the iced serum tends to form a stable condition which the powerful action of agar can disturb, but which the weaker peptone is unable to do. A similar condition is seen when normal rat serum, which has been heated at 50 C. for 15 minutes, is subjected to the action of agar, or of distilled water (Part IX).

By way of illustrating the statement just made, the following results of a series of experiments made with one and the same pool of serum are presented. The blood from 20 rats bled in the morning and early afternoon, each being separately defibrinated, was pooled and centrifuged.

gated at 8000 r.p.m. and the 76 c.c. of serum thus obtained were pooled in a small beaker and placed in cracked ice. The experiments were performed in the evening, the first being made 6½ hours after the last bleeding. For this first experiment, a mixture of 2 c.c. of 10% peptone and 16 c.c. of the serum was made and iced for 10 minutes, after which it was placed at 40 C. The test dose of the mixture was 2.25 c.c., and the injection time 4 to 7 seconds. The tests were made in pairs, one just before, and the other after incubation for 5, 15, and 30 minutes, making 4 pairs or 8 tests. After incubation for 5 minutes, one test gave a severe shock while its companion was less affected; at 15 minutes, both tests resulted in acute death; at 30 minutes, one test was fatal while the other showed but slight effects.

The results of this first experiment were very good, since the serum had become toxified within 15 minutes. By way of control to show that the observed toxicity was not due to the serum, a like number of guinea-pigs (8) were injected at once with the same pooled serum, each receiving 2 c.c.; the effects were slight or nil, as was expected. Accordingly, as a check, the first experiment was repeated exactly, with the same serum, peptone, and time conditions. Of the 4 pairs of tests or 8 guinea-pigs, only 2 gave severe shocks and there were no deaths. This second experiment, which proved a complete failure, was begun 1½ hours after the first, and as the result was so unexpected, it was repeated a third time under identical conditions, the only variation being in the age of the iced serum. The test was begun 2½ hours after the first. Of 8 guinea-pigs, tested as 4 pairs, 5 had but slight effects, 2 received a severe shock, and 1 had an atypical shock and died in 5 minutes, 30 seconds.

As a further control test, 3¼ hours after the first experiment, a mixture of 1 c.c. peptone and 12 c.c. of the same pooled serum was made; 2 tests were made at once, and 2 after incubation for 15 minutes. The dose injected was 3.25 c.c., representing 3 c.c. of serum. The effect in the first pair was practically nil, while that of the second was moderate. This control and the 2 check experiments clearly showed that the serum was not as reactive as it was at first. Apparently, prolonged exposure to air and to 0 C. was responsible for the change.

In order to test this conclusion, on the following day, a pooled rat serum was prepared, and after being kept in cracked ice for 5 hours, a portion of it was treated with peptone and incubated under the same

conditions as prevailed in the experiments of the day before. After incubation for 5 minutes, the paired test gave 2 severe shocks; at 15 minutes there was 1 acute death and 1 near-kill, and at 30 minutes both guinea-pigs died of acute shock. This experiment was very satisfactory as a demonstration of poison-production, since the mixture tested on a pair of guinea-pigs before it was incubated had very little effect. As a check, the experiment was repeated under the same conditions as before, except that the serum had been iced now for 6 hours. The peptone-serum mixture after being iced for 10 minutes was unexpectedly active, since the usual dose of 2.25 c.c. caused 1 acute death and 1 very severe shock. On incubation for 5 minutes, the mixture gave 2 severe shocks; at the 15 minute test, there was 1 death and 1 moderate shock; at the 30 minute test, there was again 1 death and 1 fair shock. Evidently, in this second test, the full amount of poison was produced very early, since incubation showed no increase. Seemingly, the serum which had been iced for from 5 to 6 hours was fairly reactive to peptone, the result being very much like that of the first experiment of the preceding day.

For the next experiment, it was decided to use as fresh a serum as possible. For this purpose 17 rats were bled in half an hour; each blood was separately defibrinated with a rod, and the pooled mass was then centrifugated at 8000 r.p.m. The pooled serum was at once iced and was used for the first experiment within  $2\frac{3}{4}$  hours after the start of bleeding. The results of this experiment are noteworthy, especially since the rats were controlled in every possible way (Table 85).

Whatever the cause, the serum in this experiment proved to be very reactive, since the mixture of peptone and serum became toxified during the icing period of 10 minutes. On subsequent incubation, apparently the full amount of poison was soon produced, since all the test animals died of typical shock. This result was so striking that it was at once controlled with 4 series of checks, in which the same pooled serum or peptone were used. Control 1 was started within 45 minutes after the injection of No. 1; Control 2 within 1 hour 45 minutes; Control 3 within 1 hour; and Control 4 within 1 hour 25 minutes, the entire experiment being put through inside of 2 hours from the first injection.

Control 1 is noteworthy, since it shows that in Nos. 7 and 8 a lethal dose of anaphylatoxin was produced within a very short time, 45 seconds. This speed of production was such that it raised the question as

to whether the poison was made in the mixture before injection, or was developed in vivo after such injection. In order to determine this point, Control 2 was made.

In Control 2, the guinea-pig was given an injection of 2 c.c. of the serum, the syringe was then detached from the needle, which remained

TABLE 85  
ACTION OF CLEAR HEATED PEPTONE SOLUTION (10%) ON NORMAL FRESH, RAT SERUM

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:80	1	200	10	—	2.25	Good shock
	"	1a	200	"	"	"	4'
	"	2	200	"	5	"	3'
	"	2a	200	"	"	"	3'56"
	"	3	195	"	15	"	3'
	"	3a	195	"	"	"	22'
	"	4	200	"	30	"	3'34"
	"	4a	197	"	"	"	4'20"
Control* 1	1:80	5	185	—	—	2.25	Slight
	"	6	200	—	—	"	Moderate
	"	7	180	—	—	"	4'29"
	"	8	198	—	—	"	3'39"
Control† 2	1:80	9	194	—	—	2 + 0.25	Practically nil
	"	10	195	—	—	" "	41'
	"	11	204	—	—	" "	Slight
	"	12	192	—	—	" "	Slight
Control 3		2 c.c. of serum were injected into each of 5 guinea-pigs; result nil or slight					
Control 4		2.25 c.c. of peptone-salt solution (1:8) injected into each of 5 guinea-pigs; result nil					

\* For each test the mixture was made separately (2 c.c. serum + 0.25 c.c. peptone). The time of reaction, that is, from start of mixing to end of injection, was but 41 to 45 seconds.

† In this control set, the serum and peptone were injected consecutively, the injection time for the 2 c.c. of serum was 4 to 10 seconds, while that for the 0.25 c.c. of peptone was but 2 seconds; the total time from the start of the injection of the serum until the end of that of the peptone was 13 seconds for Nos. 10 and 12; 14 seconds for No. 11; and 18 seconds for No. 9.

in the vein, and another with the peptone solution was connected. The speed of the entire operation is indicated in the footnote to the table. It will be seen from this control set that the very rapid and consecutive injection of the two fluids should give a summation of toxicity of the two fluids and in addition should express that which might result from the in-vivo reaction of the two components. While some

in-vivo production is indicated (No. 10), the results conclusively show that the acute deaths in Exper. A, and also those of Control 1 (Nos. 7 and 8) are due to the in-vitro interaction of the peptone and serum. Controls 3 and 4 were made to show the innocuousness of the serum and of the peptone when given separately.

It does not seem possible to interpret the results of Exper. A, in view of the controls made, in any other way than that the peptone itself is not toxic, but that when brought into contact with a reactive serum it gives rise to a poison, namely, anaphylatoxin.

It has been shown above that the reactivity of the serum depends in part upon the age of the serum, and perhaps on the length of exposure to air. An additional factor, however, is involved, for, in the next experiment, which was made with a serum, 35 minutes after the start of bleeding (as fresh a serum as possible), a test of 2.25 c.c. at the end of an incubation of 5 minutes was nil, while of a pair of tests made at 15 minutes, one gave an acute death and one but a slight effect. In another experiment with a serum, within  $2\frac{1}{2}$  hours after start of bleeding, the result was somewhat better, for, after icing the mixture (1:8) for 10 minutes, one test gave a severe shock, while that of its companion was also marked; after incubation for 5 minutes, one of a pair of tests gave acute death, the other but a slight effect; after 15 minutes a similar result was obtained, one death and one slight shock.

The excellent results of the experiment, recorded in Table 85, contrasted with those just mentioned, showed that the age of the serum was not the sole factor. Some other condition contributed to the reactivity of the serum; this might be inherent in blood of the individual animal, or it might lie in the mode of defibrination.

Peptone is at best a weak toxifying agent, and the poisonous effects noted in Table 85 and elsewhere must be looked upon as the sum of the primary or initial toxicity of the serum, and of the anaphylatoxin produced by the action of the peptone. Were the primary toxicity zero (which it never is, since the untreated serum is often fatal in dose of 6 c.c.), then the peptone would have to induce the production of a full dose of poison; on the other hand, if the primary toxicity amounts to two-thirds of a fatal dose, then the peptone would have to produce only the remaining fraction, thereby raising the toxicity over the threshold, as it were.

It will be shown in Part VIII that contact of a serum with its clot tends to increase its toxicity; this fact was made use of in the next

and final experiment. The blood of 6 rats was transferred to a common cylinder (50 c.c.) and defibrinated with a rod, after which the blood with the clot was kept at room temperature for 1 hour; it was then centrifugated, yielding 18 c.c. of pooled serum, which was then iced. A mixture, consisting of 1.5 c.c. peptone and 12 c.c. of the serum, was made, and after icing for 10 minutes, a pair of guinea-pigs were injected with the usual dose of 2.25 c.c., with only a slight or moderate effect. The first injection was made within 2½ hours after the start of bleeding. The mixture then incubated and tested after 5 minutes gave 1 acute death and 1 good shock; retested after 15 minutes, it gave 2 acute deaths. Three control tests, similar to Nos. 5 to 8 of Table 85, showed at most only a slight effect. The results of this experiment were excellent, almost as good as those given in Table 85. A fresh serum and a certain degree of primary toxicity appear to be conditions which assist in bringing out the action of peptone.

*Guinea-Pig Serum.*—The statement has been repeatedly made by Friedberger<sup>29</sup> that he and Mita obtained anaphylatoxin with Witte's peptone. The reference given by him does not appear to contain any information on that point other than the mere assertion that "Witte's peptone by the addition of complement becomes more poisonous." Besredka, Ströbel, and Jupille<sup>30</sup> in their work on "peptotoxine" gave some experiments in which 0.5 c.c. of 10, 2, and 0.2% peptone solutions were incubated, each with 3.5 c.c. of guinea-pig serum. The mixtures, after being kept for an hour at 37 C., and for 18 hours at room temperature, were found to be nontoxic, and from this they concluded that peptotoxin was not formed by the action of serum on the liquid peptone, and that the latter had to be in a particular physical state in order to be acted upon by the serum. According to their view, which corresponds with that of Friedberger, peptone is changed by the serum into peptotoxin, and this they practically identified with the bacterial anaphylatoxin. Without doubt, the peptotoxin of Besredka is anaphylatoxin produced by agar action.

It follows from this that the production of peptone anaphylatoxin in guinea-pig serum has not been demonstrated, though Friedberger claims to the contrary. Furthermore, it will be shown that this result is not as easily attained as in the case of rat serum, and the reason for this comparative failure must be made clear.

<sup>29</sup> Deutsch. med. Wehnschr., 1911, 37, p. 429. Ztschr. f. Immunitätsf., 1911, 10, p. 381; 1913, 17, p. 507; 1914, 22, p. 522.

<sup>30</sup> Ann. de l'Inst. Pasteur, 1913, 27, p. 192.

It has been demonstrated that relatively large amounts of peptone must be injected into guinea-pigs in order to produce fatal results. The clear peptone solution in dose of 0.6 c.c. may kill one-half of the test animals (Table 75), and the same result is obtained with 1 c.c. (Tables 71 and 72); even 1.5 c.c. may occasionally fail to produce a fatal shock. On the assumption that anaphylatoxin is produced when peptone is injected into the animal, it follows that 0.6 to 1 c.c. produces only an average lethal dose of the poison, since but one-half of the animals die. It may be assumed that the test guinea-pigs weighing 200 gm. contain 20 gm. of blood or 10 c.c. of plasma which furnishes the matrix for an average lethal dose of the poison. If serum is as reactive as plasma, it should be possible to make this amount of poison in vitro by bringing together 0.6 c.c. of peptone and 10 c.c. of the serum; but the injection of this amount of serum is out of question, since 6 c.c. and even less of normal guinea-pig serum at times may be fatally toxic.

The addition of 0.6 c.c. of 10% peptone to 10 c.c. of plasma represents a ratio of 1:166 (dry peptone), and since this gives rise to but an average lethal dose of poison in corpore, it follows that a mixture of this amount of peptone and serum might be expected to produce the same dose or less, and this being the case, the injection of half of such mixture (5.3 c.c.) would have little or no effect. In order to obtain results with in-vitro mixtures, it is therefore necessary to increase the amount of peptone which is added to the serum (that is, the ratio of dry peptone to serum) and to use as large a dose of such mixture as possible.

The amount of peptone which can be added to a serum is limited, however, by the fact that it is fatally toxic in a certain dose. Thus, 0.3 to 0.5 c.c. of a 20% solution when given by itself will kill one-half of the test animals, and in view of this it would be necessary to use the peptone in less amount were it not for the fact, already pointed out in Tables 75 and 76, that serum has a distinctly neutralizing action as regards the peptone. A mixture of serum and peptone may therefore actually be less toxic than the peptone solution when injected by itself. Just how far it is safe to increase the amount of peptone in such a mixture cannot be stated, but the limit is probably near 0.75 c.c. of a 20% solution, which, if injected alone, would be an almost surely fatal dose. It follows from this that the amount of peptone present in a given dose of mixture must be at or below this figure, and furthermore,

rigid controls must show that such mixture is initially nontoxic (see controls in Table 85).

The dose of serum to be employed in these tests should be as large as possible (3 to 5 c.c.), for it has been shown hitherto that guinea-pig serum is less easily toxified by agar than is rat serum. It is quite futile to attempt to toxify this serum with peptone so that it will be fatal in dose of 1 c.c. — an experience gained quite early in this investigation. On the other hand, when large doses are to be injected, it must be remembered that the serum possesses a primary toxicity, which is a variable depending in part on the animal, and in part on the mode of defibrination and treatment. M. Wassermann and Keysser<sup>31</sup> were perhaps the first to note that a pooled guinea-pig serum could be toxic in dose of 4 or 3 or even 2 c.c., but their statement, being contrary to usual experience, was not given credence. It will be shown in Part VIII that normal guinea-pig serum can be obtained which will be fatal in dose of 3 or even 2 c.c. That rat serum may be toxic in dose of 3 c.c. was shown in Part I.

One or two tests are not sufficient to control the toxicity, especially when it concerns a dose of 5 or 6 c.c., for it must be borne in mind that guinea-pigs vary greatly as to their susceptibility to anaphylatoxic serum (Table 45), to peptone (Table 72), and to normal sera (Part VIII). If possible, fully as many animals should be used for the control tests as for the experiment proper. The trio of pitfalls to guard against in this work is the toxicity of the peptone, that of the serum, and the susceptibility of the guinea-pig.

A pooled serum, to a certain extent, neutralizes the differences which are inherent in the individual sera, but it does not follow that even pooled sera will behave alike. It has been pointed out on page 695 that different pools of rat serum show an unlike behavior to peptone; the reactivity depending upon the freshness, and upon the degree of primary toxicity since the visible reaction represents the sum of the auto-anaphylatoxin, formed in the blood especially during coagulation, and of the poison produced by the peptone. Hence, the apparent production of a fatal dose of poison by peptone is easier when the amount of serum is fairly large and the inherent toxicity is relatively high; under these conditions an increase in toxicity is more readily detected than would be possible if the initial toxicity were strictly nil.

<sup>31</sup> *Folia serologica*, 1911, 7, p. 243. *Ztschr. f. Hyg. u. Infektionskr.*, 1911, 68, pp. 541, 544. *Centralbl. f. Bakteriol.*, 1911, 50, Beiheft, pp. 52, 72, 78.

Though the mode of action of peptone as regards rat serum has been established by the results just given, it is equally important to show that guinea-pig serum behaves in the same way. If it was difficult to arrive at conclusive results with rat serum, it was even more so, with that of the guinea-pig. Because of this fact it was deemed best to present at some length the consideration of the conditions underlying the problem, and for the same reason it is desirable to give all of the experimental data.

In Table 75 will be found the results of an attempt at toxifying a serum, using 0.3 c.c. of peptone solution per 3 c.c. of serum, the ratio for dry peptone being 1:50. On comparison with Table 76, where the peptone ratio was 1:30, it will be seen that, contrary to expectation, the latter concentration appears to be less active than the former; but since a different pool was used for each set, it is more likely that the cause of the variation lies in the sera.

In the belief that an increase in the amount of serum would be advantageous, the peptone remaining the same, Experiments A, B, and C of Table 86 were then made. The same pooled serum was used for Experiments A and B. It will be seen that there is no particular difference in the results of the two experiments, though the mixture in Experiment A was iced and then incubated, while that in Experiment B was placed directly at 40 C. Experiments C and D were made with another pooled serum, 2 days later, and concerning the former, it is to be noted that the outcome was practically nil. Hence the results of these 3 experiments were not as good as those given in Table 75. This may be ascribed either to a difference in the sera or to the fact that the ratio of dry peptone to serum (1:78) was less than before. Experiment D is noteworthy when contrasted with Experiment C, since in this case the same serum was rapidly toxified by a slight increase in the amount of peptone (1:45); the concentration was, therefore, approximately that used in Table 75, but the dose was increased to 5 c.c. It would appear from this that good results can be expected when large doses of serum are used.

When used in increased dose, the peptone-serum mixture 1:78 may, however, give very fair results, as seem to be indicated in Experiment B, Table 87, where the conditions were similar to those in A and C of the preceding table. The first 2 tests of Experiment B served as preliminary controls and, as such, developed only a slight shock; it might be assumed from this that the subsequent 4 fatal results indicate the for-

mation of anaphylatoxin. While probably this did occur and the poison formed was but an average lethal dose, still in the absence of a large number of controls (9), the conclusion is not quite justified. Meticulous care must be taken to exclude the factor of individual susceptibility in the test animals. Attention should be called to Exper. A, where the same serum was used, but in a greater dose, the ratio being 1:95; seemingly there was a rapid increase in toxicity in 1a as compared with No. 1. This same concentration and dose was used in the experiment given in Table 88, and the result there obtained when contrasted with that just mentioned shows clearly that the variation is due to a difference in the sera.

TABLE 86

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. THE DOSE REPRESENTS 4.7 C.C. GUINEA-PIG SERUM AND 0.3 C.C. 20% PEPTONE, EXCEPT IN EXPER. D., IN WHICH IT IS 4.5 AND 0.5 RESPECTIVELY

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:78	1	192	18	—	5	Practically nil Slight Moderate Severe 6'52"
	"	1a	"	"	—	"	
	"	2	203	"	7	"	
	"	3	"	"	15	"	
	"	4	205	"	30	"	
B	1:78	1	205	—	—	5	Slight Moderate " 7'5" Very slight
	"	2	194	—	15	"	
	"	3	197	—	30	"	
	"	4	"	—	45	"	
	"	5	192	—	60	"	
C	1:78	1	190	17	—	5	Nil " " Practically nil Very slight " "
	"	1a	"	"	—	"	
	"	2	200	"	7	"	
	"	3	195	"	15	"	
	"	4	193	"	30	"	
D	1:45	1	195	16	—	5	3' 3'55"
	"	1a	197	"	—	"	

The same pooled serum was used for A and B; it was from 5 guinea-pigs bled about 5 hours before. Another pool was employed in C and D, and was from 8 guinea-pigs bled also about 5 hours before. Injection time, 5 to 9 seconds.

A further attempt at toxifying with the same amount of peptone (0.3 c.c.), but increasing the dose of serum giving the ratio 1:95, is given in Table 88. The conditions were essentially the same as in Exper. A of the preceding table, and as pointed out, the difference in

results can only be due to a variation in the quality of the two pooled sera employed in these experiments. Although the amount of serum in this case was 5.7 c.c., it was lacking in something, since the peptone (0.3 c.c.) was able to toxify but 1 of 8 tests.

TABLE 87

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. THE DOSE REPRESENTS 0.3 C.C. 20% PEPTONE AND 5.7 OR 4.7 C.C. GUINEA-PIG SERUM

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:95	1	200	1	—	6	1 hr. 3'57"
	"	1a	204	2	—	"	
B	1:78	1	193	1	—	5	Slight
	"	1a	194	2	—	"	"
	"	2	201	30	—	"	Moderate
	"	2a	200	"	—	"	4'20"
	"	3	205	"	7	"	Slight
	"	3a	200	"	"	"	4'16"
	"	4	200	"	15	"	Moderate
	"	4a	194	"	"	"	3'10"
	"	5	200	"	30	"	3'40"
	"	6	198	"	45	"	Good
	"	7	"	"	60	"	Moderate

A single pooled serum from 6 guinea-pigs was used for this set. Exper. A was made within 3 hours after bleeding, and B 3 hours after A. Injection time, 5 to 8 seconds.

TABLE 88

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. THE DOSE REPRESENTS 0.3 C.C. 20% PEPTONE AND 5.7 C.C. GUINEA-PIG SERUM

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:95	1	200	2	—	6	Very slight
	"	1a	205	"	—	"	"
	"	2	205	28	—	"	3'37"
	"	3	206	"	7	"	Very slight
	"	3a	201	"	"	"	"
	"	4	192	"	15	"	Slight
	"	5	203	"	40	"	"
	"	6	206	"	60	"	Very slight

In this experiment, the pooled serum from 4 guinea-pigs was used about 4 hours after start of bleeding. Injection time, 8 to 12 seconds.

In the experiment given in Table 76, where the dose consisted of 0.5 c.c. peptone and 3 c.c. serum, ratio 1:30, the results were rather indifferent; this also could be ascribed to a feebly reactive serum and

the small amount used. Essentially the same results are met with in Expers. C and D of Table 90, where the same dosage was employed. On the other hand, in Exper. D of Table 86, where the dose of peptone was the same but the amount of serum increased, ratio 1:45, the results were striking, and because of this the experiment given in Table 89 was planned. In the belief that less peptone and more serum would be advantageous, the ratio of 1:100 was employed, but the result was not marked and might well be explained by the individual susceptibility of the animals.

TABLE 89

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. THE DOSE REPRESENTS 0.5 C.C. OF 10% PEPTONE AND 5 C.C. OF GUINEA-PIG SERUM

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:100	1	190	10	—	5.5	Moderate
	"	1a	194	"	—	"	Very slight
	"	2	190	"	5	"	3/18"
	"	2a	"	"	"	"	Fair
	"	3	195	"	15	"	Slight
	"	3a	"	"	"	"	Very slight
	"	4	193	"	30	"	4/8"
	"	4a	198	"	"	"	Severe
Control* 1		5	200	—	—	5.5	Very slight
		6	202	—	—	"	Severe

\* For each control the mixture was made separately and injected at once; the time of reaction, that is, from start of mixing to end of injection, was 48 and 52 seconds respectively. These tests were made preliminary to the experiment proper and not at the dose.

A pooled serum from 5 guinea-pigs was used 3 hours after start of bleeding. Injection time, 8 to 12 seconds.

For Expers. A and B of Table 90, the amount of peptone was increased to 0.75 c.c., a rather dangerous procedure, since this amount, given by itself, is usually fatal, as will be seen on reference to Controls 2 and 3 of Table 91. The serum, however, has a distinct neutralizing action as is evident from Exper. B, where the mixture was incubated directly without previous icing. The subacute deaths and one failure to kill show that an antagonism exists. This condition is also to be seen in Control 1 of Table 91; unfortunately, no control of this kind was made in the experiment of Table 90 on account of the pool of serum being exhausted. Expecting that the mixture of 0.75 c.c. peptone and 3 c.c. of serum would have little effect or at most cause subacute deaths, when injected as soon as made, it seemed that on further icing or incubation the peptone would induce the production of enough

anaphylatoxin to make it acutely fatal. This result was apparently obtained in Exper. A of Table 90, for it will be observed that while the first test resulted in a delayed death, the other three tests produced acutely fatal shocks. This result is in marked contrast with that of Exper. B, where incubation at 40 C. resulted in a distinctly weaker action, indicating that the temperature of 40 was unfavorable for the poison-production. Expers. C and D of the same table were made with the same serum and peptone, hence the results are comparable with those of A and B; it appears that 0.5 c.c. of peptone is not as active as 0.75 c.c., the amount of serum being the same.

TABLE 90

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. THE DOSE REPRESENTS 3 C.C. OF GUINEA-PIG SERUM AND 0.75 AND 0.5 C.C. OF 20% PEPTONE, RESPECTIVELY

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:20	1	196	15	—	3.75	62'
		2	197	19	—	"	6'55"
		3	195	21	—	"	4'
		4	195	24	—	"	3'50"
B	1:20	5	195	—	15	"	14'7"
		5a	193	—	"	"	Slight
		6	197	—	30	"	18'51"
		6a	200	—	"	"	1 hr. 45'
C	1:30	7	203	16	—	3.5	Fair
		7a	203	"	—	"	Good
D	1:30	8	190	—	12	"	Severe
		8a	190	—	"	"	"
		9	190	—	30	"	Moderate
		9a	193	—	"	"	Very slight

A single pooled serum from 5 guinea-pigs was used for these experiments. The tests were made 4 to 5 hours after starting to bleed. The injection time was 3 to 8 seconds.

In the hope that a more decisive result could be obtained by the employment of mixtures consisting of 0.75 c.c. of 20% peptone and 4.5 or 5.5 c.c. of guinea-pig serum, a more extensive experiment was planned. For this purpose, the pooled serum from 11 guinea-pigs (101 c.c.) was used, and the first tests were made 4 hours after starting to bleed. Of 5 preliminary controls, similar to Nos. 5 to 8 of Table 91, each receiving the dose of 5.25 c.c. as soon as mixed, 1 died of acute shock and 1 of subacute shock. Accordingly, a mixture of 6 c.c. of peptone and 36 c.c. of the pooled serum was made (ratio 1:30); this was iced and tested in pairs at 15, 30, 45, and 60 minutes, but of the

8 tests only 2 resulted in acute death. In the belief that a better result might be obtained by increasing the amount of serum, another mixture of 3 c.c. peptone and 22 c.c. of the same pooled serum was made (ratio, 1:37) and iced; of a pair of tests made with 6.25 c.c. of the mixture, after icing for 30 minutes, 1 gave an acute death; of another pair of tests made after icing for 35 minutes and incubating at 40 for 15 minutes, none developed more than a slight effect.

The disappointing results of this series clearly indicated that the serum was not as reactive as in Expers. D of Table 86, or A of 87, or A of 90. A better outcome was looked for in the next trial, where a perfectly fresh pooled serum was used within 1¾ hours after starting to bleed. The results are given in Table 91. The mixture employed in Exper. A consisted of 3 c.c. of peptone and 24 c.c. of the pooled serum, ratio 1:40, and the dose was 6.75 c.c. Although 6 c.c. of serum, given by itself, may cause acute death in a fraction of the test animals, and similarly 0.75 c.c. is an almost surely fatal dose (Nos. 9 to 14), yet the two mixed and injected at once (Control 1) caused but 1 acute death, the others being delayed (see Table 76). It may be added that these controls were made while the mixture was icing for Test 1. The results obtained in the control tests 5 to 8 were just what was desired, since it was hoped that a further slight increase in toxicity, due to an inducing action of peptone, would push the effect over the threshold, as it were, and yield a number of acute kills. This expectation was realized in Tests 2 and 3; whether the delayed death in No. 4 was due to the incubation at 40 C. or to a more resistant animal cannot be stated.

In all of these tests when incubation was resorted to, it was done at 40 C. in the belief that a higher temperature would be more favorable. The results, however, point to a distinctly destructive action upon the poison, probably due to the action of the peptone. It will be seen from Tables 86, 87, 90, and 91, that the mixtures appear to become toxic, even without incubation, and given a reactive serum, better results may be expected by keeping mixtures at 30 instead of at 40 C.

*Summation.*—Since peptone, when injected intravenously, produces acute shock and rapid death, it must be concluded that the anaphylatoxin production is rapid within the animal, and if so, it should be nearly as rapid *in vitro*. Peptone, however, is not very poisonous, or better stated, it does not readily produce poison; this is seen in the fact that relatively large doses must be injected into animals in order to produce a result. It has been shown on page 683 that its action on

animals, compared with that of agar, is weak. It was shown that in the case of the guinea-pig, 33 times as much of peptone as of agar must be given; similarly, the rat required 74 times as much. This great difference in the action of the two substances is reproduced in the test tube, and it must be correlated with the physical state of each. While the agar is in suspension, the peptone is in true solution, and in this state it does not possess a very marked action. The fact that a peptone suspension is more toxic than a clear solution was pointed out in connection with Table 70. It was also pointed out that Nathan made a similar observation concerning inulin which in suspensoid state toxified serum, but when dissolved by heating at 68 C. for 2 minutes, it was without effect.

TABLE 91

ATTEMPT AT ANAPHYLATOXIN-PRODUCTION. THE DOSE REPRESENTS 0.75 C.C. OF 20% PEPTONE AND 6 C.C. OF GUINEA-PIG SERUM

Exper.	Ratio	Guinea-Pig		Mixture			Result
		No.	Weight	Incubation		Intra-venous Injection (c.c.)	
				At 0 C. (min.)	At 40 C. (min.)		
A	1:40	1	187	30	—	6.75	16'40"
		2	190	45	—	"	3'45"
		3	190	50	7	"	2'50"
		4	188	"	19	"	28'50"
Control 1	1:40	5	187	—	—	"	23'
		6	195	—	—	"	12'24"
		7	200	—	—	"	14'44"
		8	187	—	—	"	5' 6"
Control 2		9	189	—	—	"	3' 9"
		10	180	—	—	"	2'32"
		11	190	—	—	"	3'40"
Control 3		12	185	—	—	0.75	3' 9"
		13	187	—	—	"	3'32"
		14	184	—	—	"	7'17"

A single pooled serum from 5 guinea-pigs was used for these tests, No. 1 of which was made 1½ hours after start of bleeding. The injection time for the large dose was 10 to 13 seconds, and about 8 seconds for the other.

For each test of Control 1, the mixture was made separately (6 c.c. serum + 0.75 c.c. peptone); the time of reaction, that is, from start of mixing to end of injection was from 51 to 68 seconds, that of No. 7 was 1 min. 26 seconds.

For each test of Control 2, a separate mixture of 6 c.c. of salt solution and 0.75 c.c. peptone was made. For Control 3, the undiluted peptone was used.

In order that the clear solution of peptone shall produce the disturbance in vivo which leads to poison-production, it must be used in considerable amount, and the same condition holds true for its in-vitro action. The rat serum which is extremely reactive with agar can also be toxified with peptone, but relatively the amounts of serum and peptone must be greatly increased. Thus, the excellent results in Table 85

were obtained with a peptone-serum mixture in which the ratio of dry peptone to rat serum was 1:80, but the amount of poison produced was not large, since 2 c.c. of the serum had to be injected in order to kill. When it is recalled that an agar-serum mixture (No. 3) in which the ratio is 1:40000 (Table 49) can toxify rat serum in 15 minutes so that 0.25 c.c. is fatal, it will be realized that peptone is decidedly inferior to agar as a toxifying agent. And, further, in view of this fact, it should be clear that very marked results with guinea-pig serum can hardly be expected.

A comparison of the agar-serum ratio 1:40000 with that of the peptone-serum mixture (1:80) used in the afore-mentioned experiment will make it evident that agar is 500 times as active as peptone as to in-vitro mixtures. Again, it has been pointed out in Part II that guinea-pig serum can probably be toxified in dose of 3 c.c. by a sol-gel mixture, No. 3, in which the ratio of agar to serum is 1:40000. Certainly, Mixture 4 (Table 23), having a ratio of 1:8000, readily toxifies it in 1.5-c.c. dose (Tables 24, 25). On comparing these ratios with that employed in Table 91 (1:40), it will be seen that, as to guinea-pig serum, agar is from 200 to 1000 times as active as peptone.

#### THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN BY PEPTONE

As in the case of agar, it was essential to show that the symptoms and autopsy findings after peptone injection were due to an altered state of the blood—in short, to anaphylatoxin. If the blood became toxic as a result of the action of peptone, then transfusion of a sufficient amount to a guinea-pig should produce a typical anaphylactic shock. On the other hand, if the toxicity was due to a direct action, or to a cleavage of the peptone, the transfusion of blood containing but a small fraction of the original peptone should yield no effect.

For the experiments made with this object in view, the peptone solution was prepared as usual, the concentration being 10%, unless otherwise stated. The procedure was the same as for the corresponding agar tests (Table 65). The results agree very closely with those where agar was injected, thus showing that the same mechanism is brought into being.

*Anaphylatoxin in Rats.*—In Table 92 are presented the results of 8 transfusion experiments with rats which received an intravenous injection of 2 to 3 c.c. of 10% peptone solution. It will be observed that here, as in like experiments with agar, the immediate transfer of

blood from an injected rat has either no effect or but a very slight one (Nos. 1 and 2). This is an important fact, for, if the effects seen in the later tests were due to a carrying over of a lethal dose of peptone, then such transfer should certainly be more likely in the immediate tests. As it is, the effect observed was practically nil. Furthermore, it may be added that a simple calculation, based upon the amount of blood present in a rat, the amount transferred, and the amount of peptone injected, will show that the quantity of peptone which could possibly be present in the transfused blood is but one-fourth to one-half of that contained in an average lethal dose of peptone (0.06 gm. per 200 gm. guinea-pig).

As the time of reaction within the blood increased, a rapid production of poison occurred. It will be seen that this reaction time for No. 3 was but 1 minute and 18 seconds. The accompanying chart (11) is based upon the reaction times, and it will be noted that Test 5

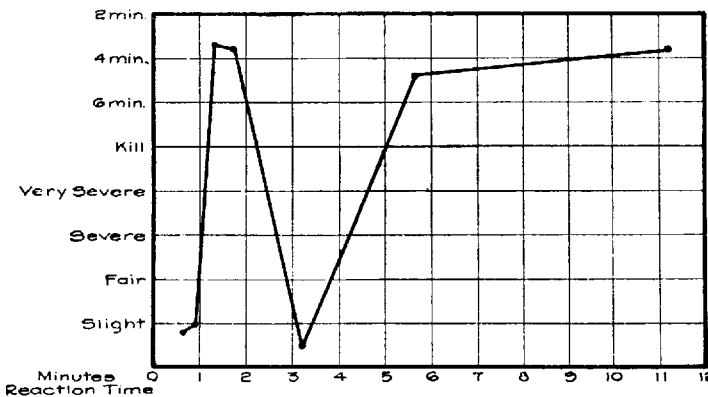


Chart 11. The in-vivo production of anaphylatoxin in rats injected with peptone (Table 92).

gave but a slight reaction which might be taken to indicate that a drop in the poison-production occurred about this time. A similar drop was obtained in the transfusion experiments with rats injected with agar (Chart 9). It was noted in connection with Test 5 that, just before the rat was operated on, the deep respiration had changed to about normal, which fact would show an individual resistance on the part of the animal, thus accounting for an apparent decrease in the poison. Further, in these tests, as in others with anaphylatoxin, it is to be remembered that the recipient may be unusually resistant. In Test 8,

where but 1 c.c. was transfused, a very severe shock was obtained. Apart from the one exception, it will be seen that the blood of the injected rats was toxic to guinea-pigs, and that this condition persisted for 11 minutes (No. 7). Control experiments, given in connection with like transfusion tests (Table 66; see also Part IX), show that as much as 4 to 5 c.c. of rat blood can be carried over into a guinea-pig without any effect, provided the transfer time is less than 1 minute.

TABLE 92  
THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN RATS INJECTED WITH 10% PEPTONE

Rat		Peptone	Guinea-Pig	Blood Transfused (c.c.)	Total Time	Transfer Time	Result
No.	Weight	Intra-venous Injection (c.c.)	Weight				
1	130	2.6	175	2.0	1'10"	29"	Slight
2	148	3.0	212	"	1'15"	19"	"
3	155	"	170	"	1'44"	26"	3'21". Typical shock
4	145	"	182	"	2' 7"	28"	3'32". " "
5	152	"	196	"	3'28"	26"	Very slight
6	151	2.0	192	"	6' 2"	22"	4'55". Typical shock
7	149	"	176	"	11'39"	29"	3'81". " "
8	155	"	182	1.0	5'50"	33"	Very severe typical shock

The difference between total time and transfer time represents the reaction time within the body of the rat.

It was possible that in these experiments, the amount of blood transferred (2 c.c.) contained more than 1 lethal dose. In order to have some information on this point, a similar series of tests was made, in which 1 c.c. of blood was transfused from the injected rat to a guinea-pig. The total time for these tests was 2'29", 3'35", 3'51", 4'36", and 5'50". The effect in the first test was practically nil; in the second, slight; in the third, moderate; in the fourth, fair; while in the fifth and last one (included as No. 8 in Table 92), they were very severe. It will be seen, therefore, that the minimal lethal dose, formed in vivo is between 1 and 2 c.c. of blood. This means that in a rat of 150 gm. with about 15 c.c. of blood, there is produced somewhat more than 7.5 guinea-pig lethal doses of anaphylatoxin. This amount, however, cannot possibly account for the anaphylactic shock and death in rats injected with peptone, since it has been shown (Part V) that rats, compared with guinea-pigs, weight for weight, will tolerate 100 fatal doses of anaphylatoxin. It is possible that the peptone injection brings about a lowered cell resistance and thus renders the rat more susceptible to the anaphylatoxin; it is also possible that the cellular as well as blood plasma reacts to the peptone by poison-production.

*Anaphylatoxin in Guinea-Pigs.*—In the first series of attempts to demonstrate the formation of anaphylatoxin, the peptone was injected in dose of 2 gm. per kilo body weight, a 20% solution being employed. The results are to be found in Table 93. It will be seen that 7 of 12 tests ended fatally with typical shock, giving a percentage of 58. This large dose of peptone was employed for the express purpose of producing as severe a shock as possible with the object of inducing a maximal disturbance, thereby facilitating the detection of the poison.

It is deserving of note that in No. 12 with a reaction time of only 43 seconds, acute shock resulted; a like outcome was obtained in No. 10, where the reaction time was 57 seconds. This would indicate that under these conditions the poison-production occurs at great speed.

TABLE 93  
THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN GUINEA-PIGS INJECTED WITH 20% PEPTONE:  
2 GM. PER KILO

Donor		Peptone Intra- venous Injection (c.c.)	Time of Death	Recipient		Total Time	Transfer Time (sec.)	Result
No.	Weight			Weight	Blood Trans- fused (c.c.)			
1	560	5	3'	202	3	5' 7"	62	Very slight
1a				196	"	5' 51"	106	"
2	615	6.2	3' 40"	190	5	5' 32"	57	3' 13". Typical shock
3	650	6.5	3' 8"	205	"	4' 40"	45	Fair shock
4	505	5	3' 3"	190	"	5' 12"	47	Good shock
5	525	5.25	4' 25"	205	"	6' 54"	49	3' 46". Typical shock
6	475	4.75	3' 45"	207	"	6' 22"	57	Severe
7	510	5.1	2' 50"	198	4	6' 12"	58	6' 54". Typical shock
8	520	5.2	3' 17"	195	5	6' 32"	60	6 hr.
9	540	5.4	3' 10"	195	"	6' 27"	73	8' 5". Typical shock
10	600	6		205	"	1' 32"	35	3' 15". " "
11	550	5.5		190	"	1' 28"	40	Moderate
12	570	5.7		200	"	1' 19"	36	4' 9". Typical shock
13*	400	4		190	"	5' 45"	40	Nil

\* No. 13 is a control which received an injection of 4 c.c. of salt solution. For other controls, see Parts VIII and IX.

The other tests all show marked toxicity with the exception of No. 1, where duplicate tests of 3 c.c. gave but very slight effects. On account of the drop in blood pressure, it was not possible to make duplicate tests with larger amounts of blood; in fact, to secure 5 c.c., required an appreciable time, as will be seen from the transfer time of the tables. In Nos. 10 to 12, where the heart was exposed immediately after the injection had been made, the desired amount of blood was quickly obtained, because the effect on blood pressure had not time to manifest itself.

Inasmuch as the dose of peptone employed in the first series of tests was large and might justify the objection that the amount of blood transfused carried with it a lethal amount of peptone, a second series with but 1 gm. of peptone per kilo was made, the results of which tests are given in Table 94. Of the 13 tests in this series, 3 were acutely fatal while 1 was protracted, although the immediate effects were severe. It is to be noted that the very early transfusions in Nos. 14 to 16 gave very slight results as compared with like tests in Table 93. The percentage of fatal shocks was less than with 2 gm., namely, 31%.

TABLE 94  
THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN GUINEA-PIGS INJECTED WITH 20% PEPTONE:  
1 GM. PER KILO

Donor		Peptone Intra- venous Injection (c.c.)	Time of Death	Recipient		Total Time	Transfer Time	Result
No.	Weight			Weight	Blood Trans- fused (c.c.)			
14	650	3.25		192	4	1'45"	55"	Slight
15	500	2.5		200	5	1'26"	43"	"
16	490	2.45		189	"	1'31"	41"	Moderate
17	500	2.5	2'45"	200	"	4'36"	1' 3"	"
18	410	2.1	4'42"	190	4	6'35"	1'10"	2'30". Typical shock
19	500	2.5	3'	185	5	5' 5"	1'30"	Slight
20	570	2.9	?	185	"	3'28"	48"	Very severe
21	460	2.3	?	180	"	3'34"	54"	6 hr. Severe
22	550	2.8	3'	205	"	6' 3"	1'18"	Slight
23	600	3	3' 8"	180	"	6' 7"	1'	14'15". Typical shock
24	430	2.15	3'12"	205	3	7' 3"	1'18"	Fair
25	550	2.75	3'10"	180	5	6'45"	1'12"	3'40". Typical shock
26	525	2.6	4'20"	191	"	7' 8"	1' 8"	Slight

The only control test made at this time was No. 13. It had been learned previously, however, that 5 c.c. of guinea-pig blood, drawn from the heart and kept in the syringe for 2 minutes, produced little or no effect on subsequent injection (Table 117). This fact is also evident from the transfusion tests after injection of agar (Table 67), as well as in like tests after specific shock (Tables 132, 133).

In interpreting the results of the tests given in Tables 93 and 94, it is necessary to bear in mind that the individual guinea-pigs vary considerably in their resistance to anaphylatoxin. The fact that a guinea-pig gives a slight response does not prove that the poison is absent, as will be seen on reference to Table 45. The dose injected may well represent an average lethal dose, that is, such as will be fatal to one-half of the test animals. While it would be very desirable to make duplicate tests from the same heart of a treated animal, this is

quite impossible as to the 5-c.c. dose, on account of the drop in blood pressure in the donor. Any single nonfatal test must, therefore, be interpreted with considerable caution. The failure to realize this fact is in evidence in much of the literature on anaphylatoxin and anaphylaxis.

The positive results cannot be ascribed to the disturbance caused by the mere volume of the liquid injected since the control with salt solution (No. 13) was without effect. Neither can they be credited to precoagulation toxicity, since the drawn blood invariably was fluid for several minutes after the injection of the recipient. Furthermore, as mentioned, even normal guinea-pig blood can be transfused in like amount without any ill effect.

It is possible to interpret them as due to the presence of a lethal dose of peptone in the volume of blood transfused. It may be conceded that such objection is fairly valid, although Schmidt-Mülheim and also Fano claimed that the peptone rapidly disappeared from the blood and was gone in from 5 to 6 minutes after the injection. This possible interpretation is somewhat valid for the tests given in Table 93, where the donor received 2 gm. of peptone per kilo body weight.

On the assumption that all of the peptone remains in the circulation, and that the blood constitutes 7-10% of the body weight, it will be found that recipient No. 8 was given from 0.072 to 0.1 gm. of peptone in the blood transferred; for the others in Table 93, the calculated amounts thus carried over would be from 0.09 to 0.125 gm. It has been shown hitherto that a dose of from 0.06 to 0.1 gm., when given by itself is fatal to about one-half of the test animals (Tables 71, 72, 75). On the other hand, it is to be borne in mind that in a mixture of serum and this amount of peptone the action of the latter is quickly neutralized (Tables 75, 76), and it is probable that a like action may occur *in vivo*, especially with a reaction time of 5 or 6 minutes. Be that as it may, some doubt is justifiable as to whether the deaths in Table 93 were caused by anaphylatoxin or by transferred peptone.

The possible interpretation of the results mentioned can hardly hold true for the tests given in Table 94, where the donors were given 1 gm. of peptone per kilo of body weight. A similar calculation shows that recipient No. 18 could receive but 0.038 to 0.054 gm. of peptone; for the other fatal cases in this table, the amount of peptone which could possibly be transferred with the blood would be from 0.048 to 0.068 gm. This fact would seem to justify the view that these deaths were not

due to peptone carried over, but to the anaphylatoxin formed in the blood. This conclusion is given strong support by the results of Tests 14 to 16, where the transfer was made as soon as possible after the injection; in these it might be expected that the peptone would be present, if at all, in maximal amount because of the very early transfusion. They are, therefore, essentially controls for the tests which follow. In the corresponding tests of Table 93 (Nos. 10 to 12) 2 proved fatal, and while this fact may be considered as evidence of a peptone transfer, it is conceivable that anaphylatoxin is produced more rapidly after an injection of 2 gm. than after one of half that amount.

While the results obtained in the transfusion tests with guinea-pigs are not as clean cut as those obtained with rats, still they at least make it highly probable that anaphylatoxin is produced *in vivo* when peptone is injected. It has been shown that the guinea-pig serum does not readily react with peptone *in vitro*, and this appears to be also true of the blood *in vivo*, since a relatively large dose must be given in order to produce a fatal shock. Such death may be due to the formation of but 2 or 3 lethal doses, and in that event it would be quite impossible to detect its presence by transfusion. When, however, a fair multiple lethal dose is developed, it becomes feasible to show its existence, and to arrive at some idea of the number of average lethal doses formed. Thus, in Test 23, in which 5 c.c. of blood transfused from a 600-gm. donor proved fatal, it would appear that from 8 to 12 lethal doses of poison had been produced, according as to whether the volume of blood is taken as 7 or 10% of the body weight.

*Anaphylatoxin in Rabbits.*—The difficulty in arriving at an unequivocal result in blood transfusion from guinea-pigs injected with peptone is accentuated in the case of the rabbit on account of the large dose which must be given in order to produce a fatal shock. This will be evident by an examination of the results of two such experiments, given in Table 95. Because of the large dose of peptone, and also because of the possibility that the blood of the rabbits might be inherently toxic, the amount of blood transfused was limited to 2 c.c. In each experiment, 2 preliminary controls were made before injecting the peptone, and these served to show an absence of inherent toxicity as to the dose employed. The blood was obtained for these tests by direct heart puncture. Thereupon, the rabbit was injected with the peptone solution, 5 gm. per kilo of body weight. In Exper. A the heart was

exposed and the blood was drawn into a heart pipet within 10 minutes after the injection, although the rabbit was not severely shocked; portions of 2 c.c. were then drawn up from the pipet and injected into Nos. 3 and 4. It should be added that the blood in the pipet remained perfectly fluid for 12 minutes, but clotted at 14 minutes (Table 80, No. 13).

Rabbit B of the other test was also tested for inherent toxicity (Nos. 5 and 6), after which it was injected with peptone, the dose being 5 gm. per kilo, and as this had but very slight effect, it was reinjected 26 minutes later with a like amount, death resulting in 3 minutes, 20 seconds (Table 80, No. 17). The heart was at once exposed and the blood was drawn up into a pipet within 6 minutes after this second injection; portions of this blood were then tested on Nos. 7 to 9. The condition of this blood is indicated in the footnote to Table 95.

TABLE 95  
THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN RABBITS INJECTED WITH 30% PEPTONE:  
5 GM. PER KILO

Donor		Peptone Intravenous Injection (c.c.)	Recipient		Blood Trans- fused (c.c.)	Total Time	Transfer Time	Result
No.	Weight		No.	Weight				
A	1350	22.4	1	207	2		13"	Slight
			2	208	"		13"	"
			3	200	"	11'38"	1'23"	"
			4	400	"	12'19"	1'32"	10'23"
B	1930	32	5	192	"		19"	Slight
			6	185	"		27"	"
			7	192	"	8' 2"	1'47"	27'
			8	185	"	10'15"	4'	3'45"
			9	198	"	14'17"	8' 2"	Fair

Nos. 1, 2, 5, and 6 are controls preliminary to the injection. In Nos. 7 to 9 the blood was not transfused but was taken from a heart pipet; hence the apparently long transfer time which actually is the time the blood was in the pipet. The blood was perfectly fluid and remained so for 35 minutes (see Rabbit No. 17, Table 80). The same explanation holds true for the transfer time of Nos. 3 and 4.

The results, taken as they are, would appear to show that anaphylatoxin was formed under these conditions. However, the possibility that the effects observed were due to peptone transferred with the blood must not be overlooked. As in the case of the guinea-pigs, on the assumption that all of the peptone remains in the circulation, a calculation shows that the recipients may have received from 0.08 to 0.11 gm. of peptone, in other words an average lethal dose. Under the circumstances, no positive conclusion can be drawn as to the formation of

anaphylatoxin in rabbits injected with peptone. As long as it is necessary to employ Witte's peptone in such large dosage it will be difficult to settle the question. If it were true that the peptone disappears very rapidly from the circulation it might aid the solution of the problem. Perhaps experiments with a more highly toxic protease, one which will kill in dose of 1 gm. or less per kilo, will give decisive results.

#### SUMMARY

The symptoms and findings in guinea-pigs injected with peptone are the same as those produced by anaphylatoxin, by agar, or by specific anaphylactic shock. A marked exophthalmos may be present, a symptom especially marked in rabbits.

Guinea-pigs show the same individual variation to peptone as has been demonstrated for anaphylatoxin and for agar; this variation extends to the several symptoms and findings.

Peptone in a dose of 0.75 gm. per kilo will usually kill guinea-pigs, but occasional failure has been noted even with 1 gm. per kilo. On the other hand, 0.3 gm. per kilo represents an average lethal dose, since it is fatal to about one-half of the test animals.

The speed of injection is a factor in the results obtained.

After a nonfatal injection, guinea-pigs show the same immunity or tolerance as has been observed in dogs. Repeated injections of a sublethal dose, at intervals of 15 minutes, result in death. The tolerance is often broken down by the injection of peptone solutions diluted with from 6 to 9 parts of salt solution or distilled water.

An hypertonic salt, confirming Ritz, may protect against an otherwise fatal dose of peptone. An even more marked antagonism is shown by sodium carbonate, which also acts against anaphylatoxin. This fact supplies a fundamental basis for the preventive and therapeutic action of alkali as to blood disturbances. Normal serum also possesses a decided antagonistic action.

Injections of peptone markedly affect the coagulation time of guinea-pig blood; the larger the dose, the more pronounced the effect.

The injection of peptone into white rats results in symptoms and findings identical with those caused by anaphylatoxin and by agar. The coagulation time of the blood may be retarded for an appreciable period, even up to 22 minutes.

The least lethal dose for the rat was found to be 2 gm. per kilo, an amount 7 times larger than that needed for guinea-pigs.

In rabbits, very large doses of peptone must be employed; perfectly typical anaphylactic shock and findings may be obtained. In addition to the usual symptoms such as dyspnea, spasms, convulsions, a striking exophthalmos is to be noted, also, a great drop in blood pressure, and a marked decrease in coagulation time. Considerable variation in the resistance of rabbits, like that in rats and guinea-pigs, was found.

The least amount of peptone which was fatal to the rabbit was 3 gm. per kilo, but some may withstand even 5 gm. per kilo. The rabbit is, therefore, more resistant than the rat. It is a striking fact that the rabbit is considerably more resistant than the guinea-pig to agar, peptone, anaphylatoxin, and to specific anaphylatoxic shock.

The rabbit shows an immunity or tolerance to repeated injections of peptone, the same as the dog or guinea-pig.

The change in the coagulability of the rabbit blood occurs immediately after peptone injection, even in nonfatal cases. The coagulation may be completely retarded for 35 minutes. At times the process does not go beyond the formation of a small clot (+), which soon shrinks and may be overlooked (fibrinolysis of Dastre).

Peptone is considerably less toxic than agar. The ratio expressing the relation of the least toxic dose of agar to that of peptone is 1:33 for the guinea-pig; 1:74 for the rat; and 1:188 for the rabbit.

Rat serum on treatment with peptone rapidly yields anaphylatoxin. A peptone suspension is more active than a perfectly clear solution. The fully dissolved peptone does not toxify rat serum as easily, nor does it yield as high a degree of toxicity as do the agar and trypanosome suspensions. Extreme dispersion is, therefore unfavorable for the reagent.

Rat serum in dose of 2 c.c. can be toxified in less than a minute.

With peptone, rat serum gives the best results when used as soon as prepared; even then variations due to the individual peculiarities of the animal occur. In the case of agar, a vastly more active reagent, these conditions are rarely in evidence.

Experiments made with guinea-pig serum, treated with peptone, indicate that here also anaphylatoxin is produced. The weak action of the peptone, and the feeble reactivity of the serum, as compared with that of the rat, render the demonstration of the poison more difficult. This is still more true when rabbit serum is tested with peptone.

A comparison of the effective agar-serum ratio with that of peptone-serum shows that with regard to rat serum, agar is 500 times

more active than peptone; with regard to guinea-pig serum, it is 200 to 1000 times as active.

The transfusion of the blood of rats injected with peptone demonstrates that anaphylatoxin is formed *in vivo*. In the rat somewhat more than 7.5 guinea-pig lethal doses were found.

Similar transfusions in the case of guinea-pigs likewise showed that anaphylatoxin was produced. In rabbits, on account of the large dose of peptone necessarily injected, the results were not as decisive.

Peptone itself is not toxic, but when brought into contact with a reactive serum or plasma it induces the change which results in anaphylatoxin-production.