A SPECIFIC POISON IN THE LIVER EXTRACTS OF RABBITS INOCULATED WITH TYPHOID AND PRODIGIOSUS BACILLI INTRAVENOUSLY.

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(Received for publication, May 27, 1918.)

The work of Parker and Franke[1] shows that when rabbits are inoculated with typhoid bacilli intravenously, there is not enough difference in either the killing off or localization of the bacteria in the organs, of normal and immune animals, to account for the difference in the latter's resistance to the infection. The experiments seem to indicate that the rabbits immune to typhoid bacilli might owe their resistance to the power to neutralize or destroy the poisons as they were being formed from the bacteria while normal animals either lacked this power or possessed it imperfectly. The liver, on account of its size and because it takes up so many more typhoid bacilli per gram of weight than any other organ, was thought of as operating in this connection. Indeed, the liver might not only be the main seat of the poison formation but might act as a detoxicant in the immune animal, but not in the normal animal. The work of Manwaring[2] and Weil[3] on the liver in anaphylaxis in dogs bears on this point. It was with the purpose of investigating this question that the experiments to follow were performed.

Dold,[4] Loeb,[5] and Dold and Ogata,[6] and others have studied the toxicity of aqueous extracts of normal organs when given intravenously. Their technique was practically the same as that used in our experiments except that they did not weigh the organs. Although Dold states that all normal rabbit organs (lung, spleen, liver, kidney, brain, and muscle) are toxic when given intravenously to rabbits, he does not give a single protocol in which liver extract was injected. Schenk,[7] however, gives one experiment which demonstrates the toxicity of nor-

[1] Unpublished.

[2] Manwaring, W. H., *Z. Immunitätsforsch., Orig.*, 1910–11, viii, 589.

[3] Weil, R., *J. Immunol.*, 1917, ii, 525.

[4] Dold, H., *Z. Immunitätsforsch., Orig.*, 1911, x, 53.

[5] Loeb, L., *Z. Immunitätsforsch., Orig.*, 1912, xii, 189.

[6] Dold, H., and Ogata, S., *Z. Immunitätsforsch., Orig.*, 1912, xiii, 667.

[7] Schenk, F., *Z. Immunitätsforsch., Orig.*, 1914, xxii, 229.

mal liver, but does not say how his extract was prepared and he may have used very large doses.

An experiment on normal tissue extracts is given below which confirms Dold's work except in the case of the liver.

EXPERIMENTAL.

A normal rabbit was bled to death under ether. Pieces of the organs noted (Table I) were placed in sterile test-tubes, weighed, ground in a sterile mortar with sand, and emulsified in salt solution added in the proportion of 5 cc. to 1 gm. of tissue. The mixture was shaken in large sterile tubes in the machine for 10 minutes and centrifugalized at high speed for half an hour. The slightly turbid supernatant fluid was pipetted off and injected into rabbits. This technique was used in all the following experiments and all inoculations were made intravenously.

TABLE I.

Rabbit No.	Weight.	Amount injected.	Organ extract.	Remarks.
	gm.	*cc.*		
1	1,840	10	Liver.	No symptoms.
2	1,900	5	Lung.	Dead in 1 min.
3	1,605	5	Kidney.	" " 1 "
4	1,250	5	Brain.	" " 2 "

We have made many experiments with normal liver extracts giving 10 cc. to rabbits weighing over 1,000 gm. and 5 cc. to those below this weight, and in only one instance was a reaction obtained. This was in a rabbit weighing 1,830 gm. which had been injected with 10 cc. of liver extract. Rapid breathing and prostration developed 2 minutes after the injection; recovery was complete in 20 minutes. A second rabbit, weighing 1,730 gm., received 10 cc. of extract of the same liver without reaction.

At the outset various doses of living typhoid bacilli (Rawling's strain) were injected intravenously into rabbits which were bled to death under ether when very sick. A piece of liver was then removed and extracted as described above. It was later found that the liver became highly toxic when even boiled typhoid antigen was

injected. However, most of the experiments were made with living typhoid bacilli. Two protocols illustrating the latter are given.

Experiment 1.—Rabbit 5; weight 1,250 gm. Injected with 1 agar slant of typhoid bacilli. Death occurred 5 hours later. Liver extracted as usual.

Rabbit 6; weight 1,050 gm. Injected with 5 cc. of the liver extract of Rabbit 5. Convulsion in 1 minute; death in 1½ minutes.

Experiment 2.—Rabbit 7; weight 1,290 gm. Injected intravenously with ⅗ agar slant of typhoid bacilli. No immediate effect, but 2 hours later the animal was weak, and during the day the weakness increased; bled and killed while in collapsed condition 8 hours after the inoculation. Liver removed and extracted as indicated.

Rabbit 8; weight 1,185 gm. Given intravenously 10 cc. of the extract. Immediately on conclusion of the injection the animal became prostrated, then rose, ran a short distance, and died; survived injection 1 minute.

By further experimentation it was found that in order to obtain rabbits in which the liver was definitely toxic the dose of typhoid bacilli had to be so adjusted that the animal was rendered very sick 4 to 8 hours after the injection, at which time it must be killed. It is difficult to make a definite rule as to what this dose should be, for rabbits vary widely in their resistance to typhoid bacilli. If an overwhelming injection is given (2 to 4 slants) so that the rabbit succumbs in about 2 hours, the liver is non-toxic. In order that a liver may be rendered sufficiently toxic, time must be allowed it, not only to take up the bacteria, kill, and disintegrate them, but also to elaborate the poisonous products. With the strain (Rawling's) used in this study the most constant results were obtained by injecting 1 agar slant of the bacilli.

Experiments were made with typhoid antigen which was prepared as follows: The 48 hour growth from 40 potato tubes of agar was washed off with isotonic salt solution and poured into centrifuge tubes, and the suspensions were centrifugalized at high speed. After pipetting off the clear supernatant fluid, the sediment was taken up in 100 cc. of salt solution, 0.2 N sodium hydroxide added to it, and the whole heated to 58°C. for ½ hour, and then shaken several hours a day for 5 days. Microscopic examination of this material which constituted the antigen showed the bacteria to be well broken up and staining very feebly.

In respect to the employment of the antigen for rendering the liver toxic, it is even more difficult to indicate the effective dose. On its inoculation the animal ordinarily falls sick soon after the injection and either gets progressively worse till death occurs or the condition improves after a couple of hours. In the latter instance a second injection must be given. The liver becomes toxic in a shorter time after antigen administration than after injection of the bacilli.

The following protocols are those of an experiment in which the antigen was given.

Experiment 3. One Dose of Antigen.—Rabbit 9; weight 1,230 gm. Injected with 0.5 cc. of the typhoid antigen. After 1 hour, diarrhea, and prostration which progressed. Killed 5½ hours after inoculation.

Liver extract was prepared from this rabbit as before. 10 cc. were injected into Rabbit 10, weighing 1,440 gm. There were immediate convulsion and death in 1½ minutes.

Experiment 4. Two Doses of Antigen.—Rabbit 11; weight 1,560 gm. Given 1 cc. of the antigen. 2½ hours later very sick. During next 2 hours partial recovery. Injected with 2 cc. of the antigen. Bled and killed 8 hours after first injection. The liver extract was prepared, and 5 cc. were injected into Rabbit 12, weighing 950 gm. Immediate convulsion; death in 3 minutes.

Experiment 5. Two Doses of Antigen.—Rabbit 13; weight 1,275 gm. Given 0.5 cc. and 1½ hours later given 2 cc. of the antigen. Killed 5 hours after injection. 10 cc. of the liver extract were injected into Rabbit 14, weighing 1,945 gm. Immediate convulsion and death in 3 minutes.

The boiled antigen is active but less so apparently than the unboiled. Two tests only were made with it; in neither did the liver extract kill the second animal, possibly because the first rabbits were not killed at the proper time.

A protocol in which the boiled antigen was used is given, as it illustrates the symptoms which arise from a sublethal dose of the liver poison.

Experiment 6.—Rabbit 15; weight 1,330 gm. Given 1.5 cc. of the typhoid antigen which had been boiled for 5 minutes. The animal became sick in less than 1 hour and remained so till killed 5 hours later. 10 cc. of the liver extract were injected into Rabbit 16, weighing 1,230 gm. 2 minutes later breathing was labored and slow, the head and abdomen resting on the floor. 16 minutes after injection it jumped up and ran a short distance, but fell again from weakness. After 30 minutes, breathing better, and animal stronger. 1½ hours after the injection, appeared almost normal. Well next morning.

10 cc. of a Berkefeld filtrate of the same liver extract were injected into Rabbit 17, weighing 1,340 gm. After 3 minutes labored breathing and weak. After 10 minutes flattened out on table; breathing very labored. After ½ hour recovering; well next morning. This experiment will be referred to again.

The toxic liver tissue changes at ice box temperature and after 2 days it is no longer poisonous. The next experiment indicates this fact and also shows that the normally toxic lung is not detoxicated under similar conditions.

Experiment 7.—Rabbit 18; weight 1,810 gm. Given 0.5 cc. of the typhoid antigen. Death occurred 65 minutes later. One piece of the liver and one piece of the lung were prepared as usual. A second piece of the liver and of the lung was placed in the ice box for 36 hours. 5 cc. of the usual liver extract from the liver of Rabbit 18 were inoculated into Rabbit 19, weighing 950 gm. In 1 minute convulsion, in 2 minutes death. 5 cc. of the liver extract prepared from the piece of liver which had been on ice were injected into Rabbit 20, weighing 930 gm.; no effect produced. 3 cc. of an extract from the lung which had been on ice were injected into Rabbit 21, weighing 1,540 gm. Dead in 1½ minutes. This test with liver was repeated with the same results. A sufficient number of experiments has not been done with the ordinary toxic liver extracts to know how long the extracted poisons will keep in the ice box. However, the fact that it has not deteriorated much in 2 days has been determined several times.

It seems probable that this detoxicating power of the liver is responsible for the difficulty in obtaining poisonous extracts. Probably the normal liver is able to neutralize, as they are formed, large amounts of poison, and it is only when the quantity produced exceeds the detoxicating power of the liver that the poison can be demonstrated. Possibly poisons are also formed from bacteria in other organs, but this point is difficult to prove on account of the toxicity of the normal organs. The fact that in rabbits the liver takes up from forty to fifty times as many bacteria as all the other organs together[1] argues in favor of its being the principal seat of the poison formation. It is interesting to note here that Weiss, Kolmer, and Steinfield[8] have recently noted that pneumonic lungs from man and dog are more toxic to laboratory animals than normal lungs.

Dold,[4] Dold and Ogata,[6] and Loeb[5] found that normal organs caused death because of a coagulative ferment, which leads to the

[8] Weiss, C., Kolmer, J. A., and Steinfield, E., *J. Infect. Dis.*, 1918, xxii, 469.

formation of thrombi in the right heart and pulmonary artery and veins. We have corroborated these findings. These writers also found that the ferment does not pass through a Berkefeld filter, and that it is destroyed by heating to 60°C. for 1 hour.

We have searched many times for thrombi in the right heart and pulmonary artery in rabbits that have succumbed to the liver poison, but in only four out of twenty-six animals autopsied immediately after death was a small clot found in the right heart. In one animal there was a clot also in the pulmonary artery. In the animals that succumbed to the liver poison the heart beats several minutes after the cessation of respiration, and the blood coagulates slowly.

Several tests were made with a Berkefeld filtrate of the typhoid livers, and although the filtrate was less toxic than the original extract, it was still poisonous as shown in Experiment 6, Rabbit 17.

Boiling of the toxic extract was active in one of the three tests.

Experiment 8.—10 cc. of the liver extract used in Experiment 4 were boiled for 5 minutes. The coagulum was centrifugalized off and 6.5 cc. of the supernatant fluid were injected into a rabbit, weighing 1,280 gm. Within 2 minutes the animal was prostrate, and there was "anaphylactic" breathing. In 5 minutes the symptoms abated and in 10 minutes they had passed off.

To exclude the blood as the source of the toxicity, 3.4 cc. of serum and 4 cc. of defibrinated blood taken from animals whose liver extract was toxic, were injected into two rabbits, weighing 600 gm. each. No symptoms resulted.

Experiments with Bacillus prodigiosus.

The next series of tests was made with another bacterial species in order to test the specificity of the poison produced. *Bacillus prodigiosus* was selected because of its toxicity for rabbits.

After many fruitless efforts it was found that a toxic liver extract could be obtained with *Bacillus prodigiosus* provided the injected animal was killed very soon after inoculation. Our best results were obtained from 2 or 3 slants of *Bacillus prodigiosus* and by killing the rabbit while still prostrate and from 1 to 3 hours after the injection. The effects are quite the same whether the toxic liver extract is made with typhoid or *prodigiosus* bacilli.

Experiment 9.—A normal rabbit weighing 1,665 gm. was given 2 agar slants of *B. prodigiosus;* killed while sick 1 hour and 10 minutes later. With the extract of the liver of this animal three rabbits were injected (Table II).

TABLE II.

Rabbit No.	Weight.	Amount injected.	Remarks.
	gm.	*cc.*	
22	1,610	5	Dead in ½ min.
23	1,440	3	" " 4 "
24	1,240	2	Very sick. Died during night.

Experiment 10.—Rabbit 25; weight 1,420 gm. Given 3 agar slants of *B. prodigiosus.* Killed 3¾ hours later. 5 cc. of the liver extract were injected into another rabbit, weighing 1,365 gm. Death was immediate.

Experiments on Immunity to the Liver Poison.

The question as to whether rabbits could be immunized to the liver poison was investigated. Although testing of resistance to multiple lethal doses of the poison was made difficult by reason of the large quantities of extract to be injected, yet it developed that in many instances one or more injections of the poison gave rise to tolerance to 1½ lethal doses persisting at least 10 days. In this respect also the liver poison differs from that found in extracts of normal organs by Dold and Ogata.[6] These writers also produced tolerance to the normal organ poison, but it lasted only 24 to 48 hours.

Two examples of the phenomenon mentioned follow:

Experiment 11.—Rabbit 26 was immunized to *prodigiosus* liver poison as follows:
Jan. 12, 1918. Weight 1,480 gm. 5 cc. of the liver extract (sublethal dose).
Jan. 18. Weight 1,470 gm. 7 cc. of the liver extract (1⅓ lethal doses).
Jan. 23. Weight 1,690 gm. 8 cc. of the liver extract (1½ lethal doses).
Jan. 28. Weight 1,580 gm. 8 cc. of the liver extract (1⅓ lethal doses).
Rabbit 27 was immunized to typhoid liver poison as follows:
Jan. 8, 1918. Weight 2,440 gm. 10 cc. of the liver extract (½ lethal dose).
Jan. 15. Weight 2,350 gm. 7 cc. of the liver extract (¾ lethal dose).
Jan. 19. Weight 2,500 gm. 10 cc. of the liver extract (1 lethal dose).
Jan. 23. Weight 2,480 gm. 12 cc. of the liver extract (1⅓ lethal doses).
Feb. 7. Weight 2,525 gm. 12 cc. of the liver extract (1½ lethal doses).

Moreover, it was found that rabbits actively immunized to *Bacillus typhosus* or *Bacillus prodigiosus* resist respectively the typhoid and *prodigiosus* liver extracts.

Experiment 12.—Rabbit 28, weighing 2,250 gm., was injected with 1 agar slant of *B. typhosus*; killed after 7 hours when very sick. An actively immune rabbit, weighing 2,200 gm., was given 10 cc. of the liver extract from Rabbit 28. No symptoms appeared. A normal rabbit, weighing 2,250 gm., was given 10 cc. of the same liver extract. Dead in 8 minutes after injection. The history of the actively immune rabbit is as follows:

Dec. 3, 1917. Weight 2,265 gm. $\frac{1}{10}$ culture of live typhoid bacilli (Rawling's).
Dec. 10. Weight 2,035 gm. $\frac{1}{5}$ culture of live typhoid bacilli (Rawling's).
Dec. 15. Weight 2,220 gm. $\frac{1}{2}$ culture of live typhoid bacilli (Rawling's).
Dec. 22. Weight 2,210 gm. $\frac{3}{4}$ culture of live typhoid bacilli (Rawling's).
Dec. 29. Weight 2,230 gm. 1 slant culture of live typhoid bacilli (Rawling's).
Jan. 7, 1918. Weight 2,200 gm. Injected with the liver extract.

The next step was to determine whether typhoid immune serum was capable of neutralizing or destroying the typhoid liver poison *in vitro* and *in vivo*. For this purpose a lethal dose (10 cc.) of the typhoid liver poison was mixed with 2 cc. of high titer typhoid immune serum and kept at 37°C. for 1 hour. The controls consisted of normal serum and salt solution instead of the immune serum. It developed that even the salt solution control became detoxicated by this treatment. The experiment was repeated twice, the procedure being the same except that in one instance the mixtures were kept at 23°C. and in the other at 0°C. for 1 hour. No differences were noted.

No explanation is offered at present to account for the destruction of the toxic extract on mere standing with saline solution. A modification of the method of procedure also failed to bring out neutralizing power of the immune serum. Thus three pieces of the liver taken from a rabbit dying of typhoid liver extract were treated respectively (*a*) with isotonic salt solution as usual, (*b*) extracted with 1:20 normal, and (*c*) 1:20 typhoid immune serum. The three mixtures were kept at 0°C. for 2 hours before injection. Upon inoculation it was found that the immune serum liver extract killed a rabbit in even less time than the salt solution control. The immune serum exerted, therefore, no detoxicating effect.

The *in vivo* test was made as follows: Rabbits 29 and 30 were injected each with 3 cc. of immune (1:40,000 titer) and of normal serum respectively and 2 minutes later were given a lethal dose of the liver poison. Both animals survived. This experiment was repeated several times with the same result. Then two other rabbits were given the normal and immune rabbit sera, $5\frac{1}{2}$ hours before the injection of the toxic liver extract. Both these died. Here again no explanation is offered for this phenomenon which calls for minute study and analysis.

As far as the tests described go it may be stated that typhoid immune serum does not protect specifically against the liver poison.

Specificity of the Poisons.

The opinion first entertained was that probably the liver poisons were related closely to those discovered by Friedberger and Nathan[9] or to the protein split products of Vaughan,[10] but the experiments on their specificity caused a modification of that view. Two sets of tests bearing on this point were made. In the first, rabbits were rendered refractory to the typhoid and *prodigiosus* poisons respectively and then tested against the heterologous poison as follows:

Experiment 13.—Rabbit 31, weight 1,560 gm., was given 10 cc. of a typhoid liver extract and died in 1 minute. Rabbit 32, weight 2,515 gm., had been given previously six graded doses of the typhoid liver poison. It was then given 12 cc. of the typhoid liver poison without effect. Rabbit 33, weight 1,650 gm., had been given four graded doses of the *prodigiosus* liver poison. It was then given 8 cc. of the typhoid liver poison. Death in 1 minute.

The experiment was modified and carried out in immune typhoid and *prodigiosus* animals.

Experiment 14.—A normal rabbit was inoculated with 1 agar slant of *B. typhosus* and killed when dying 4 hours later. The liver extract was inoculated into other rabbits (Table III).

The immune rabbits used in these experiments were prepared as shown in Table IV.

[9] Friedberger, E., and Nathan, E., *Z. Immunitätsforsch., Orig.*, 1911, ix, 444.

[10] Vaughan, V. C., Jr., and Vaughan, J. W., Protein split products in relation to immunity and disease, Philadelphia and New York, 1913.

It should be stated that a 24 hour agar slant of *Bacillus prodigiosus* contains at least twice as many bacteria as one of *Bacillus typhosus*.

The experiment was now revised so that the *prodigiosus* liver poison was injected into immune typhoid and *prodigiosus* rabbits.

Experiment 15.—A rabbit weighing 1,220 gm. was injected with 2 slants of *B. prodigiosus* and killed $2\frac{1}{2}$ hours later when very sick. The liver extract was inoculated into other rabbits (Table V).

TABLE III.

Rabbit No.	Condition.	Weight.	Amount injected.	Remarks.
		gm.	*cc.*	
34	Normal.	1,350	8	Dead in 2 min.
35	Typhoid-immune.	1,345	8	No symptoms.
36	"	1,645	8	" "
37	"	1,850	10	Slightly sick.
38	"	1,900	10	Convulsion in 4 min. Dead in 9 min.
39	*Prodigiosus*-immune.	1,555	8	" " $\frac{1}{2}$ " " " $2\frac{1}{2}$ "
40	"	2,280	10	" " $1\frac{1}{2}$ " " " 4 "

TABLE IV.

Rabbit No.	Organism injected.	Amount injected.	No. of injections.	Agglutination titer of serum for *B. typhosus*.
35	Typhoid.	1.2 slants.	7	1:4,000
36	"	0.7 slant.	6	1:20,000
37	"	0.9 "	7	1:40,000
38	"	0.8 "	6	1:4,000
39	*Prodigiosus*.	0.4 "	8	None at 1:100.
40	"	0.3 "	7	" " 1:100.

TABLE V.

Rabbit No.	Condition.	Weight.	Amount injected.	Remarks.
		gm.	*cc.*	
41	Normal.	1,600	8	Convulsion in 1 min. Dead in 2 min.
42	Typhoid-immune.	1,790	7	Very sick. Convulsion in 5 min. Dead in $7\frac{1}{2}$ min.
43	"	1,740	7	Sick. Convulsion. Dead in 45 min.
44	*Prodigiosus*-immune.	1,540	7	Very sick for 3 min. Recovered in 4 min.
45	"	1,540	7	Sick for 8 min. and then recovered.

The immune rabbits used in the experiment were prepared as shown in Table VI.

TABLE VI.

Rabbit No.	Organism injected.	Amount injected.	No. of injections.
42	Typhoid.	1.7 slants.	12
43	"	1.0 slant.	8
44	*Prodigiosus.*	0.5 "	8
45	"	0.4 "	8

Experiment 16.—In this experiment the typhoid liver extract was prepared from a rabbit, weighing 1,350 gm., which had been given 1 slant of *B. typhosus* and was killed when moribund 4 hours later. The *prodigiosus* liver extract was prepared from a rabbit, weighing 1,700 gm., which had been given 2 slants of *B. prodigiosus* and killed when very sick 1½ hours later.

The results of the experiment are shown in Table VII.

TABLE VII.

Rabbit No.	Condition.	Weight.	Amount injected.	Toxic liver extract.	Remarks.
		gm.	*cc.*		
46	Normal.	1,720	8	*Prodigiosus.*	Dead in 2 min.
47	Typhoid-immune.	1,830	8	"	" " 4½ "
48	*Prodigiosus*-immune.	1,720	8	"	Severe symptoms. Recovered.
49	Normal.	1,560	10	Typhoid.	Dead in 1 min.
50	*Prodigiosus*-immune.	1,840	10	"	" " 1½ "
51	Typhoid-immune.	1,985	10	"	Slight symptoms.

The immune rabbits of this experiment had been given at least five injections of either *Bacillus typhosus* or *Bacillus prodigiosus*.

The results of the experiment with actively immune animals indicate, therefore, that the liver poisons employed in them are specific. This point is an interesting one in view of the fact that specific immune sera for *Bacillus typhosus* and *Bacillus prodigiosus* do not neutralize the poisons. The explanation of this discrepancy is not at once apparent and further study is required to elucidate the phenomenon.

Possibly it is bound up with cellular in contradistinction to serum immunity.

DISCUSSION.

The experiments described in this paper make it probable that part at least of the intoxication produced in rabbits by injections of *Bacillus typhosus* or *Bacillus prodigiosus* is due to liver poisons of the nature of those dealt with here and which appear to be yielded to the blood by the liver, and then absorbed by the various tissues. It should not be surprising, perhaps, that the poisons in the quantities used in these experiments should be insufficient in amount to be demonstrable in the blood, for they are probably being removed from the blood continuously and with considerable speed by the tissues. Hence, only very small quantities are probably present at any one time in the circulation even when the animal is extremely sick.

Rabbits actively immune to *Bacillus typhosus* or *Bacillus prodigiosus* exhibit a condition of resistance apparently because of having acquired in the process of immunization a tolerance to the liver poison. For it is possible that even if several lethal doses of the bacteria are injected into the immune animal, not more than one lethal dose of the liver poison is produced in a unit of time, an amount which the immune animal can tolerate readily.

Probably the liver poisons are of cellular origin and are produced by the living liver cell and possibly by the cells of other organs also acting upon the typhoid or *prodigiosus* bacteria, or their disintegrated products, which fact explains why they are not produced outside the body of the animal.

Whether a similar poison is generated in typhoid fever in man is a question that cannot now be answered. It seems not impossible that it may play a part in causing the symptoms of toxemia in that disease.

CONCLUSIONS.

1. The livers of rabbits inoculated with cultures of *Bacillus typhosus* or *Bacillus prodigiosus* under certain conditions contain a toxic substance extractable with salt solution. When the toxic extracts are injected intravenously into normal rabbits the latter animals develop

symptoms resembling those of anaphylactic shock and succumb. The lethal doses of the toxic extracts are far smaller than those of normal liver extract.

2. The livers of rabbits injected with typhoid antigen also yield a toxic extract.

3. Boiling as well as filtration through a Berkefeld filter only partially detoxicates the extract.

4. Tolerance to one to two lethal doses of the poisonous extracts can be induced by cautious immunization.

5. Rabbits actively immunized to *Bacillus typhosus* or *Bacillus prodigiosus* usually resist one lethal dose of the homologous liver poison; and animals tolerant to the typhoid liver poison resist one minimum lethal dose at least of *Bacillus typhosus*.

6. Typhoid immune serum is not detoxicating either *in vivo* or *in vitro* for the typhoid liver poison.

7. The liver poisons are specific, since rabbits actively immunized to either *Bacillus typhosus* or *Bacillus prodigiosus* withstand at least one minimum lethal dose of the homologous but not of the heterologous liver poisons.

It is a pleasure to acknowledge the invaluable services of Mr. James May, whose skilled assistance contributed essentially to the success of the experiments.