MULTIPLICATION OF TUBERCLE BACILLI WITHIN MONONUCLEAR PHAGOCYTES IN TISSUE CULTURES DERIVED FROM NORMAL ANIMALS AND ANIMALS VACCINATED WITH BCG

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In a previous paper (1) a technique was described for the cultivation *in vitro* of monocytes derived from peritoneal exudates of guinea pigs. When the cells from normal animals were infected with tubercle bacilli, either virulent or attenuated, the organisms were found to multiply intracellularly even in culture systems in which extracellular proliferation was prevented by small concentrations of streptomycin added to the liquid culture medium (1). The present study deals with the comparative multiplication of tubercle bacilli within the monocytes derived from normal guinea pigs and rabbits and from animals previously vaccinated with BCG.

Materials and Methods

Animals.—Albino guinea pigs, males and females, were obtained from the Rockefeller Institute stock. They weighed between 400 and 450 gm. at the time of vaccination and more than 500 gm. when used for the preparation of exudates. Hybrid rabbits weighing 2000 to 2500 gm. were also obtained from the Rockefeller Institute.

Vaccination.—The technique of vaccination with BCG used in these experiments has been described in earlier publications from this laboratory (2). The culture of BCG, originally obtained from the Henry Phipps Institute, Philadelphia, through the courtesy of Dr. J. D. Aronson, has been propagated continuously for several years in a liquid medium containing 0.05 per cent tween and 0.5 per cent bovine albumin fraction V (3). Guinea pigs and rabbits were injected intradermally with 0.1 ml. of a 7 day old culture. This technique of vaccination was found to elicit in all guinea pigs a relatively uniform level of skin hypersensitivity to tuberculin (4, 5).

All control animals were injected intradermally with 0.1 ml. of fresh culture medium.

Infection and Cultivation of the Mononuclear Cells.—The technique has been described in detail in a preceding paper (1). Guinea pigs were injected intraperitoneally with 10 ml. and the rabbits with 50 ml. of a solution containing 0.01 mg. glycogen per ml. physiological saline. 5 days later the animals were bled by heart puncture and the exudate from each animal was collected in a volume of Hanks's balanced salt solution (6) sufficient to yield 25 to 30 ml. of fluid. Fresh, normal, homologous serum was added to the exudate in a final concentration of 2.5 per cent. 1.0 ml. of a centrifuged and diluted culture of tubercle bacilli was

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carefully mixed with 9.0 ml. of exudate. One sample of each exudate received 1.0 ml. of the diluent instead of the bacterial suspension to serve as control. The mixtures were then poured into Petri dishes and incubated at 37°C. for 1 hour in order to allow the monocytes to engulf the bacilli and to settle on small glass slides placed in the Petri dishes as described earlier. The slides with the monocytes were carefully washed in fresh balanced salt solution, covered with a thin film of formvar (7), and placed in screw-capped tubes containing 0.6 ml. of a liquid culture medium. The medium consisted of Hanks's solution, 80 per cent fresh homologous serum, 50 u. penicillin, and 5 γ streptomycin per milliliter. Of the 9 slides from each Petri dish, one was immediately fixed and stained, and the others transferred to two culture tubes, four in each. The tubes were incubated at 37°C. in a rotating machine, the medium being changed after 1 and 3 days. One slide was removed from each tube after 24 hours, 3, 5, and 7 days of cultivation; the cells were fixed in absolute methyl alcohol and treated with a cold stain for tubercle bacilli and counterstained with Giemsa.

The number of stained tubercle bacilli within each of 100 phagocytes was determined, and the cells were classified according to the number of bacilli that they contained (1 to 2, 3 to 5, 6 to 10, or more than 10 tubercle bacilli).

Monocytes derived from both normal and vaccinated animals were used in comparative experiments. The serum employed in the preparation of the tissue culture medium was usually that of the animal from which the monocytes had been obtained except in experiments designed to investigate the effect of "immune" serum *vs.* normal serum on the inhibition of growth of tubercle bacilli.

Strains of Tubercle Bacilli.—For the infection of the monocyte cultures, the following strains were used: H37Rv and Vallée (virulent); R1Rv, BCG-Phipps, and BCG-Tice (attenuated).[1]

Multiplication of Tubercle Bacilli within Phagocytes Derived from Normal and Vaccinated Animals.—

Guinea pigs were injected with BCG-Phipps or culture medium as described above. After 4 weeks or later, exudates for each experiment were collected simultaneously from a vaccinated animal and from a control. Normal serum was added in a concentration of 2.5 per cent to both exudates which were adjusted to the same cell content per milliliter. They were then infected with either BCG-Tice, BCG-Phipps, R1Rv, or H37Rv. The mixtures were distributed in Petri dishes and left at 37°C. for 1 hour. Then the monocytes were cultivated by the technique already described. The monocytes derived from the normal animal were cultured in medium to which normal serum was added, whereas the medium for the cells from the vaccinated animal contained serum from a vaccinated one. The results obtained in experiments using monocytes from animals 5 weeks after vaccination are presented in Fig. 1 and Table I.

These results show that the bacilli of both strains multiplied within the monocytes derived from the normal animal, but not within the monocytes of the vaccinated animal, at least during the first 5 days of cultivation; only on the 7th day was a slight increase in the number of bacilli observed in the latter cells.

[1] The designation BCG-Phipps and BCG-Tice indicates the institution from which the cultures were obtained; Phipps from The Henry Phipps Institute, Philadelphia, and Tice from The Tice Laboratory, Chicago, through the courtesy of Dr. S. R. Rosenthal.

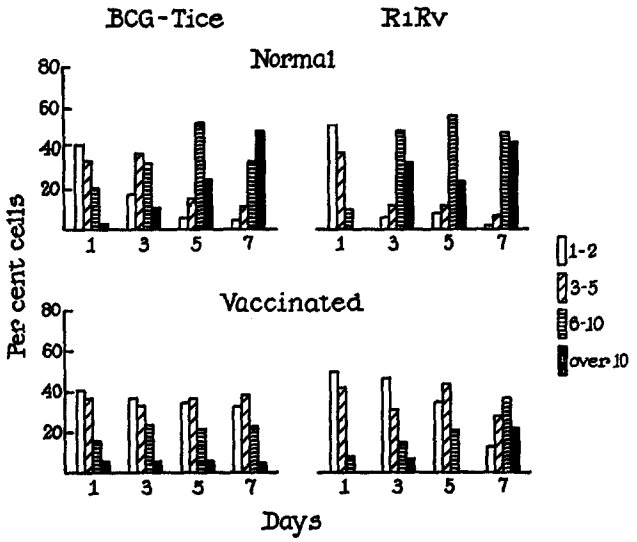

FIG. 1. Multiplication of tubercle bacilli (strains BCG-Tice and R1Rv) within monocytes derived from normal and vaccinated guinea pigs. The columns represent the percentage of phagocytes containing 1 to 2, 3 to 5, 6 to 10, and over 10 stainable bacilli.

TABLE I

Multiplication of Tubercle Bacilli (BCG-Tice and R1Rv) within Mononuclear Cells Derived from Normal and Vaccinated Guinea Pigs

Days of cultivation....	BCG-Tice								R1Rv							
	1		3		5		7		1		3		5		7	
	a*	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Monocytes from normal animals																
A (1–2 bacilli)	42‡	41	18	12	6	§	5	6	57	52	4	6	§	8	§	2
B (3–5 ")	34	36	38	30	16		12	17	38	38	15	12		12		7
C (6–10 ")	21	20	33	42	53		34	36	5	10	52	49		56		48
D (>10 ")	3	3	11	16	25		49	41	0	0	29	33		24		43
Monocytes from vaccinated animals																
A (1–2 bacilli)	41	38	37	40	35	38	33	34	50	49	47	16	35	30	13	10
B (3–5 ")	37	29	33	26	37	40	39	34	42	37	31	39	44	36	28	31
C (6–10 ")	16	27	24	27	22	20	23	26	8	13	15	27	21	24	37	37
D (>10 ")	6	6	6	7	6	2	5	6	0	1	7	18	0	10	22	22

* Columns a and b represent duplicate counts made on separate slides from two different culture tubes.

‡ 100 phagocytes containing tubercle bacilli were classified according to the number of stainable bacilli that they contained. The figures represent the percentage of phagocytes containing the following number of tubercle bacilli: 1–2 (A), 3–5 (B), 6–10 (C), and more than 10 (D).

§ No counts.

The same experiment was carried out using exudate monocytes from a normal rabbit and a rabbit vaccinated with BCG 5 ½ weeks earlier. The results presented in Table II show that bacillary multiplication within monocytes from the normal rabbit occurred at a rate similar to that observed in monocytes from normal guinea pigs whereas it was markedly inhibited in the monocytes of the vaccinated rabbit.

Degeneration of cells was noted in tissue cultures from normal guinea pigs infected with R1Rv and in rabbit cells infected with Vallée. With monocytes from vaccinated animals, there was no indication of any cytotoxic effect of the

TABLE II

Multiplication of Tubercle Bacilli (BCG-Phipps and Vallée) within Mononuclear Cells Derived from Normal and Vaccinated Rabbits

Days of cultivation....	BCG-Tice								Vallée							
	1		3		5		7		1		3		5		7	
	a*	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Monocytes from normal animals																
A (1–2 bacilli)	41*	47	19	13	7	5	8	7	62	61	36	26	6	8	12	14
B (3–5 ")	30	29	41	36	19	8	12	12	29	33	51	46	21	15	18	19
C (6–10 ")	18	18	28	27	50	30	27	28	9	6	11	21	52	57	28	29
D (>10 ")	11	6	12	24	24	57	53	53	0	0	2	7	21	20	42	38
Monocytes from vaccinated animals																
A (1–2 bacilli)	63	50	63	65	66	54	42	43	70	71	60	60	57	54	35	43
B (3–5 ")	19	24	21	20	24	29	23	26	25	24	30	31	35	33	35	38
C (6–10 ")	13	22	13	11	8	13	18	26	4	5	9	8	7	11	22	14
D (>10 ")	5	4	3	4	2	4	17	5	1	0	1	1	1	2	8	5

* See legend Table I.

bacilli, probably as a result of the fact that intracellular multiplication of bacilli was greatly inhibited in these cultures.

As more monocytes were usually present in the peritoneal exudate of vaccinated animals than in that from the normal, it was necessary to adjust them to the same cellular density. However, there was no apparent difference in phagocytic activity between the two groups of cells, as can be seen in Fig. 1 and Tables I and II. The two types of monocytes did not differ in their capacity to transform into macrophages and to proliferate.

Influence of Serum upon the Multiplication of Tubercle Bacilli within Monocytes from Normal and Immunized Animals.—In the experiments thus far reported, the monocytes had been cultivated in media containing serum derived from the same animal. An attempt was made to determine the com-

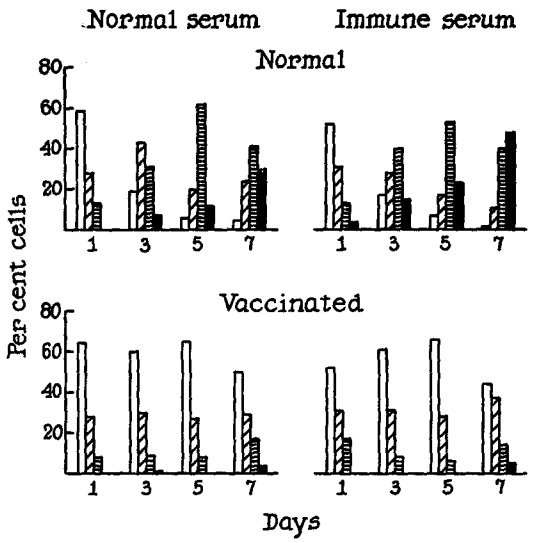

FIG. 2. Multiplication of tubercle bacilli (BCG-Phipps) within monocytes derived from normal and vaccinated guinea pigs in media containing either normal or "immune serum."

TABLE III

Multiplication of Tubercle Bacilli (R1Rv) within Mononuclear Cells from Normal and Vaccinated Guinea Pigs in Culture Medium Containing either Normal or Immune Serum

Days of cultivation....	Normal serum								Immune serum							
	1		3		5		7		1		3		5		7	
	a*	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Monocytes from normal animals																
A (1–2 bacilli)	52*	53	17	15	7	7	1	3	59	53	19	17	6	11	5	5
B (3–5 ")	31	35	28	34	17	18	11	16	28	32	43	44	20	24	24	16
C (6–10 ")	13	12	40	42	53	55	40	33	13	15	31	26	62	58	41	46
D (>10 ")	4	0	15	9	23	20	48	48	0	0	7	13	12	7	30	33
Monocytes from vaccinated animals																
A (1–2 bacilli)	64	64	56	60	66	65	55	50	52	53	61	50	66	61	44	49
B (3–5 ")	33	28	34	30	25	27	31	29	31	32	31	31	28	32	37	24
C (6–10 ")	3	8	10	9	9	8	12	17	17	14	8	19	6	7	14	20
D (>10 ")	0	0	0	1	0	0	2	4	0	1	0	0	0	0	5	7

* See legend Table I.

parative effect of normal serum or of serum from a vaccinated animal on the intracellular multiplication of bacilli within monocytes from normal or vaccinated animals.

The procedure was the same as described above with the exception that the monocytes of both normal and vaccinated animals were cultivated in presence of either normal serum or serum derived from a vaccinated animal. The results obtained with guinea pig monocytes are recorded in Fig. 2 and Table III, and those with rabbit monocytes in Table IV. In both cases, the animals had been vaccinated 8 weeks prior to the culture tests. It is clear that the cell-parasite relationship was not influenced by the source of the serum, under the conditions of the test. Serum from a vaccinated animal did not affect the multiplication of the bacilli within monocytes from a normal ani-

TABLE IV

Multiplication of Tubercle Bacilli (BCG-Phipps) within Mononuclear Cells from Normal and Vaccinated Rabbits in Culture Medium Containing either Normal or Immune Serum

Days of cultivation	Normal serum						Immune serum					
	1		3		7		1		3		7	
	a*	b	a	b	a	b	a	b	a	b	a	b
Monocytes from normal animals												
A (1–2 bacilli)	50*	48	19	16	‡	6	46	43	15	13	8	7
B (3–5 ")	32	34	35	32		11	32	31	30	31	13	16
C (6–10 ")	16	15	39	39		30	15	19	40	39	22	27
D (>10 ")	2	3	7	13		53	7	7	15	17	57	50
Monocytes from vaccinated animals												
A (1–2 bacilli)	42	42	27	29	25	25	38	41	34	31	23	25
B (3–5 ")	28	25	28	32	31	24	30	28	31	30	25	24
C (6–10 ")	22	22	25	27	26	24	21	20	20	27	26	24
D (>10 ")	8	11	20	12	18	27	11	11	15	12	26	27

* See legend Table I.

‡ No count.

mal, nor did normal serum alter the inhibition of bacillary multiplication within monocytes from a vaccinated animal.

Time Required for the Appearance of the Growth-Inhibitory Property of Monocytes Following Vaccination.—The degree of immunity in tuberculosis is usually determined by comparing the resistance of the immunized animals to virulent infection with that of control animals. Under these conditions, the degree of protection observed may be due to the immunity acquired after vaccination as well as during the course of the challenge infection. In consequence, little information is available about the time schedule of the development of immunity. The tissue culture technique used in the present studies permits the separation of the immunizing effect of previous vaccination from

that of the infection during the test itself. More specifically, it allows determination of the time required for the development of the ability by monocytes to inhibit the intracellular multiplication of tubercle bacilli.

Sixteen male guinea pigs were injected intradermally with a culture of BCG and another group received normal culture medium instead. The animals were then used as sources of exudates, according to the following schedule: The first animal of each group was injected with glycogen the day following vaccination and the others 3, 6, 10, 15, and 22 days later. The exudates were collected 5 days after injection, that is, 6, 8, 11, 15, 20, and 27 days after vaccination. In all experiments the culture BCG-Phipps was used for the infection of the

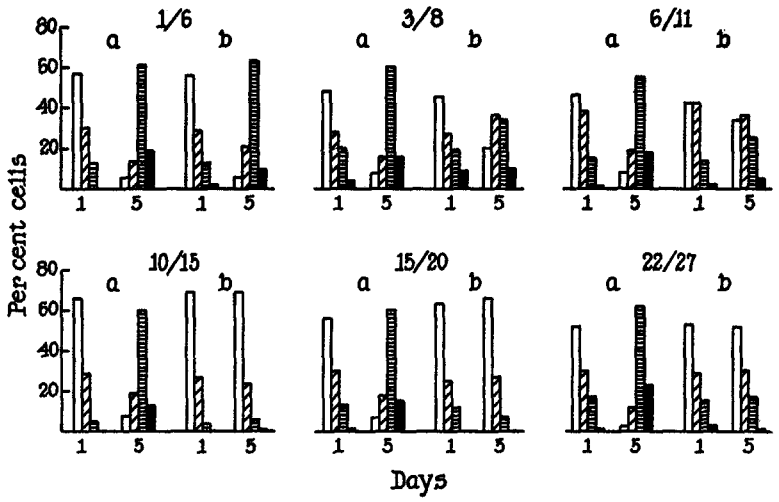

FIG. 3. Multiplication of tubercle bacilli (BCG-Phipps) from normal (*a*) and from vaccinated guinea pigs (*b*) at different intervals after vaccination. The figures indicate the number of days following vaccination after which glycogen was injected (1st figure) and the exudate was collected (2nd figure).

monocytes, and the serum used for the cultivation of monocytes was obtained either from normal animals in the case of the controls, or from vaccinated animals in the case of cells from the vaccinated ones. The results of microscopic observations made on preparations of cultures after 24 hours and 5 days of cultivation are given in Fig. 3 and Table V.

The first evidence of inhibition of the growth was observed in the cultures of monocytes derived from the exudate collected 8 days after vaccination. No multiplication whatever was observed within monocytes derived from exudates collected 15 days after vaccination or later. It is noteworthy that the multiplication of the BCG bacilli within the monocytes taken from the control animals at different periods was uniform throughout the experiment.

The time required for the appearance of skin hypersensitivity to tuberculin after vaccination was determined in the following experiment.

TABLE V

Time after Vaccination of Guinea Pigs Required for the Appearance of the Ability of Mononuclear Phagocytes to Inhibit Intracellular Multiplication of Tubercle Bacilli (BCG-Phipps)

Days of cultivation	Days after vaccination																							
	1/6*				3/8				6/11				10/15				15/20				22/27			
	1		5		1		5		1		5		1		5		1		5		1		5	
	a‡	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Monocytes from normal animals																								
A (1–2 bacilli)	57‡	53	6	9	47	48	7	8	35	46	8	§	64	66	9	8	52	56	9	7	54	52	8	3
B (3–5 ")	30	33	14	21	31	28	17	16	36	28	19		28	29	22	19	28	30	17	18	29	30	14	12
C (6–10 ")	13	11	61	63	19	20	59	60	26	15	55		8	5	59	60	17	13	64	60	14	17	53	62
D (>10 ")	0	3	19	7	3	4	17	16	3	1	18		0	0	10	13	3	1	10	15	3	1	25	23
Monocytes from vaccinated animals																								
A (1–2 bacilli)	48	56	6	15	50	45	17	20	37	42	33	34	68	69	71	69	59	63	§	66	53	47	52	59
B (3–5 ")	33	29	21	24	27	27	34	36	40	42	37	36	27	27	24	24	30	25		27	29	29	30	26
C (6–10 ")	18	13	63	56	18	19	30	34	21	14	25	25	5	4	5	6	10	12		7	15	20	17	13
D (>10 ")	1	2	10	5	5	9	19	10	2	2	5	5	0	0	0	1	1	0		0	3	4	1	2

‡ See legend Table I.

* The figures indicate days after vaccination. The first refers to the injection of glycogen and the second to the date of the experiment when the exudate was collected.

§ No count.

TABLE VI

Skin Reaction of Guinea Pigs to the Intradermal Injection of Old Tuberculin at Various Intervals of Time after Vaccination with BCG

Group	Time between vaccination and test	Size of the area of the skin reaction*											
		a	b	a	b	a	b	a	b	a	b	a	b
	days												
1	2	2‡	—	3	—	2	—	3	—	3	—	2	—
2	5	2	—	8	—	5	—	—	—	—	—	11	—
3	10	9	7	12	10	11	9	8	8	7	5	9	10
4	15	16	10	12	9	12	12	14	11	17	15	16	11
5	21	14	14	20	17	15	14	17	12	16	14		
6	37	20	12	16	10	16	14	14	14	13	12		

*Results obtained with 6 guinea pigs in each group

‡ Diameter in millimeters of the area with swelling and redness. Measurements taken (*a*) 24 hours and (*b*) 48 hours after injection of old tuberculin.

Thirty-four female guinea pigs were injected as described above, with 0.1 ml. of a BCG culture. The animals were divided into 6 groups, 4 consisting of six animals each, and 2 of

only five. Those in each group were tested for skin hypersensitivity by injecting intradermally 0.1 ml. old tuberculin Lederle diluted 1:1000, 2, 5, 10, 15, 21, and 37 days after vaccination. The area of swelling and redness was measured after 24 and 48 hours. The results are given in Table VI.

Only doubtful reactions were observed in the animals of groups 1 and 2, whereas the animals tested on the 10th day after vaccination showed a skin reaction which persisted for 48 hours or longer and could, therefore, be considered as tuberculin-positive. The reaction was more intense in the groups tested later.

DISCUSSION

Reinfection with tubercle bacilli differs from primary infection in many respects, notably in the rapidity of spread of bacilli from the site of infection and in the extent of bacillary proliferation in the tissues. The different theories which have been formulated to account for these differences have been reviewed critically in recent publications (8, 9) and can be classified as follows:—

(*a*) It has been repeatedly claimed and denied that the body fluids or blood from immunized animals possess bacteriolytic properties (10–12, and 13–17). (*b*) Experimental evidence has been adduced to support the view that tubercle bacilli degenerate or are destroyed within giant cells (18), polymorphonuclear leukocytes (19), monocytes and macrophages (20, 21), or clasmatocytes (22). But this statement has not remained unchallenged (17, 23, 24). (*c*) A combined effect of humoral and cellular factors has also been invoked (25–27). (*d*) The fixation of the tubercle bacilli at the site of reinfection has been held by many workers to be the result of increased inflammatory reaction as a result of hypersensitivity (28). This dependence of immunity on hypersensitivity has also been denied (29).

By permitting the separation of some of the multiple factors which come into play during reinfection, the *in vitro* experimental system used in the present experiments has made possible a study of their respective influence upon the intracellular parasitism of tubercle bacilli. It has been shown that multiplication of attenuated and virulent tubercle bacilli occurs readily within the phagocytic cells of normal guinea pigs and rabbits—especially in monocytes and macrophages, but is greatly retarded or inhibited within phagocytes from vaccinated animals. This inhibition appears to be independent of the presence of humoral factors in the culture medium, a fact which lends support to the assumption that as a result of vaccination the phagocytes themselves become endowed with the ability to interfere with the intracellular multiplication of tubercle bacilli.

The results reported in this and a previous paper are in accord with Lurie's concept of the role of the monocytes in immunity to tuberculosis. By injecting monocytes which had phagocytized virulent tubercle bacilli into the anterior

chamber of the rabbit eye, this author found that the infection of the eye progressed rapidly when monocytes from a normal rabbit were used whereas it was retarded in experiments utilizing monocytes from immunized rabbits (30). In Lurie's experiments, as in the ones described in the present paper, inhibition of bacillary multiplication appeared to be independent of the addition of immune serum to the system.

In the present experiments, the growth-inhibiting property of the phagocytes of guinea pigs could first be recognized *in vitro* on approximately the 10th day after vaccination. It is commonly found *in vivo* that the first signs of inhibition and destruction of bacilli occur 10 to 14 days after infection (31). This similarity in findings suggests that the *in vitro* system can be used for the analysis of the mechanisms of acquired immunity in tuberculosis.

SUMMARY

When monocytes derived from normal guinea pigs or rabbits were infected with tubercle bacilli and cultivated *in vitro*, the bacilli multiplied abundantly within the cytoplasm of these cells.

By contrast, intracellular multiplication of the bacilli was retarded or completely inhibited within the monocytes of rabbits or guinea pigs vaccinated with BCG. This inhibition of growth was observed with both virulent or attenuated strains of tubercle bacilli.

Under the conditions used in the present study, the ability of monocytes to inhibit bacillary proliferation was the same whether serum from a normal animal or from vaccinated animals was used in the tissue culture medium. Moreover, the serum of vaccinated animals did not inhibit multiplication of tubercle bacilli within monocytes derived from a normal animal.

The ability of guinea pig monocytes to interfere with intracellular bacillary proliferation was first perceptible 8 days after vaccination.

BIBLIOGRAPHY

1. Suter, E., *J. Exp. Med.*, 1952, **96**, 137.
2. Fenner, F., and Dubos, R. J., *J. Exp. Med.*, 1950, **91**, 269.
3. Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, 1947, **56**, 334.
4. Dubos, R. J., Schaefer, B., and Suter, E., unpublished results.
5. Boyden, S. V., and Suter, E., *J. Immunol.*, 1952, **68**, 577.
6. Hanks, J. H., and Wallace, R. E., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71**, 196.
7. Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, **81**, 233.
8. Lurie, M. B., *Am. J. Med.*, 1950, **9**, 591.
9. Rich, A. R., Pathogenesis of Tuberculosis, Springfield, Illinois, Charles C. Thomas, 2nd edition, 1951, 509, 571.
10. Deycke, G., and Much, H., *Münch. med. Woch.*, 1909, **56**, 1985.
11. Kraus, R., and Hofer, G., *Centr. Bakt., 1. Abt., Ref.*, 1912, **54**, 191.
12. Manwaring, W. H., and Bronfenbrenner, J., *J. Exp. Med.*, 1913, **18**, 601.

13. Baldwin, E. R., *J. Med. Research*, 1904, **12**, 215.
14. Burnet, É., *Ann. Inst. Pasteur*, 1915, **29**, 119.
15. Baatz, *Centr. Bakt., 1. Abt., Orig.*, 1920, **84**, 81.
16. Kirchner, O., *Z. Immunitätsforsch.*, 1932, **74**, 56.
17. Hanks, J. H., and Evans, B., *Am. Rev. Tuberc.*, 1940, **41**, 605, 620.
18. Metchnikoff, E., *Arch. path. Anat. u. Physiol.*, 1888, **113**, 63.
19. Rist, E., Léon-Kindberg, M., and Rolland, J., *Ann. Méd.*, 1914, **1**, 310, 375.
20. Metalnikov, S., and Secreteva, V., *Ann. Inst. Pasteur*, 1927, **41**, 301.
21. Lurie, M. B., *J. Exp. Med.*, 1929, **50**, 747.
22. Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 627.
23. Bartel, J., and Neumann, W., *Centr. Bakt., 1. Abt., Orig.*, 1906, **40**, 723.
24. Borquist, M., and Rowe, C., *Am. Rev. Tuberc.*, 1931, **24**, 172.
25. Much, H., *Ergebn. Hyg., Bakt., Immunitätsforsch., u. exp. Therap.*, 1917, **2**, 622.
26. Clawson, B. J., *J. Infect. Dis.*, 1936, **58**, 64.
27. Lurie, M. B., *J. Exp. Med.*, 1939, **69**, 555.
28. Krause, A. K., *Am. Rev. Tuberc.*, 1926, **14**, 211.
29. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1930, **47**, 189.
30. Lurie, M. B., *J. Exp. Med.*, 1942, **75**, 247.
31. Lurie, M. B., *J. Exp. Med.*, 1932, **55**, 31.