

THE FATE OF TYPHOID BACILLI INJECTED INTRAVENOUSLY INTO
NORMAL AND TYPHOID IMMUNE RABBITS.*

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Introduction. — Hopkins and Parker,¹ in their work on the fate of hemolytic streptococci injected into susceptible and insusceptible animals, found that most of the organisms were taken up by the lung, especially in insusceptible animals as the cat — or in the partially immunized rabbit. On account of the unavoidable interruption of this work, preliminary observations on the effect of immunization could not be extended far enough to lead to a definite conclusion. It was with the purpose of investigating this phase of immunity that the present work was undertaken. As it is difficult to produce a real immunity in rabbits to the streptococcus hemolyticus, we decided to work with typhoid bacilli even though we were dealing then with a toxemia rather than an infection.

Technic. — Suspensions in sterile saline solution of typhoid bacteria from a twenty-four hour agar slant were injected intravenously into normal and immune rabbits. These were killed after varying intervals of time by bleeding from the carotid under ether. Pieces of organs to be studied were placed in sterile test tubes, weighed, ground in a sterile mortar with sand and emulsified in salt solution added in the proportion of 1 cubic centimeter to .1 gram of tissue. Plate cultures were made in meat extract agar of amounts of organ emulsion representing from .1 to .00001 gram and the colonies counted after eighteen hours. For the sake of clearness, however, all figures given in the tables have been calculated for .1 gram of organ tissue; this number being the average of those obtained in all of the dilutions.

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Experimental. — Each type of experiment reported has been performed a number of times, but only a few tables illustrating the different classes will be given in detail — as the results in the duplicates were consistent with those here recorded. Many preliminary experiments were first performed in order to obtain as clear a picture as possible of the localization and degree of killing of typhoid bacilli in normal rabbits killed at varying intervals after their injection with the different amounts of that organism.

Table I. illustrates an experiment where a normal rabbit weighing 2,725 grams was injected intravenously with *B. typhosus* No. 10. Blood from the ear four minutes after injection showed approximately 133,200 bacteria per cubic centimeter. The rabbit was bled to death under ether ten minutes after the injection, and plate cultures made from the blood and the organs in the manner described above. Portions of each tissue excised were incubated at 37° C. for four hours before plating to determine the bactericidal action *in vitro*.

TABLE I.

Normal rabbit. Weight: 2,725 grams. Injected intravenously with B. typhosus No. 10.

	Lung. Colonies .1 gm.	Liver. Colonies .1 gm.	Spleen. Colonies .1 gm.	Kidney. Colonies .1 gm.	Psoas. Colonies .1 gm.	Bone Marrow. Colonies .1 gm.	Adrenal. Colonies .1 gm.	Blood. Colonies 1.0 c.c.
Excised 10 min. after injection and plated . . .	98	58,500	18,000	20	0	510	850	7
Excised 10 min. after injection. Plated after 4 hrs. incuba- tion.	5	47,200	5,600	15	0	320	—	—

Table II. records the results obtained when a normal rabbit (No. 1) weighing 1,780 grams was injected intravenously with half of an agar slant of *B. typhosus* (Rawling's) and killed ten minutes later as compared with those obtained when a normal rabbit (No. 2) weighing 1,775 grams was injected

with the same amount of the same culture, but killed three and a half hours later. The bactericidal action of the tissues of Rabbit No. 1 was also determined *in vitro* for the four- and seven-hour periods.

TABLE II.

	Lung. Colonies 0.1 gm.	Liver.	Spleen.	Kidney.	Psoas.	Bone Marrow.	Adrenal.	Lymph Node.	Blood. Colonies 1 c.c.
Rabbit No. 1 excised 10 min. after injection. Plated immediately. . .	35,000	780,000	175,000	160	0	2,000	5,000	0	550
Plated after 4 hrs. incubation.	2	2,500,000	1,400,000	3,600	0	*	7,200	3,200	—
Plated after 7 hrs. incubation.	38,000	13,000,000	9,000,000	70,000	60	*	Omitted	6,000	—
Rabbit No. 2 excised 3½ hrs. after injection. Plated immediately. . .	1	14,000	600	20	0	1,240	100	0	0

* Indicates that the colonies in the plate from .01 gram were too numerous to estimate and that no plates were made from smaller amounts of tissue.

These representative experiments demonstrate that when the animal is killed within a half hour after receiving a lethal dose or more of typhoid bacteria intravenously, enormous quantities of the organisms are found in the liver and spleen. As indicated by the plate culture method, in the primary distribution of the invading bacteria at least twice as many are deposited in the liver as in the spleen and approximately one hundred times as many as in the lung or in the bone marrow. Relatively few are found in the blood, kidneys and adrenals, and practically none in the lymph nodes and muscles.

The lung is the only tissue which shows slight bactericidal power *in vitro* during the first four hours of incubation; the high degree of killing indicated in Table II. for the lung during the "four-hour period" is, however, exceptional and probably due to some error in technic. More typical figures indicating the degree of destructive action usually obtained from lung tissue are given below.

TABLE III.
Colonies per .1 gram lung tissue.

	Rabbit 2.	Rabbit 3.	Rabbit 11.
Plated immediately	100	4,250	3,300
Plated after 3½-4-hour incubation . .	5	500	2,200

In vivo, — that is, in rabbits killed from three and a half to six hours after injection, — the bacteria have largely disappeared from the various organs except in the case of the bone marrow, where they remain in approximately the same numbers. Tables II., V. and VI. illustrate this. This apparent constancy in the bacterial content of the bone marrow led us to investigate this matter further.

A normal rabbit weighing 1,250 grams received an intravenous injection of one slant of *B. typhosus* (Rawling's), and was bled to death under ether fifty-three hours later when almost moribund.

TABLE IV.

	Lung.	Liver.	Spleen.	Bone Marrow.	Blood 1 c.c.
Plated immediately .	720	790	1,220	1,350,000	0

This experiment was repeated with the same results. It seems possible therefore that in rabbits that are slowly dying of typhoid the bone marrow may constitute a focus of infection from which the organisms are continually swept into the blood stream and from there are taken up by the organs.

Experiments with rabbits immunized to typhoid as compared with the normal were now undertaken. Since the dosage used in immunization varied slightly with different animals, the method used will be given in detail in each experiment.

The only constant difference between the normal and immune rabbits was the primary localization of the bacteria in the lung, the number taken up by the immune animal being somewhat greater than in the normal; this difference is,

however, not as pronounced as that found by Hopkins and Parker between insusceptible and susceptible animals when injected with hemolytic streptococci. The bactericidal powers of the organs *in vitro* and *in vivo* were the same in both normal and in immune animals. In Tables V. and VI. the results of two comparative experiments are given.

Comparative study of normal and immune rabbits.— Normal rabbit No. 1, weighing 2,210 grams, and immune rabbit No. 2, weighing 1,960 grams, were inoculated with 616 and 560 million bacteria respectively and were bled and killed fifteen minutes after their injections. Normal rabbit No. 3, weighing 1,665 grams, and immune rabbit No. 4, weighing 1,875 grams, were inoculated with 532 and 560 million bacteria respectively and were killed three and a half hours after their injections.

The bactericidal suspension injected was varied so that each rabbit received an amount proportional to its body weight. A number of bacteria per cubic centimeter of suspension was determined by the dilution plate method.

TABLE V.

	Lung.	Liver.	Spleen.	Kidney.	Bone Marrow.	Blood 1 c.c.
Rabbit No. 1, normal. Excised 15 min. after injection. Plated immediately	3,300	650,000	335,000	140	715	50
Plated after 4 hrs. incubation .	2,200	360,000	700,000	500	500	—
Rabbit No. 2, immune. Excised 15 min. after injection. Plated immediately	46,250	888,000	388,000	2,000	17,000	400
Plated after 4 hrs. incubation .	30,500	625,000	455,000	980	11,000	—
Rabbit No. 3 normal. Excised 3½ hrs. after injection. Plated immediately	132,500	23,000	6,000	7	646	9
Rabbit No. 4, immune. Excised 3½ hrs. after injection. Plated immediately	222,500	6,700	440	27	13,700	63

Rabbit No. 2 was immunized as follows:

10/13	Weight 1,770 gm.	1/120	Killed agar slant No. 10.
10/23	" 1,850 "	1/80	" " " " "
10/30	" 1,695 "	1/40	" " " " "
11/5	" 1,835 "	1/20	" " " " "
11/10	" 2,040 "	1/10	" " " " "
11/16	" 1,930 "	1/10	Alive " " " "
11/23	" 2,020 "	1/10	" " " Rawling.
11/27	" — "	1/5	" " " "

Used 12/13. Weight 1,960 gm.

Rabbit No. 4 was immunized as follows:

10/13	Weight 1,840 gm.	1/60	Killed agar slant No. 10.
10/23	" 1,915 "	1/80	" " " " "
10/30	" 1,845 "	1/40	" " " " "
11/5	" 1,760 "	1/10	" " " " "
11/10	" 1,775 "	1/10	" " " " "
11/16	" 1,890 "	1/10	" " " " "
11/23	" 1,940 "	1/10	" " " Rawling.
11/27	" 2,045 "	1/5	" " " "
12/3	" 1,990 "	1/2	" " " "

Used 12/13.

Normal rabbit No. 1, weighing 1,900 grams, and immune rabbit No. 2, weighing 1,885 grams, were bled and killed ten minutes after injection. Normal rabbit No. 3, weighing 2,130 grams, and immune rabbit No. 4, weighing 2,160 grams, were bled and killed four hours and ten minutes after injection. Normal rabbit No. 5, weighing 2,000 grams, and immune rabbit No. 6, weighing 2,025 grams, were bled and killed nineteen and a half hours after injection. All six rabbits received 39,627,000 typhoid bacteria intravenously.

TABLE VI.

	Lung.	Liver.	Spleen.	Bone Marrow.	Blood i c.c.
Normal rabbit No. 1. Excised 15 min. after injection. Plated immediately	5,600	270,000	176,000	68,000	270
Immune rabbit No. 2. Excised 15 min. after injection. Plated immediately	229,000	509,400	387,400	5,240	273
Normal rabbit No. 3. Excised 4 hr. 10 min. after injection. Plated immediately	32	15,300	7,200	18,300	29
Immune rabbit No. 4. Excised 4 hr. 10 min. after injection. Plated immediately	5,000	4,070	440	740,000	27
Normal rabbit No. 5. Excised 19½ hr. after injection. Plated immediately	32	77	560	67,000	15
Immune rabbit No. 6. Excised 19½ hr. after injection. Plated immediately	20,000	150	97	10,000	3

Rabbit No. 2 was immunized as follows:

11/27 Weight 2,085 gm. 1/10 Killed agar slant Rawling's.
 12/3 " 1,920 " 1/5 " " " "
 12/10 " 1,830 " 1/5 Live " " "
 12/15 " 1,950 " 1/2 " " " "
 12/22 " 1,950 " 3/4 " " " "
 Used 1/15.

Rabbit No. 4 was immunized as follows:

11/27 Weight 2,545 gm. 1/10 Killed agar slant Rawling's.
 12/3 " 2,400 " 1/5 " " " "
 12/10 " 2,330 " 1/5 Live " " "
 12/15 " 2,340 " 1/2 " " " "
 12/22 " 2,340 " I " " " "
 12/29 " 2,240 " I " " " "
 Used 1/16.

Rabbit No. 6 was immunized as follows:

11/27 Weight 2,445 gm. 1/10 Killed agar slant Rawling's.
 12/3 " 2,390 " 1/5 " " " "
 12/10 " 2,135 " 1/5 Live " " "
 12/22 " 2,075 " 1/2 " " " "
 12/29 " 1,900 " I " " " "
 Used 1/15.

Discussion. — An attempt was made in this work to demonstrate some difference in either the killing or localization of typhoid bacteria in normal and immune rabbits. Our results were entirely negative in regard to the killing of the bacteria. Both in normal and immune animals the bacteria are taken up and killed in the organs with extraordinary speed. If a medium-sized rabbit is inoculated with a half slant of typhoid, it is usual to find, four hours later, only about ten per cent of the inoculated number of organisms still alive in its organs and tissues. The liver, because of its relatively large weight and because of its ability to take up more bacteria per gram than any other organ, must play the principal part in this destruction. By weighing the organs of normal rabbits and taking the blood as ten per cent and the muscle as forty-two per cent of the body weight we calculated that the liver usually takes up from forty to sixty times as many bacteria as all the other organs and tissue together. It can be seen from this that the liver would probably play an important part in the toxemic manifestations of typhoid in rabbits.

Although no constant difference in the killing of bacteria can be demonstrated in normal and immune rabbits, these animals vary normally a good deal in their resistance to typhoid. For this reason only those differences can be counted which are large and regular. An extreme instance of this variation is given below as it bears out the belief of one of the writers as to the production of typhoid toxemia in rabbits.

A normal rabbit weighing 1,775 grams was inoculated with 530 million typhoid bacteria (one-half slant) along with four other rabbits (see Table V.). He was very sick a half hour later and died one and a half hours after the inoculation. No blood could be collected. The organs were cultured as usual. The following table shows the number of bacteria in the organs calculated for .1 gram of tissue.

Lung.	Liver.	Spleen.	Kidney.	Bone Marrow.
255	3,800	5,000	7	800

If these results are compared with rabbits No. 3 and No. 4 in Table V., it will be seen that in the latter animals, although they were killed three and a half hours after the injection, the number of viable bacteria in their organs was much greater than in the organs of the rabbit who died one and a half hours after a proportional injection. This suggests that the great susceptibility of this rabbit was due to the greater bactericidal powers of its organs and tissues, thus producing a larger amount of poison than it could cope with.

In regard to the localization of the bacteria in the organs,—only a few words need be said. Although there appears to be a slight difference in the lung — the immune lung taking up a greater number than the normal — it does not seem likely that this difference can play any important part in the resistance of immune animals to typhoid, as the number of bacteria taken up even in the immune lung is only a very small part of the number taken up by the other organs, especially by the liver.

CONCLUSIONS.

1. There is no difference in the bactericidal powers of normal and immune rabbits to intravenous injections of typhoid bacilli.
2. The lungs of immune rabbits take up a slightly greater number of bacteria than do normal lungs.

REFERENCE.

1. Journ. Exper. Med., 1918, xxvii, 1.