THE FATE OF HUMAN AND BOVINE TUBERCLE BACILLI IN VARIOUS ORGANS OF THE RABBIT.

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In a recent study of the susceptibility of various organs of the rabbit to tuberculosis (1) attention was drawn to the fact that their "native" cellular reaction to the intravenous injection of heat-killed tubercle bacilli differed greatly. From this and from other data the inference was drawn that different organs in a given species possess an inherently different power to destroy tubercle bacilli before any allergic manifestations are apparent. It was thought desirable to examine this problem more thoroughly.

Now, while dead bacilli elicit the cellular reaction to the chemical constituents of the organism, they do not reproduce all the conditions of infection with living bacilli. Dr. Eugene L. Opie suggested that the problem be attacked with living bacilli by cultural methods. Obviously this approach complicates the problem, but the results so obtained are more significant.

Cultural methods have often been used for the study of infectious diseases and for the isolation of tubercle bacilli from various sources, but the only instance of their use we know of, for the quantitative estimation of tubercle bacilli in a given source, is Lange's attempt (2) to estimate the number of living bacilli by the number of colonies that developed from a given suspension of tubercle bacilli planted on serum egg medium. After a few trials with (3) Boissevain's plasma method, which was found to be unsuitable for our purpose, preliminary experiments were performed with Dorset's egg medium and Petroff's gentian violet medium. It soon appeared that, while the method does not give the absolute number of living tubercle bacilli in a given specimen of tissue, it gives the relative numbers with sufficient accuracy. During the progress of this work Madsen and Mørch (4) have reported

a close correspondence between Petroff's method and guinea pig inoculation in their studies on rabbits treated with sanocrysin.

Method.

At varying intervals of time after the intravenous injection of varying quantities of different types of tubercle bacilli, the rabbit is killed by a blow on the head. The hair on the entire ventral surface of the animal is removed by a depilatory mixture and the skin washed and covered with an antiseptic. With rigorous precautions for sterility, the skin is reflected. The abdominal cavity is opened, a separate pair of sterile instruments being used for each step. The first organ that presents itself in the wound is the liver. 1 gm. quantity is weighed accurately into a sterile Petri dish. In a similar manner like weighed amounts of spleen and kidney are excised as they are successively uncovered. The thoracic cavity is now opened and 1 gm. quantity of lung, representative in its gross pathological condition of the entire organ, is removed. Aseptically, the right femur is crushed with forceps and a weighed amount, as near a gm. as possible, of bone marrow is removed. The specimens of tissue are then ground in sterilized mortars with sterilized sand as finely as possible consistent with sterility and are suspended in a given quantity of fifteenth molar disodium phosphate (5). From these suspensions, containing a known weight of a given tissue, dilutions are made in the same alkaline medium. 1 cc. quantity of each dilution of each organ is shaken in a sterilized tube containing glass beads and seeded directly on three tubes of Dorset's medium and three tubes of Petroff's medium, divided as equally as possible and spread as evenly as possible on the surface of the medium by means of a pipette. The suspended material is allowed to settle for at least $\frac{1}{2}$ hour, often much longer. Uniform distribution of the inoculated material is secured by allowing the tubes to recline so that the surface of the medium is in a horizontal plane. The tubes are now incubated. Another 1 cc. specimen is treated with 3 per cent sodium hydroxide according to Petroff's method, centrifugalized, neutralized with hydrochloric acid, and seeded on three tubes of Petroff's and Dorset's medium respectively as before. After the tubes have been left in the incubator for varying periods of time, the number of tubercle bacillus colonies in each is counted and recorded at weekly or biweekly intervals. Repeated readings are necessary both to check the counts and also to prevent loss of any data that might result from subsequent contamination.

We found that at least 3 months must elapse before the ultimate number of colonies in any one tube could be determined, for often new colonies formed even as late as 10 weeks after incubation, especially with the bovine strain. Since the tubes must be maintained at incubator temperature for such a long time all precautions against drying out of the medium are necessary. Precautions must also be taken against the spreading of colonies by the liquid of condensation. For these reasons, the tubes were always read in an upright position and handled as

little as possible. When the number of colonies appearing on the surface of a tube was not more than 200 they could easily be counted directly. When the colonies exceeded this number, they were estimated by wrapping about the tube a piece of x-ray celluloid from which square windows of varying sizes had been cut out and reading these areas with a magnifying glass. Repeated readings tended to eliminate gross errors. In case of any doubt whether a given colony was of tubercle bacilli or not, smears were made and stained by the Ziehl-Neelsen method.

Strains.—Two human strains and one bovine strain were used. The human strains, P-15 B and P-48 A, were isolated in the latter part of 1923 from a nodule in the lung and from a hilum lymph node respectively of human autopsy material (6). Both were typical human strains as determined by rabbit inoculation. The bovine strain, Bovine C, was also isolated in 1923 by Dr. Joseph D. Aronson from a lymph node of a tuberculous cow. Originally it was exceptionally virulent for the rabbit (7) and it still behaves like a typical virulent bovine strain.

Dosage.—In one series of experiments the fate of the human tubercle bacillus, P-15 B, was compared with the fate of the bovine type, Bovine C, in the various organs of the rabbit in quantities of 0.1 mg. each to rabbits of approximately the same weight. In another series the behavior of the same bovine strain was compared with another human strain, P-48 A, in quantities of 0.001 mg. per kilo of body weight.

The animals were killed 2 days, 1, 2 and 4 weeks and 2 months after intravenous infection and at other intervals, as will be apparent from the tables and curves.

The figures in the tables represent the average number of colonies recovered, usually from three separate tubes, each planted with a like quantity of the same suspension of tissue of a given dilution. When two dilutions were used the figures represent the average of both. Since it required an entire day to perform a single experiment, for frequently as many as 120 tubes had to be seeded from a single animal, the same suspension of tubercle bacilli could not be used to inoculate all the rabbits of a given series. A given suspension was used to inoculate a number of rabbits corresponding to the number of intervals required, and similarly other suspensions, until the number of rabbits required for each interval was obtained. The slight variation in dosage thus introduced was lessened by preparing the suspensions as uniformly as possible from similarly aged cultures, etc. The same alkaline medium was used for making the suspension of tubercle bacilli as in suspending the tissues.

The Fate of Human Tubercle Bacilli in Large Dosage in the Various Organs of the Rabbit.

The number of colonies recovered from similar weights of lung, liver, spleen, kidney and bone marrow, 2 days, 1, 2 and 4 weeks and 2 months after injection of 0.1 mg. P-15 B into rabbits, after direct seeding upon Dorset's and Petroff's media and also after sodium hydroxide treatment following 3 months incubation, is recorded in detail in Table I.

TABLE I.

Number of Colonies Obtained from Organs of Rabbits Infected with 0.1 Mg. B. tuberculosis, Human.

Rabbit No.	Interval after injection	Lung Direct		Lung Treated		Liver Direct		Liver Treated		Spleen Direct		Spleen Treated		Kidney Direct		Kidney Treated		Bone marrow Direct		Bone marrow Treated	
		D.*	P.†	D.	P.	D.	P.	D.	P.	D.	P.	D.	P.	D.	P.	D.	P.	D.	P.	D.	P.
9-75	2 days	—	—	—	—	—	23	0	0	78		10	15	—	0	—	0	5	2	—	—
9-76	2 days	—	P	17	—	42	22	—	6	200	300	—	34	0	2	0	0	6	11	0	3
9-82	2 days	—	P	—	—	28	60	1	0	23	25	0	0	0	0	0	0	26	2	0	1
Average	2 days	—	P	17	—	35	35	1	2	100	162	5	16	0	1	0	0	12	5	0	2
9-77	1 wk.	—	—	32	35	225	—	9	0	—	—	13	4	2	4	0	—	94	0	1	0
9-84	1 wk.	—	—	—	—	225	80	—	—	825	585	—	—	—	—	—	—	13	3	—	—
9-88	1 wk.	—	—	125	22	400	150	10	4	∞	875	62	37	31	5	0	0	64	58	1	0
Average	1 wk.	—	—	78	28	283	115	9	2	1,000	730	37	20	16	4	0	0	57	20	1	0
9-85	2 wks.	—	—	275	237	600	—	32	31	∞	∞	416	600	87	172	0	0	100	96	1	0
9-90	2 wks.	∞	∞	18	20	100	—	1	1	∞	∞	2	1	—	—	0	—	—	—	7	4
9-78	2 wks.	∞	∞	275	50	—	—	22	13	∞	1,185	5	0	—	37	9	0	333	75	33	4
Average	2 wks.	∞	∞	189	102	350	—	18	15	∞	∞	141	200	87	104	3	0	216	85	14	3
9-79	4 wks.	∞	∞	420	250	640	11	2	2	700	258	1	16	85	37	13	22	277	88	15	13
9-86	4 wks.	∞	∞	1,183	1,500	27	0	1	1	366	—	1	7	630	500	0	16	33	18	12	0
R 9-92	4 wks.	∞	1,200	—	—	—	—	0	0	300	20	—	1	545	283	—	4	57	16	1	0
Average	4 wks.	∞	∞	801	875	333	5	1	1	455	139	1	8	420	273	6	14	122	40	9	4
9-87‡	2 mos.	13,750	10,000	555	—	1	0	0	—	2	0	0	—	816	1,066	21	—	213	18	0	—
10-12‡	67 days	66	—	16	45	0	0	0	0	1	0	0	6	0	0	0	0	0	0	0	0
15-01‡	60 days	50	18	2	1	12	0.5	6	3	22	—	20	6	68	38	41	3	5	1	1	0
Average	2 mos.	4,622	5,009	191	23	4	0	2	1.5	8	0	6	3	294	368	20	1.5	72	6	0.3	0

* D. = Dorset's medium.

† P. = Petroff's medium.

‡ Computed from dilutions 1:10 and 1:100 instead of 1:6 as in all remaining figures in this table.

Mathematical accuracy is not to be expected in a biological experiment of this nature, especially in dealing with the capricious growth proclivities of an organism like the tubercle bacillus. Furthermore, variations in the susceptibility of individual rabbits, in the pieces of tissue selected, and in the dispersion of the tubercle bacilli in the inoculum, caused variations in the results obtained. The method is nevertheless adequate for the problem on hand.

The arithmetical averages of the number of bacilli recovered from each organ of three separate rabbits are plotted in the accompanying curves (Charts I and III).

Original Deposition.—2 days after the intravenous injection of bacilli, the number of colonies recovered from like quantities of the various organs of the rabbit is similar to the relative distribution of India ink after intravenous injection (8).
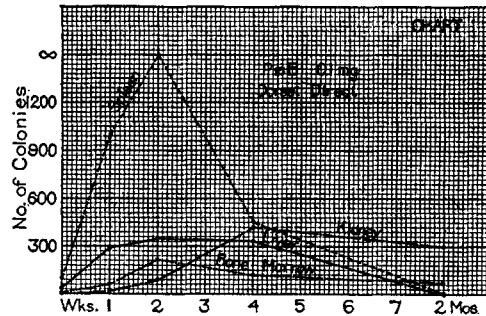

CHART I.

Thus from the spleen, an average of 100 colonies was recovered from 25 mg. of tissue on Dorset's medium, then 35 colonies from the liver, next 12 from the bone marrow, and none from the kidney. Essentially parallel results were obtained from Petroff's medium after direct seeding and from both media after sodium hydroxide treatment. Of course smaller numbers of colonies were recovered after treatment, but since we are not concerned with absolute but with relative quantities each of these data serves as a check for the other.

It is difficult to isolate tubercle bacilli in pure culture from the lung without sodium hydroxide, until pathological changes have taken place. For this reason it is not certain from the data in the table what is the relation of the lungs to the other organs as regards the deposition of tubercle bacilli. Apparently it is other than can be accounted for by the distribution of particulate matter.

1 week after the infection there is a rapid multiplication of the bacilli in the different organs, but not to the same degree in all the organs. The spleen shows

the most rapid multiplication; the liver much less.   An increased number of bacilli
is also discernible in the bone marrow and kidney.

This increase in all the organs continues unabated in the *2nd week* after injection.
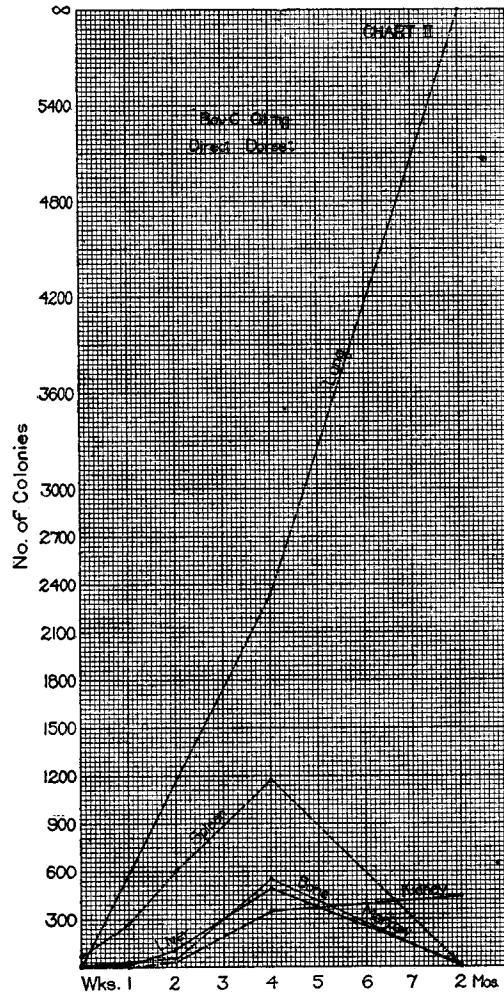

CHART II.

At this time, the relative numbers of colonies to be recovered from a given organ
bear only a superficial relationship to the original deposition in that organ (see
Table I).

4 weeks after infection, a striking change takes place. The picture differs greatly in the spleen, liver and bone marrow on the one hand, and in the lung and kidney on the other. In the first group of organs, especially in the spleen, there is not only a cessation of growth but a marked destruction of tubercle bacilli, as is indicated by the far fewer numbers of colonies recoverable from them after 4 weeks than after 2 weeks. There are still, however, large numbers of tubercle bacilli remaining in these organs. In the lung and kidney, on the contrary, the increase in the number of bacilli continues unabated.

2 months after infection the destruction is almost complete in the liver and spleen and comparatively few bacilli can be isolated from the bone marrow. But it is apparent that the rate of destruction between 4 and 8 weeks is much slower than
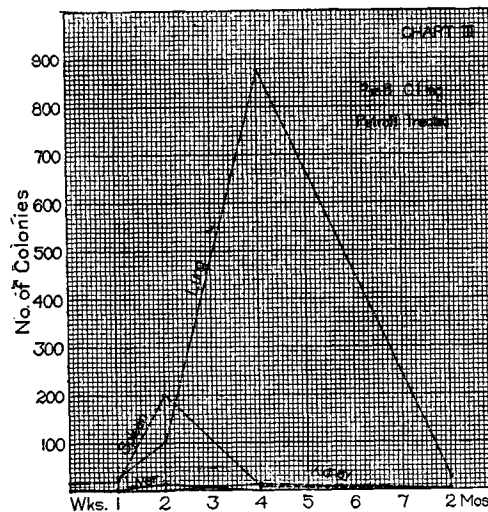

CHART III.

between 2 and 4 weeks. Destruction also becomes apparent in the lung, and cessation of growth, if not destruction, in the kidney.

The factor of individual resistance of the rabbit to tuberculous infection is especially noticeable in the 2 month interval. Compare the number of colonies isolated from the several organs of Rabbit 98–7 on the one hand and Rabbits 10–12 and 15–01 on the other. Such variations sometimes make the interpretation of data difficult.

It is seen then, that during the first 2 weeks after infection with 0.1 mg. of P-15 B the tubercle bacilli multiply in all the organs. Although their rate of increase differs in the different organs, there is as

TABLE II.

Number of Colonies Obtained from Organs of Rabbits Infected with 0.1 Mg. B. tuberculosis, Bovine.

Rabbit No.	Interval after injection	Lung Direct D.*	Lung Direct P.†	Lung Treated D.	Lung Treated P.	Liver Direct D.	Liver Direct P.	Liver Treated D.	Liver Treated P.	Spleen Direct D.	Spleen Direct P.	Spleen Treated D.	Spleen Treated P.	Kidney Direct D.	Kidney Direct P.	Kidney Treated D.	Kidney Treated P.	Bone marrow Direct D.	Bone marrow Direct P.	Bone marrow Treated D.	Bone marrow Treated P.
9-98	2 days	—	—	0	0	10	15	0	0	66	7	—	0	0	0	0	0	0	0	0	0
17-62‡	2 days	5	0	0	0.8	10	0	4	4	70	0	0	2	—	—	0.5	0	20	0	0	0.5
Average	2 days	5	0	0	0.4	10	7	2	2	68	3.5	0	1.0	0	0	0.2	0	10	0	0	0.2
9-94	1 wk.	—	0	0	0	—	0	0	0	57	4	0	1	0	0	0	0	18	2	1	0
9-99	1 wk.	—	0	0	0	20	0	1	0	261	0	0	0	1	0	0	0	78	0	1	0
10-00	6 days	—	—	0	0	3	1	0	0	501	5	0	0	1	1	0	0	1	0	0	0
Average	1 wk.	—	0	0	0	12	1	1	0	273	3	0	1	1	1	0	0	32	1	1	0
9-95	2 wks.	—	—	2	2	118	0	1	3	416	1	0	0	—	—	0	0	49	1	0	1
10-01	2 wks.	—	—	12	45	61	0	1	10	800	0	1	3	22	44	3	0	28	17	0	1
10-05	2 wks.	—	—	0	0	180	0	1	0	600	0	0	0	57	0	0	0	88	0	0	0
Average	2 wks.	—	—	5	16	119	0	1	4	605	1	1	1	39	22	1	0	55	6	1	1
99-6	4 wks.	795	0	46	0	27	0	23	12	225	0	3	0	4	0	0	0	167	1	—	0
10-02‡	4 wks.	1,200	1,200	16	105	34	2	0	0	918	100	3	4	241	0	0	0	103	100	1	7
10-06‡	4 wks.	5,000	12,200	330	383	1,396	216	65	33	2,375	0	33	166	798	0	0	0	1,385	276	40	120
Average	4 wks.	2,331	4,466	130	162	485	72	29	15	1,172	33	13	57	347	0	0	0	551	125	20	42
9-97‡	2 mos.	1,666	1,120	233	85	27	0	4	3	16	0	0	0	21	0	4	1	0	0	0	0
11-59‡	64 days	∞	∞	∞	75,000	6	0	0	0.5	2	0	3	0	883	510	595	945	0.5	0	0	0
Average	2 mos.	∞	∞	∞	∞	16	0	2	1.7	9	0	1.5	0	452	255	299	473	0.2	0	0	0

* D. = Dorset's medium.
† P. = Petroff's medium.
‡ Computed from figures for 1:10 and 1:100 dilutions; all other figures represent dilution 1·6.

yet no effective opposition to the growth of the virus. Furthermore, the subsequent arrest in multiplication followed by destruction of the bacilli takes place about simultaneously in the three physiologically and anatomically very different organs, the spleen, liver and bone marrow. The only function these organs have in common is to remove particulate matter or bacteria from the blood stream, a func-
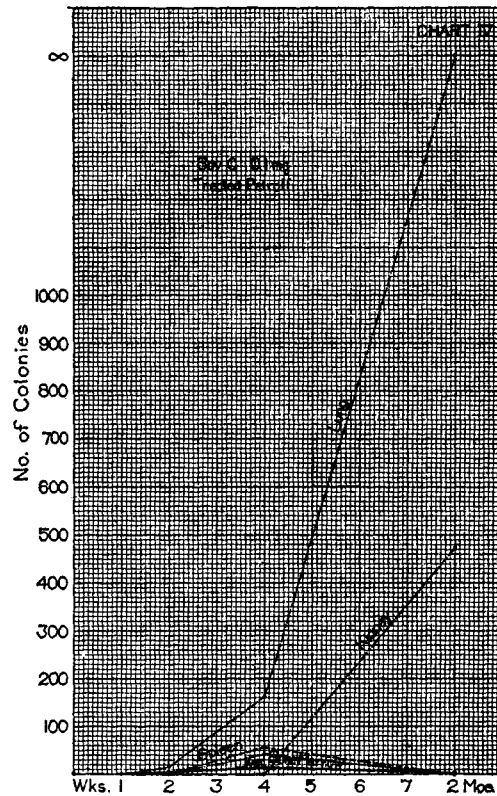

CHART IV.

tion shared to a much smaller degree by the lung and kidney, where destruction takes place later. Again this turn in the fate of tubercle bacilli does not depend upon the quantity of tubercle bacilli accumulated in a given organ. Thus in the spleen they reach uncountable numbers before destruction begins but only moderate numbers in the liver and bone marrow.

The majority of the animals recovered from the disease at the end of 2 months, though they were far from completely eliminating the bacilli.

The Fate of Bovine Bacilli in Large Dosage in the Various Organs of the Rabbit.

Table II and Charts II and IV show the fate of bovine tubercle bacilli in various organs of the rabbit at different intervals of time after the intravenous injection of 0.1 mg. of Bovine C, which is comparable in all respects to the fate of P-15 B.

One striking difference is apparent at once: 2 days after bovine infection far fewer colonies were isolated from like quantities of tissue. Yet the relative distribution in the various organs is again found to be similar to that of particulate matter; namely, spleen, liver, bone marrow and kidney. In Rabbit 17–62 the deposition in the lung was less than in the bone marrow.

It was found that the growth in vitro of this virulent bovine organism is much more subject to unfavorable influences than the human type P-15 B. Yet when these two strains are transplanted on glycerol agar in the usual way, they grow equally fast and luxuriantly. It is possible that this difference in behavior may be attributed not to different in vivo relations but simply to the original dysgonicity of the bovine bacillus, which comes into play when the bacilli are sufficiently dispersed. It may be for this reason that fewer colonies are isolated from tissues infected with Bovine C, and not because fewer viable bacilli are present. This problem is being investigated separately. However, as can be seen from the tables, very large numbers of colonies can be isolated even from Bovine C at times.

1 week after infection there is an increase in the number of colonies to be recovered from the various organs, but the relative rise is much less than with P-15B.

Without attaching too much significance to the actual numbers, it is permissible to draw attention here to the following relationships. 1 week after infection, 5 days after the first interval, the human strain, seeded directly on Dorset's medium, showed an increase of about eight times in the liver, ten times in the spleen, five times in the bone marrow, and sixteen times in the kidney, while the bovine strain similarly cultured showed hardly any increase in the liver and kidney, and an increase of three times in the bone marrow and four times in the spleen.

Now even if we admit that the bovine strain is dysgonic on reisolation from the tissues so that we start with a lower base line for this strain, still if its rate of growth in the body were the same as the human strain's, the relative increase would be the same in the various organs. Therefore we are forced to the conclusion that during the 1st week the bovine strain grows more slowly in the body of the rabbit than the human strain. With both strains the rate of multiplication of the bacilli in the spleen is much faster than in the liver.

2 weeks after infection the increase continues in all the organs, just as with the human strain.

The bovine bacilli reach their highest numbers in the liver, spleen and bone marrow *4 weeks* after infection, at a time when the human bacilli show a decided decrease.

A large percentage of the rabbits thus infected died before the close of the next interval. Out of seven rabbits, only two survived 2 months. The remaining five died on an average of 38 days after infection. Two died on the 24th and 25th day after infection, at a time when the multiplication of the bacilli in all of the organs was going on unchecked. Thus it is seen that, with large doses of bovine tubercle bacilli, death may take place before any effective opposition to the growth of the parasite in any of the organs is realized. Individual rabbits of greater resistance may survive long enough for the altered reaction of the tissues toward the virus to become apparent.

2 months after infection we find the disappearance, almost but not quite complete, of the tubercle bacilli in the liver, spleen and bone marrow, the organs that showed the earliest change in behavior toward the human infection. At the same time the animal is dying from pulmonary and renal infection. For in contradistinction to the fate of the human strain, the lung and kidney still show an unhindered multiplication, especially the lung, where the tissue becomes veritably a pure culture of tubercle bacilli. Thus even the resistant animal succumbs.

The chief difference between the human and bovine types, therefore, is the slower multiplication of the bovine bacilli and the later appearance of their destruction. Although the difference is quantitative rather than qualitative it is sufficient to cause death after bovine infection, because of the continued multiplication in the lung and kidney, and regression of the lesion and survival after human infection.

The Fate of Human Bacilli in Small Dosage in the Various Organs of the Rabbit.

The human tubercle bacillus, then, grows more rapidly in the body of the rabbit than the bovine, and this more rapid growth is followed

TABLE III.

Number of Colonies Obtained from Organs of Rabbits Infected with 0.001 Mg. B. tuberculosis, Human, per Kilo.

Rabbit No.	Interval after injection	Lung Direct D.*	Lung Direct P.†	Lung Treated D.	Lung Treated P.	Liver Direct D.	Liver Direct P.	Liver Treated D.	Liver Treated P.	Spleen Direct D.	Spleen Direct P.	Spleen Treated D.	Spleen Treated P.	Kidney Direct D.	Kidney Direct P.	Kidney Treated D.	Kidney Treated P.	Bone marrow Direct D.	Bone marrow Direct P.	Bone marrow Treated D.	Bone marrow Treated P.
11-40	30 min.	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
11-61	30 min.	30	0	5	5	3	1	1	0	6	3	1	1	0	0	0.15	0	0	0	0	0
11-71	3 hrs.	3	2	0	1	0	0	0	1	1	0	3	2	0	0	0	0	0	0	0	0
11-62	1 day	—	—	1	1	2	0	1	2	4	1	1	1	0.15	0	0	0	0	0	0	0
12-25	1 day	9	11	5	2	1	0	2	3	9	10	7	10	0	0	0	0	0.15	0.3	0.3	0.15
11-46	2 days	—	3	0	0.5	1	0	0	0	3	0	0	0	0	0.15	0	0	0.15	0	0	0
12-28	2 days	15	9	3	6	—	0	2	5	8	3	3	3	0	0	0	0	0	0	0	0
Average	Short intervals	11.4	4.1	2.0	2.2	1.5	0.1	0.8	1.5	4.5	2.4	2.1	2.4	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
11-42	1 wk.	3	3	0	0	—	0	0.3	0	8	0	0	0	0	0	0	0	2	0	0	0
11-47	1 wk.	6	6	3	0	3	0	0.3	0.3	6	6	0.6	1.6	0	0	0	0	0	0	0	0
12-26	1 wk.	43	105	9.5	3	3	0.3	0	0	29	10	4.3	2.6	0	0	0	0	8	1.3	0.3	0.6
Average	1 wk.	17	38	4.1	1	3	0.1	0.2	0.1	14.3	5.3	1.6	1.4	0	0	0	0	3.3	0.4	0.1	0.2
11-48	2 wks.	63	50	33	15	12	0	2.6	1.3	211	30	11	18	0	0	0	0	0	0.3	0	0
11-74	2 wks.	—	—	46	47	30	34	14.8	8.3	844	390	150	143	1.3	1.8	0.3	1	17	16	1.3	4.5
10-86	2 wks.	870	330	78	129	88	86	9	16	1,850	1,630	244	180	0.4	0.6	0.1	0.3	25	32	14	11
Average	2 wks.	466	190	52	63	43	40	8.8	8.5	968	683	135	113	0.4	0.6	0.1	0.4	14	16.1	5.1	5.1
11-73	4 wks.	10,660	8,000	5,400	4,530	139	120	22	36	6,330	4,160	870	1,236	1.6	0	0.3	1.0	220	270	43	38
11-75	4 wks.	415	416	236	202	1.7	0	1	2	498	280	158	142	0	0	0	0	13	11	8	13
11-51	4 wks.	—	—	2,560	3,100	3	0	2	0	204	163	44	16	5	6.6	9	2	9	5.5	2.5	1.6
Average	4 wks.	5,537	4,208	2,732	2,610	47	40	8	12	2,344	1,534	357	464	2	2	3	1	80	95	17	17

11-57	2 mos.	151	94	98	121	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
9-41	2 mos.	4,600	6,400	3,250	2,700	—	0	0	0	15	0	0	1.6	210	50	81	145	0	0	0
12-27	2 mos.	2,230	4,750	2,500	4,430	0	0	0	0.3	190	1.5	21	35	246	196	180	280	0	0	0
Average	2 mos.	2,327	3,748	1,949	2,417	0	0	0	0.1	69	0.5	7	12	152	82	87	141	0	0	0
13-09	4 mos.	—	—	—	0.5	—	—	0	0	—	—	0	0	—	0	0	0	0	0	0
13-19	4 mos.	—	785	690	390	—	—	—	0	0	0	—	—	—	—	0	0	0	0	0
14-48	4 mos.	—	20	17	0	0	—	0	0	—	—	0.3	2	—	0	0	0	—	0	0
Average	4 mos.	—	402	353	130	0	—	0	0	0	0	0.15	1	—	0	0	0	0	0	0
14-56	6 mos.	—	200	28	21	0	0	0	0	52	33	33	9	0	0	0	0	0	0	0
14-52	6 mos.	50	53	—	29	0	0	0	0	36	2	37	45	—	0	0	0	0	0	0
14-47	6 mos.	10	—	3	—	—	—	0	—	0	—	0	—	0	—	0	—	0	0	—
Average	6 mos.	30	126	15	25	0	0	0	0	29	17	23	27	0	0	0	0	0	0	0

* D. = Dorset's medium.
† P. = Petroff's medium.

by a more rapid destruction. The question arose whether the phase of destruction would be deferred if the accumulation of human tubercle bacilli were retarded by injecting smaller doses.

A series of rabbits was inoculated intravenously with 0.001 mg. per kilo of a human strain, P-48 A. This dose was chosen because it was thought that human
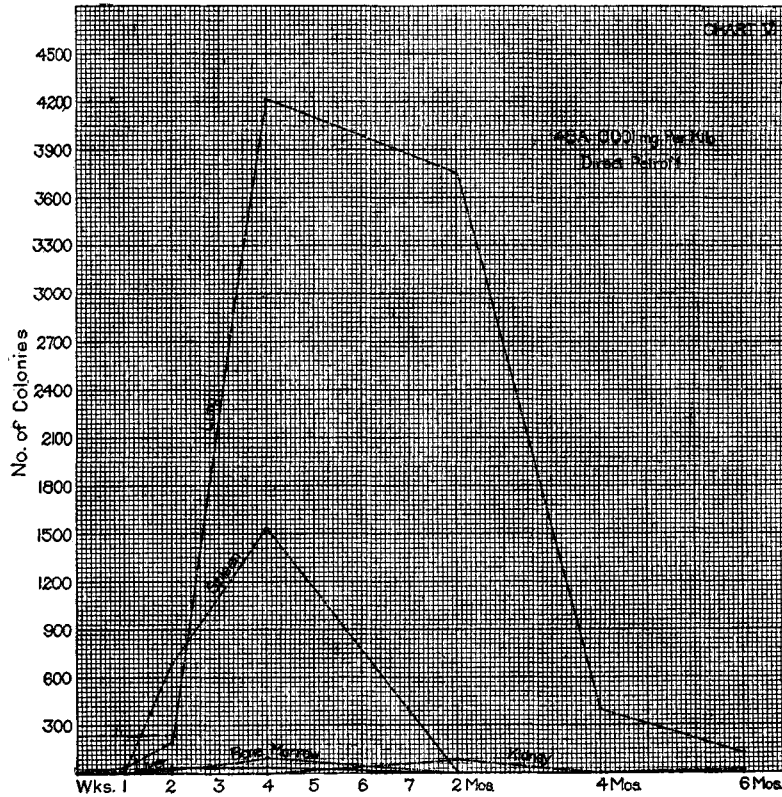
CHART V.

bacilli in a smaller quantity might be destroyed by virtue of the native reaction, and this strain, to see whether the relations would hold for a different strain.

The animals were killed at the following intervals: 30 minutes, 3 hours, 1 and 2 days, 1, 2 and 4 weeks, 2, 4 and 6 months. With the exception of the short periods at least three rabbits were used for each interval. The organs were cultured as above described. The detailed results are given in Table III and the averages are plotted in the curves of Chart V.

Original Distribution.—During the first *2 days* the greatest number of colonies was recovered from the lung, next from the spleen, next from the liver, and as a rule none from the bone marrow and kidney. No significant difference in distribution was noted if the rabbits were killed 30 minutes, 3 hours, 1 day or 2 days after infection. It is noteworthy that again, as apparently with the large doses of human bacilli, more colonies are recovered from the lung than from the spleen and liver, a distribution consistently at variance with the distribution of particulate matter. The relative distribution to the other organs follows that of manganese dioxide and carbon, namely, spleen, liver, bone marrow and kidney. Since even 30 minutes after injection the bacilli were recovered in greater numbers from the lung than from the other organs, the phenomenon cannot be due to a rapid and immediate multiplication in the lung and a slower initial multiplication or more rapid destruction in the other organs, but must be due to a greater deposition in the lung. No explanation can well be offered with the data at hand.

1 week after infection there is scarcely any rise in the number of bacilli recoverable from the various organs. This is in contrast to the sharp rise in the 1st week after the injection of 0.1 mg. P-15 B, and shows the same latent period noted after the injection of 0.1 mg. Bovine C.

2 weeks after infection there is a marked rise in the number of bacilli recoverable from the spleen and lungs. In the liver and bone marrow the increase is much less, and it is scarcely perceptible in the kidney. Here the striking difference in the behavior of tubercle bacilli in the several organs comes to the fore. The slight rise in the bone marrow and in the kidney can easily be attributed to the very slight original localization of bacilli in these organs. But the increase in the liver cannot, for here the original deposition was only 3 to 4 times less than in the spleen, and by the end of the 2nd week an average of 968 colonies was recovered from the spleens of three animals on Dorset's medium as against an average of only 43 colonies from their livers.

4 weeks. At this interval not only is there no destruction of the tubercle bacilli, such as was found in the spleen, bone marrow and liver 4 weeks after the introduction of 0.1 mg. P-15 B, but the bacilli are increasing in all the organs. However, the rate of increase in the interval from the 2nd to the 4th week is much slower than in the interval from the 1st to the 2nd week. It is noteworthy that the peak is reached in the spleen with over 2000 bacilli, but in the liver with only 47, showing again that the liver of the rabbit is far more unfavorable soil for tubercle bacilli than the spleen. Tremendous numbers of tubercle bacilli are recovered from the lung.

2 months after infection there is complete destruction of the bacilli in the liver and bone marrow, a marked reduction in the number recoverable from the spleen, and a considerable reduction in the lung. The drop in these organs appears to be simultaneous but probably only because of the length of time between intervals. It was unfortunately not forseen that observations 6 weeks after infection would have been desirable. In the kidney, the bacilli are still on the increase. It is

noteworthy that with the much smaller quantity of human bacilli, 0.001 mg., the destruction is not so complete at 2 months in the spleen as with the much larger dose, 0.1 mg. With the latter, an average of only eight colonies was recovered from the spleens of three rabbits at this time, but with the small dose an average of 69 colonies was recovered from somewhat smaller quantities, 16 mg., of the spleen on Dorset's medium. On the other hand the destruction is more complete in the bone marrow than with the larger dose.

Thus the chief differences appear in the behavior of a small quantity of human tubercle bacilli as compared with a large quantity of an organism of the same type though not the same strain. (1) There is a latent period of a week with the small dose; with the large dose the increase is immediate. (2) The destruction of tubercle bacilli begins later with the small dose than with the large dose. (3) At a given time after infection, more complete destruction is apparent after the large dose than after the small dose, at least in the spleen. Some organs, such as the liver and bone marrow, will show only slight numbers of bacilli even when their numbers are at their height after the small dose; after the larger dose the same organs will show considerable numbers.

Since none of the animals infected with the small dose of bacilli died from their tuberculous affection it seemed advisable to follow the fate of the bacilli in the various organs to their extinction, if possible.

4 months after infection the bacilli had been greatly reduced in the lung; they could no longer be isolated from the liver, kidney and bone marrow. Only a single stray colony could be recovered occasionally from the spleen, in which again, even 4 months after infection, the destruction of bacilli is not quite complete. Unfortunately these organs were cultured during the summer heat and many tubes appeared to be incompletely sterilized, although the methods used had heretofore been totally satisfactory in securing sterile media. However, sufficient data were obtained to assure us of the general trend of events.

6 months after infection the number of bacilli recoverable from the lungs is still further reduced. It is noteworthy that in the interval between the 2nd and 4th months the rate of destruction of the tubercle bacilli in the lung is very much faster than during the 2 months that follow. Thus on Petroff's medium after direct seeding the decrease in the 2nd to the 4th month was about 9 times, during the next 2 months a little more than 4 times. From the liver, kidney and bone marrow no tubercle bacilli were isolated 6 months after infection, just as none were recovered from these organs 4 months after infection. However, in the spleen a second slight increase became apparent.

Thus whatever force is responsible for the change bringing about the destruction of the tubercle bacillus weakens as time progresses. As a result the destruction is rapid at first, and becomes slower with time, and as early as 6 months after infection with 0.001 mg. P-48 A per kilo the bacilli have again begun to increase in the spleen while they are still decreasing in the lung. Possibly an adaptation of the parasite to the host has been effected.

The Fate of Bovine Tubercle Bacilli in Small Dosage in the Various Organs of the Rabbit.

A series of animals was infected intravenously with 0.001 mg. Bovine C per kilo. The detailed findings are presented in Table IV, and the averages are plotted in the accompanying curves, Chart VI.

With the smaller as with the larger dosages, fewer tubercle bacilli were isolated from the organs of rabbits infected with the bovine, than from those infected with the human strain. In harmony with this, and also with the relative distribution of particulate matter, the tubes seeded with suspensions of bone marrow and kidney 1 and 2 days after bovine infection with small doses, remained sterile in each case, and only occasionally and irregularly was a stray colony isolated from the lung and liver.

1 week after infection a slight rise in the number of colonies recovered was perceptible in the lung and spleen, while the liver, kidney and bone marrow gave practically all negative results in each of the three rabbits. This essentially parallels the latent period observed with a similar dose of a human strain.

2 weeks after infection there is a considerable rise in the number of colonies recoverable from the various organs. But the rise is much slower than with human bacilli in the same dosage. Thus between the 1st and 2nd weeks after infection with the human bacilli in small doses, the increase in the number of colonies recovered from the spleen was 69 times on Dorset's medium and 136 on Petroff's medium after direct seeding, but with the bovine, it was 8 times on Dorset's medium and 31 times on Petroff's. Like relations obtained in the other organs. It is noteworthy that very few bacilli were recovered from the liver of rabbits infected with small doses of either human or bovine strain; with the latter the number was negligible. On the other hand, the bacilli attain large numbers in the spleen of even bovine-infected animals and reach very large numbers in the spleens of rabbits infected with human bacilli. This discrepancy is altogether out of proportion to the relative original deposition in these two organs and seems to justify the conclusion that even in the "native" state the liver of the rabbit can check the growth of the bacilli much more completely than the spleen. At the end of the 2nd week some multiplication is also apparent in the lung and bone marrow. The kidney, however, still fails to show any bacilli.

TABLE IV.

Number of Colonies Obtained from Organs of Rabbits Infected with 0.001 Mg. B. tuberculosis, Bovine, per Kilo.

Rabbit No.	Interval after injection	Lung Direct D.*	Lung Direct P.†	Lung Treated D.	Lung Treated P.	Liver Direct D.	Liver Direct P.	Liver Treated D.	Liver Treated P.	Spleen Direct D.	Spleen Direct P.	Spleen Treated D.	Spleen Treated P.	Kidney Direct D.	Kidney Direct P.	Kidney Treated D.	Kidney Treated P.	Bone marrow Direct D.	Bone marrow Direct P.	Bone marrow Treated D.	Bone marrow Treated P.
11-83	1 day	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13-15	1 day	—	—	0	0	0	0	0	0	1.6	0	0	0	0	0	0	0	0	0	0	0
11-86	1 day	—	—	0.3	0.3	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0
11-85	2 days	—	—	0	0	0	0	0	0	0	0	0.3	0	—		—	0	0	0	0	0
13-35	2 days	—	—	0	0	0	0	0	0	1	0	0	0	—		0	0	0	0	0	0
12-30	2 days	1	—	0	0	0.3	0	0	0	7.5	0	0	0	—		0	0	0	0	0	0
Average	1 and 2 days	—	—	0	0.1	0.3	0	0	0	1.7	0	0.0	0	0	0	0	0	0	0	0	0
12-34	1 wk. (8 days)	—	—	0	0	0	0	0	0	1.3	0	0	0	0	0	0	0	0	0	0	0
13-29	1 wk.	—	0	0	0.3	0	0	0	0	4.3	0	2.3	0	0	0	0	0	0	0	0	0
12-32	1 wk.	47	0	7.6	20	0	0	0	0	20	0			—		—	—	1.6	0	0	0
Average	1 wk.	47	0	2.5	6.7	0	0	0	0	8.5	0	0.7	0	0	0	0	0	0.5	0	0	0
12-17	2 wks.	—	22	1	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
13-30	2 wks.	—	20	3	1.8	0	2	0	2.3	5.6	0	0	0	0	0	0	0	0	0	0	0
12-33	2 wks.	39	40	10	24	30	0	0	0	185	93	22	33	—	0	0	0	9.5	10	1.8	1.3
Average	2 wks.	39	27	4.6	9.2	10	0	0.6	0.7	64	31	7.3	11	0	0	0	0	3.1	3.3	0.6	0.4
11-89	4 wks.	60	85	8.5	2.5	0	0	0	0	30	16	35	0	0	0	0	0	0.5	0	0	0
13-36	4 wks.	30	—	13	29	0	0	0	0	705	625	40	107	44	0	9	6.5	21	6	0	7
12-36	4 wks.	257	750	52	91	3	0	0	3	750	916	305	385	105	0	8	0	5	0	2	3
Average	4 wks.	115	417	24	40	1	0	0	1	495	519	126	164	49	0	5.6	2.1	8.8	2	0.6	3.3

12-38	2 mos.	800	600	583	500	—	0	0	0	0	330	90	325	195‡	—	6	340	177	0	0	0
12-31	2 mos.	—	290	23	28	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12-37	2 mos.	70	—	4	13	0	0	0	0	0.6	1.5	—	0	0	—	90	3	3	—	—	—
15-26	2 mos.	1,155	4,465	197	770	0	0	0	0	0.1	110	45	81	49	105	32	85	45	0	0	1.50
Average	2 mos.	675	1,785	202	328	0	0	0	0.5						52				0	0	0.30
15-04	4 mos.	—	650	220	117	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
15-08	133 days‡	5,250	6,000	4,400	2,800	0	0	1	0	0	4	1	1	0	2,160	1,000‡	605	965	0	0	0
Average	4 mos.	5,250	3,325	2,310	1,458	0	0.5	0.5	0	0.5	2.5	0.5	0	0	1,080	500	302	482	0	0	0

* D. = Dorset's medium.
† P. = Petroff's medium.
‡ Probable figure.

4 weeks after infection, the bacilli have reached their highest numbers in the spleen and bone marrow. The growth in the lung continues unabated. For the first time since infection the bacilli appear in considerable numbers in the kidney. They were isolated from the liver of only one rabbit. In general the fate of the bacilli of the bovine and human strains is similar at this time, with, however, the following exceptions. With the human strain the number of bacilli in the lungs has gone far beyond those of the spleen. With the bovine strain the bacilli in
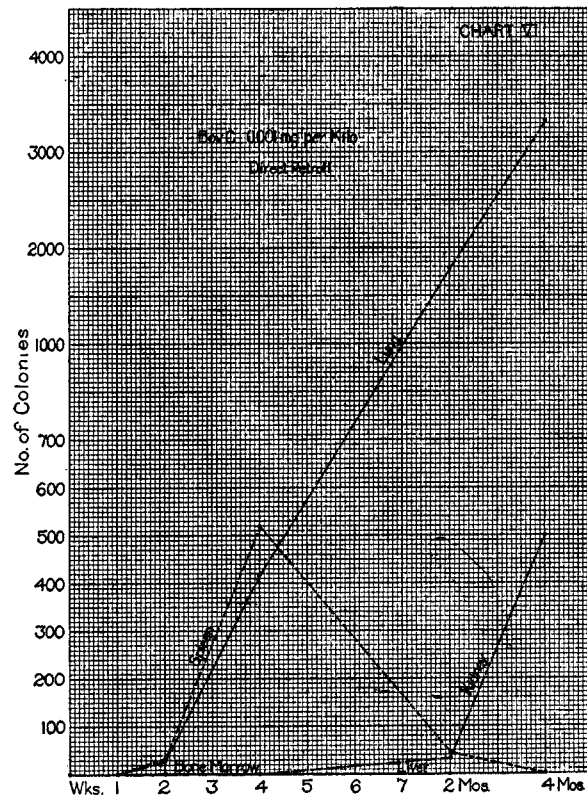
CHART VI.

the lungs still lag considerably behind those in the spleen, pointing again to the relatively slower multiplication of the bovine bacilli. Furthermore, the bacilli of the human strain reach higher numbers in the liver and bone marrow than do the bovine, so that the latter may be negligible while the former, even in the liver, attain considerable numbers. On the other hand, in the kidney the bovine bacilli have reached considerable numbers at this time, at least on Dorset's medium, whereas the human bacilli still are negligible.

2 months after infection the difference between the behavior of the human and bovine strains in this dosage is very striking.   In the human strain the lungs have not only checked the further growth of the bacilli but they show considerable reduction in their numbers.   The bovine strain, on the other hand, still continues to grow and multiply without any effective opposition.   In both cases the spleen has effectively and markedly reduced the number of viable bacilli recoverable from similar quantities of tissue; however, the destruction is much more complete in the spleen of the rabbit infected with the human strain than with the bovine strain.   With both types the bacilli have almost completely disappeared from the bone marrow and liver, in which the destruction is much more complete than in the spleen although these three organs generally parallel each other in behavior. In the kidney, both types of bacilli continue to increase.

4 months after infection the difference in the fate of the two types in small doses is most pronounced in two organs, the lung and kidney.   While the bacilli of the P-48 A strain continue to be destroyed in the lung in this period the bovine bacilli are still increasing in numbers.   Thus the drop in the lung begins in the 2nd month with the human infection and continues from then on, but the bovine bacilli continue to increase in number even 4 months after infection.   Again the kidney after human infection shows complete disappearance of the bacilli by the 4th month, while bovine bacilli continue to multiply in this organ unabated.   The destruction of bovine bacilli is also not quite so complete in the spleen as the destruction of human bacilli in the same interval.   However, in both instances, the destruction is quite complete in the liver and bone marrow.   The majority of the rabbits developed an extensive tuberculosis at the time of killing from which they would probably have died.

Thus we see, with the small doses of bovine as with the large doses, that one group of organs more or less completely destroys the bacilli— the liver, bone marrow and spleen—and the second group—the lung and kidney—is unable to check their growth.   As between the bovine and human strains in small doses, we see the same difference that appeared between the human and bovine strains in large doses: slower growth and slower destruction of the bovine bacilli.

DISCUSSION AND SUMMARY.

In this study the attempt has been made to follow the fate of tubercle bacilli in the lung, liver, spleen, kidney and bone marrow of rabbits infected intravenously with large and small doses of human and bovine tubercle bacilli by determining the number of colonies recoverable from similar quantities of tissue on egg media at varying intervals during the course of infection.   This method offers certain possibili-

ties for the elucidation of this problem precluded by the modes of attack used hitherto. Histological methods, while giving precise data in regard to tissue changes produced by the tubercle bacilli, are poor instruments for determining the fate of the bacilli in a given organ. Without stressing the notorious difficulties in staining the organism at all times, histological technique can give no definite answer to the question whether certain stained bacilli are living or dead, and it is the number of living bacilli that is of importance. Again animal inoculation, while an excellent index of the presence of living virulent bacilli, is a very inaccurate index of the number of living bacilli in a given specimen of tissue, for it is possible to infect guinea pigs with even a very few bacilli.

Lewis and Sanderson (9) have studied the histological changes in the rabbit's lung at varying intervals of time after the intravenous injection of similar quantities of human and bovine tubercle bacilli. They found that up to the 8th day tubercle bacilli are not abundant in either series. "After the 8th day bacilli become very difficult to find in the human series. In the bovine series they become increasingly numerous and very rapidly so." They conclude that "the natural resistance of the rabbit to infection with the tubercle bacillus of human type is apparently referable to a failure of this type of bacillus to multiply in the body of this species to any considerable extent. Exceptionally there are localized lesions (kidneys and lung nodules) which are associated with abundant growth and which show that this failure to multiply may be due to some positive growth restraining factor rather than to a failure of suitable nutritive materials." These authors, then, feel that the human tubercle bacillus does not grow very much in the body of the rabbit due to some undefined native restraining force.

Austin (10) approached this problem by treating rabbits inoculated intravenously with bovine and human bacilli with saline extracts of rabbit lungs. He found that fewer pulmonary lesions develop as a result of this treatment, especially in the bovine-infected group. He concludes that the "difference in the resistance of the rabbit lung to the two types of infection may be largely due to a quantitative difference in the defensive factors that are needed."

Rogers (11) studied the fate of human and avian bacilli in the liver of pigeons by histological methods after intravenous injection. He concluded that the bacilli are rapidly and extensively phagocytized by the endothelial cells lining the venous sinusoids of the liver and that the greater portion of them are rapidly destroyed by these cells in a time commensurate with the destruction of pneumococci. He found no evidence of mutiplication.

In summarizing the results obtained by cultural methods, it appears that the original localization of human or bovine tubercle bacilli in

the various organs of the rabbit follows the distribution of particulate matter (12), being greatest in the spleen, next in the liver, next in the bone marrow, and least in the kidney. The relative position of the lung among these organs is still doubtful owing to the difficulty of isolating tubercle bacilli in pure culture without treatment at the beginning of the infection. But in some work, to be described in a later communication, with 1 to 2 mg. of BCG given intravenously the deposition in the various organs of rabbits was found to be as follows: spleen 610, liver 322, lung 202, bone marrow 72 and kidney 6. These figures represent the number of colonies recovered from similar quantities of tissue after direct seeding on Dorset's medium 1 day after intravenous inoculation. They are in agreement with Bull's finding with typhoid bacilli (13).

The fate of the bacilli thus localized depends upon many factors: the dosage, whether large or small, the type, whether human or bovine, and doubtless many conditions not touched on here. Their original localization, although a factor, is far from being the controlling factor.

At first the tubercle bacilli grow and multiply in all the organs. The human type grows very fast, the bovine type much more slowly. Moreover, the rate of multiplication of both types is very different in the various organs. Thus in the spleen it is much more rapid than in the liver; it may even be negligible in the liver while very rapid in the spleen with small enough dosage, especially of bovine bacilli. But as yet in all the organs no effective opposition is offered to the multiplication of the tubercle bacillus.

Soon, however, a remarkable change takes place. The liver, spleen and bone marrow begin to destroy the tubercle bacilli. The time at which the destruction begins in these three organs depends on the dose and the type of bacillus. With large doses of human bacilli it becomes apparent between the 2nd and 4th weeks, when the bovine bacilli, in similar quantities, have reached their highest numbers. Their destruction does not become apparent until the interval between the 4th and the 8th weeks. If small quantities of human bacilli are injected, their destruction in these three organs is also delayed to the interval between the 4th and the 8th weeks.

By the 8th week after infection with a large dose of human bacilli the destruction is more complete than after a small dose. By the 8th

week after infection with a small quantity of bovine bacilli the destruction is much less complete in the spleen than after a small quantity of human bacilli. This change in the behavior of these three organs does not depend upon the actual number of bacilli accumulated in a given organ. All these facts speak clearly for the contention that the native power of the various organs to destroy tubercle bacilli is insufficient to check their growth effectually and, as a result of a stimulation most probably associated with the growth of the bacilli, an altered behavior toward the tubercle bacilli is brought about so that they are destroyed. However, while this change is sufficient in the liver and bone marrow to destroy the bacilli completely, it is insufficient to eliminate them completely from the spleen. This especially applies to small dosage.

Furthermore, the behavior of the tubercle bacilli in the lung and kidney is quite different from their behavior in the organs just mentioned. Here with large doses of human bacilli the destruction becomes apparent in the interval between the 4th and the 8th weeks instead of the interval of the 2nd to the 4th weeks as in the liver, spleen and bone marrow. With bovine bacilli in similar dosage the bacilli in the lung and kidney continue to increase in number until the death of the animal. With small doses of human bacilli the lung shows a beginning of destruction by the 8th week, when there is almost complete destruction in the liver, spleen and bone marrow. By the 4th month they have completely disappeared from the kidney and they are still decreasing in the lung. With the same dosage of bovine bacilli the organisms continue to multiply in the lungs and kidney even 4 months after infection. It is noteworthy that throughout the study it was found that after the phase of destruction has been brought about, the rate of destruction in the several organs is at first very rapid, but soon the force spends itself so that as time progresses the bacilli are more and more slowly destroyed, and a few lingering bacilli remain alive. In fact, in the spleen, 6 months after infection, a slight secondary rise was noticed. It is to be emphasized that the group of organs where tubercle bacilli are first destroyed is the liver, spleen and bone marrow, whose common function is to remove foreign particulate matter or bacteria from the body; and again, that of these three, the spleen lags behind the bone marrow and liver in its destruction of both bovine and human bacilli.

In the study cited above (1) on the cellular reaction of the organs of the rabbit toward tubercle bacilli it was found that the normal, non-immune rabbit destroyed heat-killed tubercle bacilli most effectively in the liver, less effectively in the bone marrow, much less in the spleen, and least of all in the lungs.

A correlation is thus seen between the native reaction of the several organs toward dead bacilli and the rate of destruction of living human and bovine bacilli in the same organs. The liver and bone marrow, which destroyed bacilli more effectively than the spleen in the "native" state, also destroy them more completely in the "immune" state, and these organs much more rapidly than the lung and kidney. Further-more, the native ability of the liver and bone marrow to destroy tubercle bacilli is almost sufficient to inhibit their growth altogether in rabbits infected with small doses of both the human and bovine types, especially the liver after infection with the bovine strain. Again throughout this study it was seen that tubercle bacilli, whether human or bovine, whether in large or small doses, always grow faster in the spleen than in the liver. This is perhaps to be connected with the liver's having a greater native ability than the spleen to destroy tubercle bacilli.

These facts offer some confirmation for the hypothesis (14) that "acquired resistance is only a specific increment of natural resist-ance." The natural resistance in itself is always insufficient to check the growth of the bacilli in all the organs.

The acquired ability of the organs to destroy tubercle bacilli be-comes increased as a result of the growth of the bacilli and the accumu-lations of their products in the body. The acquired ability is then added to the natural ability. In this light we can understand, to some extent at least, the fate of the bacilli in the various organs. The work of Lewis and his collaborators (15, 16) on the different hereditary powers of resistance of guinea pigs to tuberculous infection and the correlation that they have drawn between antibody production and this resistance would point in the same direction.

Römer (17) has shown that the allergic state in tuberculosis as repre-sented by the tuberculin reaction can be brought about more quickly with larger doses of tubercle bacilli than with smaller doses. This finding has recently been confirmed by Lewis and Aronson (18).

Since it was found that the human tubercle bacilli grow faster than the bovine and that their destruction in the various organs is brought about earlier, furthermore, since with smaller doses of human bacilli the altered body reaction is brought about later than with larger doses, again, since the destruction is more complete after the same time in certain organs with large doses of both types of bacilli than with small doses, the conclusion is forced upon us that perhaps the virulence of a given type of tubercle bacillus for the rabbit is closely related to the original growth of the bacillus. This is an inverse relation. The more rapid the growth, the less virulent. For the more rapidly a given organism grows in the body the more rapidly is a resistance stimulated and the more rapidly are the bacilli destroyed. On the other hand, the more slowly an organism grows the later is this brought about. Thus in the bovine infection, while the bacilli are effectively destroyed in the liver, spleen and bone marrow, the unabated growth of bacilli in the lungs and kidney brings about the death of the animal by the replacement of tissue consequent upon the unchecked tubercle formation. Doan and Sabin (19) have recently pointed out that regression of the tuberculous process takes place in the bone marrow and, to a lesser degree, in the spleen while the disease progresses in the lung and kidney even in bovine-infected rabbits.

However, this immunity is not complete; even as late as 6 months after the infection the bacilli have not entirely disappeared from the lungs of rabbits infected with 0.001 mg. of human bacilli per kilo, and apparently they are still persisting in the spleen if not increasing.

The results obtained in the study, not yet published, of the fate of BCG in the various organs of the rabbit, are altogether in harmony with the conception that the virulence of mammalian tubercle bacilli for the rabbit is inversely related to their multiplication in the body. With these bacilli the process of quick growth followed quickly by destruction is even more rapid than with the human type.

It is pertinent to note that animals that are susceptible to the mammalian bacillus are usually more susceptible to the bovine type than to the human type as determined by experimental inoculation. Thus (20) the ox, sheep, goat, cat, pig and rabbit are much more susceptible to the bovine infection than to the human. Even the guinea pig and monkey are somewhat more susceptible to the bovine type than to

the human. The dog is apparently moderately susceptible to both, although some maintain that the dog also is more susceptible to the bovine type (21). The only species for which, it is thought, the human type is more virulent is man. But even this conclusion is doubtful (Neufeld (22)). For man is infected by the bovine bacillus largely by the intestinal route, for which much larger dosages are necessary than for the respiratory route, by which tuberculosis is far more commonly spread among human beings. It is possible, therefore, that the mechanism of virulence may be the same in all species.

CONCLUSIONS.

1. The original distribution of tubercle bacilli of both human and bovine types to the various organs of the rabbit after intravenous inoculation follows the distribution of particulate matter in the following order per gm. of tissue; spleen, liver, lung, bone marrow and kidney. The relative position of the lung amongst these organs is less certain than that of the others.

2. At first the tubercle bacilli both of the human and bovine types grow in all the organs without any effective opposition.

3. The rate of growth of both types differs in the various organs. It is much faster in the spleen than in the liver. With small doses very little growth takes place in the liver and bone marrow, especially with the bovine type.

4. The human type of tubercle bacillus grows faster in the several organs of the rabbit than the bovine type.

5. This more rapid growth of the human type is followed by an earlier and more complete destruction of the human type than of the bovine.

6. With both types destruction occurs first in the liver, spleen and bone marrow. In the lung and kidney destruction of the human type takes place later, and unchecked multiplication of the bovine type continues in these organs until the death of the animal.

7. With smaller doses of human bacilli destruction is brought about later in the liver, spleen and bone marrow than with larger doses. With both types, in a given time, destruction is more complete in some organs after a large dose than after a small dose.

8. The destruction in the various organs is rapid at first and pro-

gresses more slowly as time passes. So that even 6 months after intravenous injection of small doses of human tubercle bacilli they have not yet completely disappeared from the lung and spleen.

BIBLIOGRAPHY.

1. Corper, H. J., and Lurie, M. B., *Am. Rev. Tuberc.*, 1927, xi, 237.
2. Lange, B., *Z. Tuberk.*, 1926, xlvi, 455.
3. Boissevain, C. H., *Am. Rev. Tuberc.*, 1926, xiii, 96.
4. Madsen, Th., and Mørch, *Acta Tuberc. Scand.*, 1926, ii, 99.
5. Jennings, F. B., *J. Infect. Dis.*, 1926, xxxix, 310.
6. Opie, E. L., and Aronson, J. D., *Arch. Path. and Lab. Med.*, 1927, iv, 1.
7. Mudd, S., and Furth, J., *J. Immunol.*, 1927, xiii, 369.
8. Corper, H. J., and Lurie, M. B., *Am. Rev. Tuberc.*, 1926, xiv, 680.
9. Lewis, P. A., and Sanderson, E. S., *J. Exp. Med.*, 1927, xlv, 291.
10. Austin, R., *Arch. Path. and Lab. Med.*, 1927, iv, 687.
11. Rogers, J. C., *Am. Rev. Tuberc.*, 1928, xvii, 192.
12. Drinker, C. K., and Shaw, L. A., *J. Exp. Med.*, 1921, xxxiii, 77. Lund, C. C., Shaw, L. A., and Drinker, C. K., *J. Exp. Med.*, 1921, xxxiii, 231.
13. Bull, C. G., *J. Exp. Med.*, 1915, xxii, 475.
14. Smith, T., *Am. Rev. Tuberc.*, 1926, xiv, 485.
15. Wright, S., and Lewis, P. A., *Am. Naturalist*, 1921, lv, 20.
16. Lewis, P. A., and Loomis, D., *J. Exp. Med.*, 1928, xlvii, 437.
17. Römer, P., *Beitr. Klin. Tuberk.*, 1909, xiv, 1.
18. Lewis, P. A., and Aronson, J. D., *Am. Rev. Tuberc.*, 1923, vii, 404.
19. Doan, C. A., and Sabin, F. R., *J. Exp. Med.*, 1927, xlvi, 315.
20. Cobbett, L., The causes of tuberculosis, Cambridge, 1917, 248.
21. Opie, E. L., *J. Exp. Med.*, 1908, x, 419.
22. Neufeld, F., *Am. Rev. Tuberc.*, 1927, xvi, 66.