STUDIES ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS

The Mobilization of Mononuclear Phagocytes in Normal and Immunized Animals and Their Relative Capacities for Division and Phagocytosis

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Plates 35 and 36

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It is generally recognized that the lesion of reinfection in tuberculosis differs from that of the primary infection by the acceleration and intensification of the immediate inflammatory reaction, by the quickened formation of nodule and tubercle and by their abortive nature. In previous studies (1) it was concluded that the most significant factor in the mechanism of immunity to tuberculosis is the rapid mobilization of mononuclear phagocytes with an increased physiological capacity to destroy or inhibit the growth of tubercle bacilli; the more rapid formation of epithelioid tubercles and their ready resolution were attributed to these factors. Recently Dienes and Mallory (2) have also shown that normal guinea pigs respond with an exudation of polymorphonuclears to the introduction of tubercle bacilli, but, following tuberculous infection, and synchronously with the development of hypersensitivity to tuberculin, the reinjection of this microorganism elicits a predominantly mononuclear reaction. These investigators therefore consider the quickened mobilization of these phagocytes as the result of the allergic state.

The chief present endeavor was to elucidate the mechanism of this accelerated mobilization of mononuclears which characterizes the response to reinfection of the tuberculous or allergic animal.

The old studies of Müller (3), the more extended observations of Opie (4), and the recent investigations of Weiss (5) have indicated

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that the proteolytic enzyme of polymorphonuclears, leukoprotease, is active in a neutral or slightly alkaline medium. Lymphoprotease, the enzyme derived from mononuclear phagocytes, is active in an acid medium. Menkin (6) correlated the leukocytic formula and the pH of the medium during the course of an acute inflammation. He found that the polymorphonuclears persist in an exudate the pH of which is above 7.0. When the pH level falls to 6.9 or 6.8 the mononuclears predominate. Therefore it seemed pertinent to determine whether the more ready mobilization of the mononuclear phagocytes in the immune animal may not be associated with a more acid reaction at the site of reinfection than at the site of a primary infection. Furthermore, since it was found that in the acellular areas of a subcutaneous agar focus containing tubercle bacilli, the microorganism fails to grow in the body fluids of the immune animal (1, 1936), it was thought possible that a local tissue acidosis (7) might conceivably develop at the site of reinfection which would account for the bacteriostasis. This seemed the more plausible since Dernby and Avery (8) found that acidity *in vitro* was bacteriostatic for pneumococci, and since, furthermore, as had been determined by Lord and Nye (9), a pH lower than 6.8 will kill pneumococci *in vitro*. Therefore the following experiments were performed.

The pH at the Site of Reinfection and the Fate of the Bacilli

6 per cent agar in saline, adjusted to pH 7.4, was melted, cooled to 50°C., mixed with a suspension of virulent bovine tubercle bacilli and injected subcutaneously into normal and tuberculous rabbits. Normal guinea pigs and guinea pigs vaccinated with the attenuated tubercle bacillus, R 1, received a similar mixture of agar and tubercle bacilli in one flank and the same amount of melted agar without tubercle bacilli in the opposite flank. At different intervals of time following the injection, the rabbits were lightly anesthetized with ether and the guinea pigs with sodium amytal. The skin over the agar focus was quickly reflected and, with dry scissors, snips of agar were removed and immediately submerged in liquid petrolatum. The pH of this agar was determined by the method of Hastings and Sendroy (10).

It can be seen from Table I that up to the 2nd week following inoculation, the pH of a subcutaneous agar focus containing virulent tubercle bacilli was distinctly lower in the tuberculous rabbit or the vaccinated guinea pig than in the corresponding normal control.

That this greater acidity at the site of reinfection than at the site of a primary inoculation is not to be associated with the varying behavior of the tubercle bacilli in the normal and immunized animals is suggested by the observation that even in the absence of tubercle bacilli the pH of an agar focus in vaccinated guinea pigs was lower than in normal animals. Similar differences were noted in the pH of the contents of collodion-impregnated silk bags placed in the peritoneal cavities of normal and tuberculous rabbits (11).

Although the hydrogen ion concentration was definitely lower at the site of reinfection than at the site of a primary inoculation, the

TABLE I

The pH of a Subcutaneous Agar Focus with and without Tubercle Bacilli in Normal and Immunized Rabbits and Guinea Pigs

Interval after injection of agar	Agar focus with tubercle bacilli in rabbits		Agar focus with tubercle bacilli in guinea pigs		Agar focus without tubercle bacilli in guinea pigs	
	Normal	Tuberculous	Normal	Vaccinated	Normal	Vaccinated
1 day	7.55	7.30	7.40	7.25	7.15	6.85
3 days	—	—	7.20	6.85	6.95	6.70
1 wk.	7.35	7.05	7.00	6.80	7.15	6.70
2 wks.	7.20	7.10	6.90	7.05	6.85	7.00

difference was only about 0.2 of a pH. It does not seem likely that this difference can play a significant rôle in the inhibition of growth of tubercle bacilli in the immunized animals, especially since Long (12) found no difference whatsoever in the growth energy of tubercle bacilli between pH 6.4 and 7.8; and even the pneumococcus is injured by a low pH only in the absence of body fluids, but, in their presence, it survives and multiplies at a pH of 5.5 (13).

The pH at the Site of Inflammation and the Mobilization of Mononuclears

While the greater acidity at the site of reinfection does not explain the inhibition of growth of tubercle bacilli in the immunized animals, does it account for the more rapid appearance of mononuclear phagocytes in these foci? If the pH of the medium in an acute inflammation is a significant factor in the mobilization of the cells it should follow from the considerations noted above that sensitized guinea pigs would respond to a non-specific irritant with an exudate containing more

mononuclears than normal animals, since it was found that even in the absence of tubercle bacilli the site of a local inflammation in a vaccinated guinea pig is more acid than a similar site in a normal animal.

TABLE II

The pH of the Exudate and Its Leukocytic Formula after an Intrapleural Injection of Aleuronat-Starch, together with the Simultaneous Leukocytic Formula of the Blood in Normal and Sensitized Guinea Pigs

Interval after injection	Guinea pig No.		pH of exudate		Leukocytic formula of exudate				Simultaneous leukocytic formula of the blood			
					Normal		Sensitized		Normal		Sensitized	
	Normal	Sensitized	Normal	Sensitized	Granulocytes	Non-granulocytes	Granulocytes	Non-granulocytes	Granulocytes	Non-granulocytes	Granulocytes	Non-granulocytes
1 day	18-6 L	18-6 B*	7.15	6.65	96.4	3.6	79.0	21.0	69.0	31.0	55.6	44.4
" "	32-2	1*	7.15	6.95	93.0	7.0	43.5	56.5	63.0	37.0	78.0	22.0
" "	A	4*	7.15	6.95	96.0	4.0	68.5	31.5	85.0	15.0	51.0	49.0
" "	19-2	30-0*	7.30	7.00	88.0	12.0	87.5	12.5	30.0	70.0	63.0	37.0
" "	10-2	10-3†	7.20	7.10	91.2	8.8	86.7	13.3	55.5	44.5	74.0	26.0
" "	11-0	10-6†	7.10	6.75	86.8	13.2	78.1	21.9	60.0	40.0	69.0	31.0
" "	49	40†	7.30	7.10	87.5	12.5	80.0	20.0				
" "		41†		7.10			67.5	32.5				
Average.....			7.20	6.95	91.2	8.8	73.8	26.1	60.4	39.6	65.1	34.9
2 days	49	40†	7.25	7.35	79.0	21.0	72.3	27.7				
" "		41†		7.15			38.5	61.5				
3 "	10-2	10-3*	7.35	7.20	88.5	11.5	78.5	21.5	30.0	70.0	39.3	60.7
" "	11-0	10-6*	7.15	6.70	71.3	28.7	66.2	33.8	41.0	59.0	34.0	66.0

* Tuberculous guinea pigs.

† R 1 vaccinated guinea pigs.

For this purpose a group of normal and sensitized guinea pigs were injected intrapleurally with a mixture containing 5 per cent aleuronat and 3 per cent starch. At different intervals thereafter the exudate was withdrawn and its pH was immediately determined by the method of Hastings and Sendroy. Simultaneously smears were made of this exudate as well as of the venous blood, in order to determine whether there was any correlation between the pH of the exudate and its leukocytic formula, and also, whether the cells in the exudate were a reflection of those circulating in the blood. The smears were prepared with Wright's stain according to Osgood's modification (14). At least 200 cells were counted for differentiating the cells.

It can be seen from Table II that actively tuberculous and R 1 vaccinated guinea pigs mobilized mononuclears much more quickly than normal animals, even in response to a non-specific irritant. Thus, in 7 normal animals, 24 hours after injection, the non-granulocytes constituted an average of 8.8 per cent of the mobilized cells. Under the same conditions these cells constituted 26.1 per cent in sensitized animals. Furthermore the lead that the mononuclears attained in the exudate of the sensitized animal on the 1st day after the onset of the inflammation was maintained on the 3rd day, when the mononuclears were still present in greater percentage in the exudate of the allergic animal. As can be seen from the last four columns of this table the cell constituents of the circulating blood bore no constant relation to the exudate cells. The latter, therefore, were not solely determined by the cells circulating in the blood.

While the pH of the exudate of vaccinated or tuberculous guinea pigs was almost always definitely, though only slightly, more acid than a similar exudate in normal guinea pigs, and though correspondingly the mononuclears were also more numerous in the exudate of the sensitized animal, the relationship was not an exact one. Thus on the 2nd and 3rd days following the onset of the inflammation the mononuclears had replaced a large number of the granulocytes originally present in the exudate of both normal and sensitized animals. However there was no corresponding reduction in the pH of the exudate, a fact suggesting that other factors also must be responsible for their appearance at the site of inflammation.

Similar observations have been made in rabbits. Table III shows that the mononuclear phagocytes were mobilized at the site of a non-specific inflammation with greater rapidity in a tuberculous than in a normal rabbit. The pH of the exudate, however, did not differ significantly. Obviously, therefore, the pH of the exudate is not the determining factor for the type of cell mobilized. Nor were the cells of the exudate a reflection of those circulating in the blood at that time. Again, as was found in the guinea pig, so also in the rabbit, even on the 3rd day following the onset of the inflammation the mononuclears were still present in greater percentage in the exudate of tuberculous than of normal animals. It is plain, therefore, that both sensitized rabbits and guinea pigs mobilize mononuclear phagocytes

more readily than normal animals, in response not only to specific reinfection but also to a non-related irritant. Furthermore the pH of the exudate bears no constant relationship to the mobilization of these cells.

TABLE III

The pH of the Exudate and Its Leukocytic Formula after an Intrapleural Injection of Aleuronat-Starch, together with the Simultaneous Leukocytic Formula of the Blood in Normal and Tuberculous Rabbits

Interval after injection	Rabbit No.		pH of exudate		Leukocytic formula of exudate				Simultaneous leukocytic formula of blood			
					Normal		Tuberculous		Normal		Tuberculous	
	Normal	Tuberculous	Normal	Tuberculous	Granulocytes	Non-granulocytes	Granulocytes	Non-granulocytes	Granulocytes	Non-granulocytes	Granulocytes	Non-granulocytes
1 day	E2-19	Bii3-12	7.15	7.15	98.5	1.5	83.5	16.5	73.0	27.0	84.0	16.0
" "	E2-18	A3i-3	7.05	7.15	90.5	9.5	60.0	40.0	68.0	32.0	51.0	49.0
" "	G3-18	Di3-13	7.25	7.10	86.5	13.5	74.5	25.5	57.0	43.0	48.0	52.0
" "	17-0	396	7.20	7.25	85.0	15.0	65.0	35.0	22.0	78.0	29.0	71.0
" "	16-9	7	7.20	7.00	84.0	16.0	91.3	8.7	27.0	73.0	48.0	52.0
" "	C-38	A3i-6	6.95	7.20	92.5	7.5	89.0	11.0	86.0	14.0	56.0	44.0
" "	—	A3i-5	—	7.15	—	—	45.0	55.0	—	—	44.0	56.0
" "	52	Bis-2	7.40	7.25	95.0	5.0	59.0	41.0	—	—	—	—
" "	G2-12	4	6.95	7.05	93.6	6.4	71.1	28.9				
" "	G2-19	5	6.95	7.10	90.0	10.0	79.7	21.3				
" "	G2-24	BH3	6.95	7.05	90.6	9.4	54.1	45.9				
" "	G2-28	—	6.95	—	87.2	12.8	—	—				
Average . . .			7.09	7.13	90.3	9.7	70.2	29.8	55.5	44.5	51.4	48.6
2 days	G2-12	4	7.10	7.15	60.9	39.1	38.6	61.4				
" "	G2-28	5	6.95	7.05	68.9	31.1	59.4	40.6				
	—	BH3	—	7.25	—	—	31.9	68.1				
3 "	G2-19	5	7.35	7.00	23.6	76.4	22.5	77.5				
" "	G2-28	4	7.20	7.15	22.1	77.9	19.8	80.2				
" "	G2-24	BH3	7.25	7.30	36.5	63.5	10.2	89.8				

Capacity for Division and Phagocytosis of Mononuclears Derived from Normal and Immunized Animals

Preliminary observations both in rabbits and guinea pigs indicated that not only do the mononuclears appear earlier at the site of inflammation in a tuberculous than in a normal animal, but the lymphocytes also accumulate more rapidly in the former. It would seem that the entire succession of cells that characterizes inflammation in general

is accelerated in the tuberculous animal irrespective of the nature of the irritant. Again the mononuclears in the exudate of a tuberculous animal more frequently contained ingested polymorphonuclears, which were more often in advanced stages of disintegration than those of a normal animal. This increased phagocytosis, however, may be due not to the enhanced physiological activity of these cells, but to the more rapid deterioration that the polymorphonuclears undergo in the commonly more acid medium of the exudate in the tuberculous animal, as suggested by the work of Evans (15). Hence more of them are available for phagocytosis in the sensitized than in the normal animal, for injured cells are more readily phagocyted than normal ones. Yet, the mobilized mononuclears in the immune animal were often larger and more frequently binucleate than those in the exudates of normal animals. These and other considerations suggested that the differences observed may all be an expression of a heightened physiological activity on the part of these cells in a sensitized animal as compared with that of the mononuclears in a normal animal.

In Vivo Cell Division and Phagocytosis by Mononuclears in Normal and Immunized Animals

To test the foregoing assumption groups of normal, actively tuberculous and R 1 vaccinated guinea pigs were given a mixture of aleuronat-starch and India ink intrapleurally. The injection was made in pairs, a normal and a sensitized animal receiving an identical amount of the same materials. On the 2nd day after the injection the exudates of the sensitized and the normal animals were withdrawn, stained in the usual manner and the following observations made: the percentage cell distribution, the per cent of mononuclears containing ingested carbon, the amount of carbon present per cell, the incidence of binucleated or multinucleated cells and the occurrence of mitotic figures.

Table IV indicates that both tuberculous and vaccinated guinea pigs responded with a greater percentage of mononuclears on irritation with a mixture of aleuronat-starch and India ink than normal animals, just as they reacted to aleuronat alone. A larger percentage of the mobilized mononuclears phagocyted carbon particles than those in a normal guinea pig. The individual mononuclears of the sensitized animals engulfed more carbon than those in the normal animal.

TABLE IV

The Leukocytic Formula, the Intensity of Phagocytosis and the Incidence of Mononuclear Division in the Pleural Exudates of Normal and Sensitized Guinea Pigs 2 Days after Injection of Aleuronat-Starch and India Ink

Guinea pig and experiment No.	Tuberculous, vaccinated or normal	Granulocytes	Non-granulocytes	Mononuclears phagocyting carbon	Phagocytes with coarse particles or agglutinated masses of carbon	Binucleated or multinucleated mononuclears	Presence of mitotic figures
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
93,1	Tuberculous	47.0	53.0	91.2	79.0	9.2	+
72,1	Normal	97.0	3.0	45.5	46.0	6.0	+
30,2	Tuberculous	57.6	42.4	92.0	87.0	8.0	+
73,2	Normal	94.6	5.4	46.0	45.0	3.0	0
69,3	Tuberculous	64.0	36.0	55.0	70.0	11.5	+
74,3	Normal	93.6	6.4	48.7	49.0	8.2	+
30-4,4	Tuberculous	72.5	27.5	88.0	79.0	28.0	+
38-9,4	Normal	84.5	15.5	28.0	35.7	15.0	0
38-5,4	Normal	82.5	17.5	52.0	46.1	7.0	−
35-7,5	Tuberculous	81.0	19.0	66.0	4.9*	19.0	+
39-7,5	Normal	81.0	19.0	45.0	5.0*	11.0	0
33-6,6	Tuberculous	61.5	38.5	63.0	9.3*	29.0	−
39-6,6	Normal	77.0	23.0	46.0	6.4*	30.0	−
34-9,7	Tuberculous	68.0	32.0	92.0	12.6*	33.0	+
39-8,7	Normal	78.5	21.5	72.0	7.5*	28.0	0
33-5,8	Tuberculous	79.0	21.0	53.0	6.2*	29.0	0
39-5,8	Normal	93.0	7.0	43.0	7.7*	25.0	+
18-3,9	Vaccinated	67.7	32.3	85.6	84.0	7.8	+
70-0,9	Normal?†	67.8	32.2	81.5	71.0	7.2	+
18-5,10	Vaccinated	57.5	42.5	83.5	46.0	16.0	+
30-3,10	Normal‡	61.0	39.0	66.0	69.1	9.0	0
18-7,11	Vaccinated	67.0	33.0	80.0	69.5	21.0	+
30-2,11	Normal‡	72.0	28.0	68.0	63.3	10.0	0
Average	Tuberculous	65.7	34.3	77.2	73.5	19.2	−
	Normal	81.9	18.1	53.5	53.7	13.3	

* Number of carbon particles per cell.

† Mononuclear nodules with numerous mitoses in the lung; fibrosis of cervical nodes.

‡ Received aleuronat-starch into opposite pleural cavity 3 days before present injection.

This ingested carbon was also found in coarser aggregates in the former (Figs. 1 and 2). The incidence of both amitotic and mitotic division was greater in the sensitized than in the normal guinea pigs (Fig. 3).

A statistical analysis of the data presented in this table showed that the probability, P, that the observed difference in the phagocytosis of carbon particles was not due to random sampling is 99.9 per cent. This and similar figures reported in this paper were calculated from the "Student" t-distribution curve as given by Fisher (16) and from the tabulated values of P as published by Yule and Kendall (17).

TABLE V

The Leukocytic Formula, the Intensity of Phagocytosis and the Incidence of Mononuclear Cell Division in Normal and Tuberculous Rabbits 2 Days after Intrapleural Injection of India Ink and Aleuronat-Starch

Rabbit No.	Normal or tuberculous	Granulocytes	Non-granulocytes	Mononuclears phagocyting carbon	Binucleated or multinucleated mononuclears
		per cent	*per cent*	*per cent*	*per cent*
DM-3	Tuberculous	45.3	54.7	25.0	23.0
20-5	Normal	75.2	24.8	12.5	1.0
Y-4	Tuberculous	43.7	56.3	38.0	8.0
20-7	Normal	63.2	36.8	40.0	1.0
DM-1	Tuberculous	53.7	46.3	33.0	35.0
20-4	Normal	70.3	29.7	16.5	1.5
FM-3	Tuberculous	51.2	48.8	40.7	24.0
20-6	Normal	44.4	55.6	46.5	4.5
Average	Tuberculous	48.5	51.5	34.0	22.5
	Normal	63.3	36.7	28.9	2.0

The value of P from the incidence of binucleated or multinucleated cells is 92.

Similar experiments in normal and tuberculous rabbits are summarized in Table V. It is seen that in rabbits also the incidence of binucleated or multinucleated mononuclears in the exudate of tuberculous animals was much greater than that found in the exudates of the normal. The value of P is 99.4. No significant difference in the phagocytosis of carbon by these cells was found.

Therefore it seems evident that the mononuclears of sensitized guinea pigs and rabbits respond *in vivo* with a greater degree of cell division to a non-specific irritant than those of normal animals.

This increased tendency for division on the part of mononuclears of a tuberculous animal may be held, at least in part, responsible for the more rapid mobilization of these cells in response both to a specific and to a non-specific irritant. That the phagocytic properties of the mononuclears of the sensitized guinea pigs are also increased is suggested by the fact that a greater percentage of these cells are engaged in phagocytosis of carbon *in vivo* and that a majority of these cells in the sensitized animal engulf a larger amount of carbon than the cells in normal animals. However, the evidence is not conclusive, for it has been demonstrated that carbon introduced into the tissues of tuberculous animals agglutinates to a greater degree than in a normal animal (1, 1936). Therefore the enhanced phagocytosis observed may be due to the different dispersion of the carbon particles in the tuberculous or vaccinated animal rather than to the increased physiological capacity for phagocytosis. Hence *in vitro* experiments with cells washed free of their body fluids, and in the presence of serum derived also from normal animals, are essential to test this conclusion.

In Vitro Phagocytosis of Carbon Particles by Mononuclears Derived from Normal and Immunized Guinea Pigs

Accordingly *in vitro* phagocytosis experiments were set up with mononuclears derived from normal, actively tuberculous and vaccinated guinea pigs. The method used is essentially that described by Lucké, Strumia, Mudd, McCutcheon and Mudd (18), with slight modifications.

Briefly, 20 to 40 cc. of light mineral oil were injected intraperitoneally into a normal, an R 1 vaccinated or a tuberculous guinea pig. The injection was executed in pairs, a normal and a sensitized animal receiving an identical amount of the same substance. From 4 to 8 days thereafter 100 cc. sterile 0.4 per cent sodium citrate in 0.85 per cent sodium chloride (19) was injected intraperitoneally. The animal was then quickly killed. The cells were removed and, after separation of the oil, were centrifuged at low speed, decanted, washed in saline and centrifuged again at the same speed. The cells derived from both animals were counted and adjusted with saline to contain the same number per cubic millimeter. Supravital preparations with neutral red were made of each exudate used for the phagocytosis experiments.

Into each of two sterile tubes, 7.5 × 1.0 cm., was pipetted a given volume of cells derived from the normal animal, and into two other tubes was placed the same volume of cells from the immunized animal. To one of each of these pairs

was added an amount of fresh or inactivated serum of a given dilution derived from the normal animal; to the other was added an identical amount of a similar serum of the same dilution derived from the sensitized animal of the same experiment. To each of these four tubes was now added the same volume of the same dilution of India ink. The tubes were stoppered with sterile paraffined corks, and rotated at 37°C. in the incubator on the machine described by Lucké (18) for 20 to 30 minutes at 5 to 8 revolutions per minute. The tubes were then immediately plunged into ice water to stop phagocytosis. Smears were made and stained as previously described. At least 200 cells were counted to determine the percentage distribution of cells in the mixture. A minimum of 100 mononuclears was counted to determine the percentage of cells engaged in phagocytosis of carbon particles, and at least 50 of the cells with ingested particles were examined for the amount of carbon they contained. Table VI presents the essential observations.

It should be noted first in appraising the results that usually the exudates derived from normal animals contained more cells than those derived from the actively tuberculous or vaccinated guinea pigs. The mononuclears of the immunized animals were usually larger, with more abundant cytoplasm, and, in supravital preparations, frequently contained more prominent neutral red granules than those derived from normal animals. Often these vacuoles were tinted more yellow in the "immune" cells, a fact indicating a higher hydrogen ion concentration within their cytoplasm. In many cases the pseudopodia of the immune cells were sensibly more prominent than those of mononuclears derived from normal animals. These observations suggest a greater physiological activity on the part of the mononuclears derived from sensitized animals.

It will be noted in the third column that the number of mononuclears in each phagocytic mixture of a given experiment was similar for the normal and immune cells in the majority of instances. The lymphocytes were usually more numerous in the exudates derived from immunized than those from normal animals, just as was found previously with aleuronat-starch as the inflammatory irritant. This fact tended to lower somewhat the reported percentage of cells engaged in phagocytosis in the mixtures derived from innumized animals. For it was not always possible to differentiate with certainty large lymphocytes, which are rarely phagocytic, from mononuclear phagocytes in stained smears, which were the basis for the estimate of their phagocytic rates.

From the fifth column it is evident that in 9 out of 12 experiments, in the presence of serum derived from the normal animal of a given pair, a significantly larger percentage of mononuclears derived from immunized guinea pigs were engaged in phagocytosis of carbon than mononuclears derived from normal animals under identical conditions, P being 93 and 99 for mononuclears derived respectively from tuberculous and vaccinated guinea pigs. Furthermore, as may be seen from the next column, the amount of carbon ingested by the average immune mononuclear was greater than that engulfed by normal mononuclears.

TABLE VI

In Vitro Phagocytosis of Carbon Particles by Mononuclears Derived from Normal and Sensitized Guinea Pigs

Guinea pig and experiment No.	Tuberculous, vaccinated or normal	Number of mononuclears per c.mm. in phagocytic mixture	Serum dilution	Phagocytosis in normal serum				Phagocytosis in immune serum			
				Mononuclears phagocyting carbon	Number of carbon particles per cell	Number of carbon particles in 100 cells	Ratio between amount of carbon phagocyted by 100 immune and 100 normal cells	Mononuclears phagocyting carbon	Number of carbon particles per cell	Number of carbon particles in 100 cells	Ratio between amount of carbon phagocyted by 100 immune and 100 normal cells
				per cent				*per cent*			
13,1	Tuberculous	12,300	Undiluted	96.0	14.0	1344	1.81	92.0	13.6	1251	0.98
6,1	Normal	5000	"	75.0	9.9	742		85.0	15.1	1283	
84,2	Tuberculous	12,350	"	89.5	22.0	1969	1.99	95.0	26.0	2470	2.29
9,2	Normal	12,800	"	82.5	12.0	990		82.0	13.3	1090	
30-0,3	Tuberculous	19,200	1:10	85.0	13.3	1130	3.91	96.0	16.4	1574	5.14
43-7,3	Normal	18,250	"	85.0	3.4	289		68.0	4.5	306	
36-0,4	Tuberculous	11,500	"*	97.0	17.1	1658	2.07	95.0	17.0	1615	3.04
41-0,4	Normal	14,300	"*	87.0	9.2	800		78.0	6.8	530	
36-2,5	Tuberculous	22,900	"*	97.0	11.0	1067	1.09	90.0	7.3	657	1.77
41-3,5	Normal	30,600	"*	96.0	10.2	979		79.0	4.7	371	
37-0,6	Tuberculous	36,000	"*	86.0	4.3	369	1.57	93.0	3.1	288	0.98
42-0,6	Normal	36,000	"*	90.0	2.6	234		84.0	3.5	294	
36-1,7	Tuberculous	3900	Undiluted*	82.0	18.5	1517	1.74	83.0	18.2	1510	1.61
41-1,7	Normal	8800	"*	67.0	13.0	871		72.0	13.0	936	
18-6,8	Vaccinated	12,200	"	94.5	†			92.0	†		
78,8	Normal	17,700	"	75.0				65.0			
18-2,9	Vaccinated	7500	"	79.0				84.8			
40-1,9	Normal	8700	"	67.6				76.9			
18-1,10	Vaccinated	18,170	"	85.0				92.2			
40-1,10	Normal	8700	"	67.6				76.9			
17-8,11	Vaccinated	9800	"	82.5	†			87.3	†		
79,11	Normal	10,100	"	68.9				70.1			
19-0,12	Vaccinated	20,700	1:10	98.0	6.7	656	1.19	95.0	9.1	864	1.93
43-6,12	Normal	15,800	"	85.0	6.5	552		86.0	5.2	447	

* Serum inactivated at 56°C. for ½ hour.

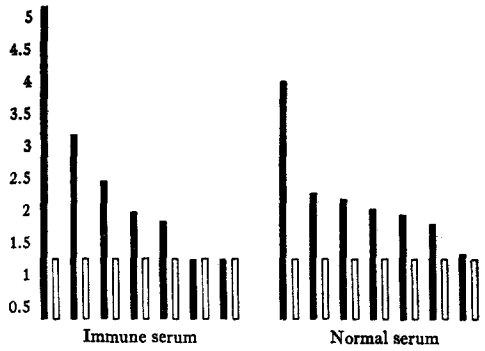
† On the average these cells contained more and coarser carbon particles than those of their corresponding controls.

The final estimate of the phagocytic capacity of these cells was obtained by determining the number of particles that were ingested by 100 unselected mononuclears derived from the normal and the immunized animal of a given experiment. This resulted from the multiplication of the percentage of cells engaged in phagocytosis by the observed average number of particles ingested, and is recorded in the seventh column. It is plain that this estimate is more significant than either the percentage of cells engaged in phagocytosis, or the average number of ingested particles per cell alone would be. For in any condition in which the fate of an animal depends on the phagocytic activity of its cells, it is not only the number of cells that are capable of phagocytosis, but also the amount of material that each of these cells will engulf, that will determine the outcome.

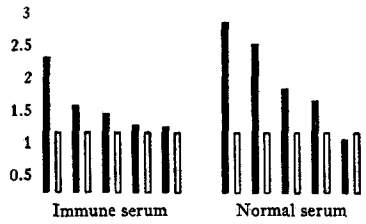
In the next column, the ratio between the amount of carbon phagocyted by 100 normal and 100 immune mononuclears is given. It is clearly seen that in the majority of instances a markedly greater capacity for phagocytosis is apparent in mononuclears derived from tuberculous or vaccinated animals as compared with that of mononuclears derived from normal animals. This ratio is graphically presented in Text-fig. 1.

Essentially similar observations are recorded in the remainder of Table VI in relation to these same phagocytic mixtures, but in the presence of the serum of the sensitized animal of the given pair. The value of P for the percentage phagocytosis by mononuclears derived from actively tuberculous and vaccinated animals is 99 for both. Figs. 4 and 5 illustrate the *in vitro* phagocytosis of carbon particles by cells derived from a tuberculous and a normal guinea pig in the presence of the serum of the tuberculous individual of the experimental pair. In Text-fig. 1 are also graphically presented the ratios between the amount of carbon phagocyted by 100 unselected normal mononuclears and 100 immune mononuclears in the presence of the immune serum of the tuberculous partner of each test.

In summary, therefore, the phagocytic capacity of mononuclears derived from tuberculous or R 1 vaccinated guinea pigs for nonspecific particulate matter such as carbon is, in the majority of instances, significantly greater than that of phagocytes derived from normal animals. Out of a total of 14 experiments with 7 tuberculous guinea pigs and their normal controls in the presence of normal and immune serum, the mononuclears derived from the tuberculous animals were significantly more actively phagocytic than those derived from normal animals in 11, or 78.6 per cent. In the remaining 3 experiments there was no significant difference in the phagocytic capacities of leukocytes derived from the normal and the tuberculous individuals.



TEXT-FIG. 1



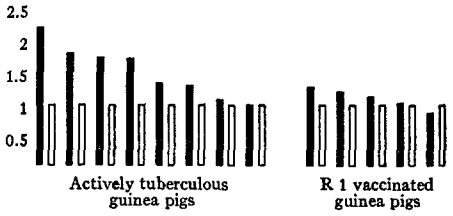
TEXT-FIG. 2

TEXT-FIG. 1. Ratio between the number of carbon particles phagocyted by 100 unselected mononuclears derived from normal and tuberculous guinea pigs in the presence of immune serum and normal serum.

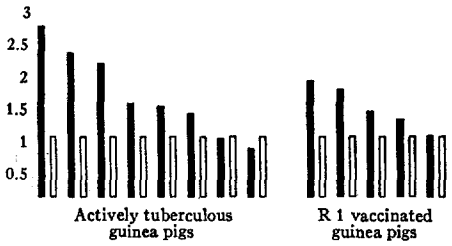
□ mononuclears derived from normal animals.

▮ mononuclears derived from sensitized animals.

TEXT-FIG. 2. Ratio between the number of staphylococci phagocyted by 100 unselected mononuclears derived from normal and tuberculous rabbits in the presence of immune serum and normal serum.



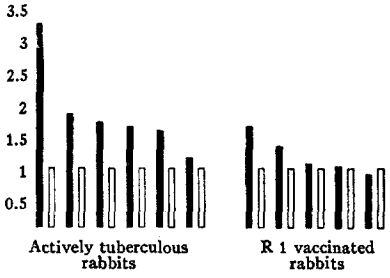
TEXT-FIG. 3



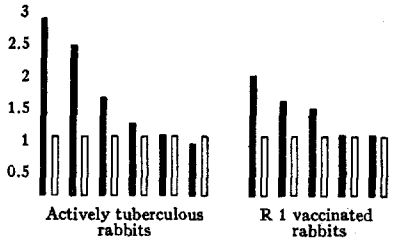
TEXT-FIG. 4

TEXT-FIG. 3. Ratio between the number of tubercle bacilli phagocyted, in the presence of immune serum, by 100 unselected mononuclears derived from normal guinea pigs and from actively tuberculous guinea pigs and R 1 vaccinated guinea pigs.

TEXT-FIG. 4. Ratio between the number of tubercle bacilli phagocyted, in the presence of normal serum, by 100 unselected mononuclears derived from normal guinea pigs and from actively tuberculous guinea pigs and R 1 vaccinated guinea pigs.



TEXT-FIG. 5



TEXT-FIG. 6

TEXT-FIG. 5. Ratio between the number of tubercle bacilli phagocyted, in the presence of immune serum, by 100 unselected mononuclears derived from normal rabbits and from actively tuberculous rabbits and R 1 vaccinated rabbits.

TEXT-FIG. 6. Ratio between the number of tubercle bacilli phagocyted, in the presence of normal serum, by 100 unselected mononuclears derived from normal rabbits and from actively tuberculous rabbits and R 1 vaccinated rabbits.

In 10 additional experiments with guinea pigs vaccinated with R 1 and controls there was a uniformly greater percentage of mononuclears engaged in phagocytosis when they were derived from vaccinated than when obtained from normal animals. Incomplete observations in this series indicated that the individual mononuclears of vaccinated animals also tended to engulf more carbon particles than individual mononuclears derived from normal animals.

In Vitro Phagocytosis of Staphylococci and Collodion Particles by Mononuclears Derived from Normal and Immunized Rabbits

In vitro phagocytosis trials with rabbit mononuclears and India ink gave no satisfactory results. Therefore experiments were set up with collodion particles and staphylococci as phagocytic material. These were carried out in exactly the same manner as was described above for phagocytosis of carbon particles by guinea pig cells except that the rabbits received larger amounts of mineral oil to elicit the mononuclear exudate and more citrated saline was used to wash the peritoneal cavity. Smears of phagocytic mixtures with staphylococci were prepared with Wright's stain, as described above, while those containing collodion particles were stained with the Ziehl-Neelsen technique, as the latter are acid-fast.

Table VII presents the pertinent observations on the relative capacity for phagocytosis of unrelated particles, staphylococci, by mononuclears derived from normal rabbits and from rabbits infected with virulent bovine tubercle bacilli, in the presence of the sera derived from these normal and tuberculous rabbits. Similar observations with collodion as the non-specific test particles for phagocytosis are also presented in Table VII.

It is clear that mononuclears derived from tuberculous rabbits usually have a greater phagocytic capacity for staphylococci in the presence of either normal or immune serum than these cells possess when obtained from normal animals. The value of P for the number of staphylococci phagocyted by 100 unselected mononuclears is 92 and 85 in the presence of normal and immune serum respectively. Likewise immune mononuclears coming from rabbits vaccinated with R 1 ingest a greater amount of collodion particles than these phagocytes do when derived from normal rabbits. The latter conclusion is less certain statistically, for the values of P here are only 61 and 87 for the same observations. Yet, in 3 out of 4 rabbits the mononuclears derived from the vaccinated animals ingested from 2 to 6 times the number of collodion particles phagocyted by cells derived from normal animals. Text-fig. 2 graphically presents the results with

staphylococci. Figs. 6 and 7 illustrate the relative phagocytosis of staphylococci by mononuclears derived from tuberculous and normal

TABLE VII

In Vitro Phagocytosis of Staphylococci and Collodion Particles by Mononuclears Derived from Actively Tuberculous, R 1 Vaccinated and Normal Rabbits

Rabbit and experiment No.	Tuberculous, vaccinated or normal	Number of mononuclears per c.mm. in phagocytic mixture	Serum dilution	Phagocytosis in normal serum				Phagocytosis in immune serum			
				Mononuclears phagocyting particles	Number of particles per cell	Number of particles in 100 cells	Ratio between number of particles phagocyted by 100 immune and 100 normal cells	Mononuclears phagocyting particles	Number of particles per cell	Number of particles in 100 cells	Ratio between number of particles phagocyted by 100 immune and 100 normal cells
Staphylococci											
				per cent				*per cent*			
20-7, 1	Tuberculous	11,500	Undiluted	97.8	24.4	2386	2.46	96.0	20.9	2006	2.24
10-6, 1	Normal	9000	"	79.0	12.3	971		81.5	11.0	896	
20-5, 2	Tuberculous	4300	"	99.5	16.1	1601	1.54	97.5	12.1	1179	1.11
10-8, 2	Normal	6600	"	91.0	11.4	1037		91.5	11.6	1061	
15, 3	Tuberculous	2350	"	88.0	12.7	1117	2.83	83.0	11.5	954	1.42
5, 3	Normal	3400	"	73.0	5.4	394		79.0	8.5	671	
AD, 4	Tuberculous	3900	"	80.0	10.8	864	1.72	79.0	9.6	758	1.30
21-3, 4	Normal	3000	"	69.0	7.3	503		60.0	9.7	582	
20-8, 5	Tuberculous	12,500	"	59.0	5.6	330	0.89	58.0	6.6	382	1.09
21-2, 5	Normal	14,400	"	63.0	5.9	371		55.0	6.4	352	
Collodion Particles											
33-3, 1	Vaccinated	5700	Undiluted	79.0	2.9	229	4.98	74.0	3.0	222	2.13
33-2, 1	Normal	5600	"	33.0	1.4	46		55.0	1.9	104	
33-4, 2	Vaccinated	49,280	1:10	32.0	3.7	118	4.21	61.0	3.1	189	2.45
33-5, 2	Normal	52,080	"	19.0	1.5	28		43.0	1.8	77	
33-7, 3	Vaccinated	40,000	"	92.5	6.6	610	4.04	97.0	11.3	1096	6.68
33-6, 3	Normal	25,000	"	63.0	2.4	151		71.5	2.3	164	
34-1, 4	Vaccinated	49,820	"	93.5	7.2	673	0.64	70.0	5.9	413	1.05
34-2, 4	Normal	46,375	"	91.0	11.5	1046		87.5	4.5	393	

rabbits in the presence of the latter's serum. Figs. 8 and 9 illustrate the phagocytosis of collodion particles by the mononuclears of a vaccinated and a normal rabbit in the presence of the former's serum.

In Vitro Phagocytosis of Tubercle Bacilli by Mononuclears Derived from Normal and Immunized Guinea Pigs and Rabbits

It is evident, therefore, that the phagocytic capacity of mononuclears derived from actively tuberculous or vaccinated rabbits and guinea pigs for substances unrelated to the disease is greater than that of phagocytes derived from normal animals, and that this is independent of the presence of normal or immune serum; in both media the phagocytes of the immunized animals ingest more particles. From the standpoint of the mechanism of immunity to tuberculosis it is important to determine whether the enhanced phagocytic capacity conferred by active tuberculosis or by vaccination upon these mononuclears applies also to the tubercle bacillus. Accordingly similar experiments were set up with mononuclears and sera derived from normal, actively tuberculous and R 1 vaccinated guinea pigs and rabbits, using living virulent human type tubercle bacilli (P 15 B) as the particle for phagocytosis.

The experiments were set up in the manner described above in detail for carbon particles. The serum, fresh or inactivated, in different dilutions, constituted one-third of the volume of the phagocytic mixtures. There were between 150,000 and 580,000 tubercle bacilli per c.mm. in the phagocytic mixtures of the different experiments. It is needless to state that in any test of the properties of normal and immune cells all the conditions were as nearly alike as was possible; the single known variable was the derivation of the phagocytes.

Table VIII presents the pertinent data for cells derived from guinea pigs. In columns 3 to 5 are included the differential cell counts of all the exudates that resulted in each one of the normal and sensitized guinea pigs from the injection of the same amount of petrolatum after the same lapse of time. It will be noted that, in general, sensitized animals, whether actively tuberculous or vaccinated, responded to the intraperitoneal injection of mineral oil with an exudate that contained a larger percentage of mononuclears and lymphocytes and a conspicuously lower concentration of granulocytes than did normal animals. In other words, as was noted above with aleuronat-starch as an irritant, the succession of cells that characterizes inflammation in general was accelerated in the immunized animal in reaction to this non-specific irritant also.

It is clear from an analysis of the remaining observations recorded

TABLE VIII

In Vitro Phagocytosis of Tubercle Bacilli by Mononuclears Derived from Normal, Actively Tuberculous and R 1 Vaccinated Guinea Pigs

Experiment No.	Tuberculous, vaccinated or normal	Differential count of cells in exudate			Number of mononuclears per c.mm. in phagocytic mixture	Serum dilution	Phagocytosis in normal serum			Phagocytosis in immune serum		
		Mononuclears	Lymphocytes	Granulocytes			Mononuclears phagocyting tubercle bacilli	Number of tubercle bacilli per cell	Ratio between number of tubercle bacilli phagocyted by 100 immune and 100 normal cells	Mononuclears phagocyting tubercle bacilli	Number of tubercle bacilli per cell	Ratio between number of tubercle bacilli phagocyted by 100 immune and 100 normal cells
		per cent	*per cent*	*per cent*			*per cent*			*per cent*		
1	Tuberculous	63.0	36.0	1.0	12,350	Undiluted	86.0	4.8	1.38	81.0	5.3	1.32
	Normal	65.5	13.5	21.0	12,800	"	68.0	4.4		72.0	4.5	
2	Tuberculous	82.5	12.0	5.5	12,300	"	85.0	13.7	1.55	79.0	10.1	1.00
	Normal	33.5	1.5	65.0	5000	"	84.0	8.9		82.0	9.8	
3	Tuberculous	74.0	8.0	18.0	18,000	"	79.0	9.2	1.50	63.0	9.3	1.36
	Normal	74.0	3.0	23.0	17,000	"	69.0	7.0		58.0	7.4	
4	Tuberculous	93.5	5.5	1.0	19,200	1:10	66.0	7.2	2.20	69.0	11.3	1.76
	Normal	89.5	5.0	5.5	18,250	"	47.0	4.6		59.0	7.5	
5	Tuberculous	65.4	31.2	3.4	11,500	"*	34.0	2.0	2.83	91.0	7.9	1.85
	Normal	78.0	2.5	19.5	14,300	"*	12.0	2.0		76.0	5.1	
6	Tuberculous	66.3	22.0	11.7	22,900	"*	38.0	2.8	0.96	66.0	4.5	1.09
	Normal	72.7	4.6	22.7	30,600	"*	37.0	3.0		62.0	4.4	
7	Tuberculous	74.5	18.0	7.5	36,000	"*	28.0	1.9	0.80	31.0	4.1	2.27
	Normal	61.1	8.4	30.5	36,000	"*	33.0	2.0		28.0	2.0	
8	Tuberculous	73.0	11.0	16.0	3900	Undiluted*	93.0	26.5†	2.39	91.0	23.4	1.77
	Normal	80.0	12.5	7.5	8800	"*	66.0	15.6		80.0	15.0	
9	Vaccinated‡	83.5	6.5	10.0	29,100	1:50	72.0	3.3	1.93	51.0	3.6	1.03
	Normal	35.5	2.0	62.5	11,500	"	41.0	3.0		47.0	3.8	
10	Vaccinated	68.5	22.5	9.0	30,140	1:10	61.0	4.6	1.42	71.0	6.3	1.23
	Normal	67.5	15.5	17.0	29,700	"	45.0	4.4		65.0	5.6	
11	Vaccinated	82.0	6.0	12.0	25,500	"	83.0	7.7	1.29	76.0	11.7	1.30
	Normal	73.0	4.5	22.5	22,700	"	73.0	6.8		76.0	9.0	
12	Vaccinated	60.5	17.5	22.0	26,100	"	64.0	5.6	1.79	90.0	11.4	0.87
	Normal	61.3	12.0	26.7	27,300	"	50.0	4.0		85.0	13.9	
13	Vaccinated	82.0	11.5	6.5	20,700	"	33.0	2.2	1.01	71.0	7.7	1.14
	Normal	61.5	7.0	31.5	15,800	"	29.0	2.5		60.0	8.0	
Average	Sensitized	74.5	16.0	9.5								
	Normal	65.6	7.1	27.3								

* Serum inactivated at 56°C. for ½ hour.

† Estimated, not directly counted because of agglutination of ingested bacilli.

‡ Vaccinated with suspension of tubercle bacilli killed by submersion in liquid petrolatum at 37°C. for 8 days. The guinea pig's sensitivity to tuberculin was ++.

in this table that the phagocytic capacity for tubercle bacilli of mononuclears derived respectively from actively tuberculous and vaccinated guinea pigs was, in a large majority, greater in the presence of either normal or immune serum than that of mononuclears obtained from normal animals, under identical conditions. In the remaining instances there was no distinct difference in the phagocytosis of tubercle bacilli by mononuclears derived from either normal or immunized guinea pigs.

A further analysis of these data shows that the observed enhancement of the phagocytic capacity of mononuclears conferred upon them by active tuberculosis has a probability of significance of 83 per cent for the number of tubercle bacilli phagocyted by 100 unselected mononuclears in the presence of either normal or immune serum.[1] However, the observed smaller increment in phagocytic capacity afforded mononuclears by vaccination is of questionable statistical significance. The value P for the corresponding observations is only 77 and 57 in the presence of normal and immune serum respectively with cells derived from such animals.

These results are graphically presented in Text-figs. 3 and 4. Figs. 10 and 11 illustrate the relative phagocytosis of tubercle bacilli by mononuclears derived from tuberculous and normal guinea pigs in the presence of the former's serum.

In Table IX are presented the pertinent data for the relative phagocytosis of tubercle bacilli by mononuclears derived from normal, actively tuberculous and R 1 vaccinated rabbits. It is evident from an examination of these protocols, that the phagocytic capacity for tubercle bacilli of mononuclears derived from tuberculous or vaccinated rabbits is greater in the majority of instances than that of mononuclears derived from normal rabbits in the presence of either normal or immune serum. In the remaining instances there was no significant difference in phagocytosis between these mononuclears. It is also apparent that active tuberculosis frequently confers upon the mononuclears a relatively greater enhancement of their phagocytic

[1] For the sake of brevity this column was omitted from Table VIII; however, the observations from which the data in this column were calculated are given there.

TABLE IX

In Vitro Phagocytosis of Tubercle Bacilli by Mononuclears Derived from Normal, Actively Tuberculous and R 1 Vaccinated Rabbits

Rabbit and experiment No.	Tuberculous, vaccinated or normal	Number of mononuclears per c.mm. in phagocytic mixture	Serum dilution	Phagocytosis in normal serum				Phagocytosis in immune serum			
				Mononuclears phagocyting tubercle bacilli	Number of tubercle bacilli per cell	Number of tubercle bacilli in 100 cells	Ratio between number of tubercle bacilli phagocyted by 100 immune and 100 normal cells	Mononuclears phagocyting tubercle bacilli	Number of tubercle bacilli per cell	Number of tubercle bacilli in 100 cells	Ratio between number of tubercle bacilli phagocyted by 100 immune and 100 normal cells
				per cent				*per cent*			
14, 1	Tuberculous	12,000	Undiluted	84.0	5.1	428	1.65	82.0	5.0	410	1.77
8	Normal	18,000	"	65.0	4.0	260		70.0	3.3	231	
15, 2	Tuberculous	2000	"	85.0	7.7	654	1.22	74.0	12.1	895	1.64
5	Normal	3000	"	80.0	6.7	536		79.0	6.9	545	
FM4, 3	Tuberculous	29,000	"	61.0	2.7	164	1.01	72.0	3.2	230	1.17
7	Normal	27,000	"	49.0	3.3	161		48.0	4.1	196	
16, 4	Tuberculous	10,000	1:10	98.0	13.2	1293	2.96	93.0	11.7	1088	1.70
31-8	Normal	14,000	"	78.0	5.6	436		76.0	8.4	638	
10-4, 5	Tuberculous	9500	Undiluted*	56.0	9.0	504	0.87	83.0	14.0	1162	1.92
10-8	Normal	9000	"*	72.0	8.0	576		65.0	9.3	604	
10-5, 6	Tuberculous	18,500	"*	58.0	8.2	475	2.51	72.0	10.3	741	3.40
10-9	Normal	18,500	"*	43.0	4.4	189		42.0	5.2	218	
33-3, 7	Vaccinated	5700	1:10	81.0	8.4	680	2.03	95.0	10.0	950	1.71
33-2	Normal	5600	"	67.0	5.0	335		73.0	7.6	554	
33-9, 8	Vaccinated	39,000	"	53.0	3.0	159	1.60	63.0	4.1	258	1.07
33-8	Normal	41,000	"	43.0	2.3	99		65.0	3.7	240	
33-4, 9	Vaccinated	49,280	"	68.0	7.9	537	1.49	78.0	5.6	437	1.37
33-5	Normal	52,080	"	60.0	6.0	360		69.0	4.6	317	
33-7, 10	Vaccinated	40,000	"	81.0	14.6	1182	1.02	85.5	17.4	1487	0.90
33-6	Normal	25,135	"	66.5	17.4	1157		72.0	23.0	1656	
34-1, 11	Vaccinated	49,820	"	69.0	8.1	558	1.02	64.9	9.9	642	1.04
34-2	Normal	46,375	"	59.0	9.3	548		69.6	8.8	612	

* Serum inactivated at 56°C. for 30 minutes.

capacities than is afforded them by vaccination with attenuated tubercle bacilli.

When these data are subjected to a statistical analysis it is found that the observed enhancement of the phagocytic capacity conferred upon mononuclears of rabbits by active tuberculosis has a probability of significance of 88 and 96 per cent for the number of tubercle bacilli phagocyted by 100 unselected mononuclears in the presence of normal and immune serum respectively. As was found above with guinea pig cells, however, the observed lesser increment in phagocytic capacity afforded rabbit mononuclears by vaccination of rabbits with attenuated tubercle bacilli is of low statistical significance. The value of P for the corresponding observations is only 68 and 57 in the presence of normal and immune serum respectively.

Text-figs. 5 and 6 summarize the data graphically. Figs. 12 and 13 illustrate the relative phagocytosis of tubercle bacilli by rabbit mononuclears derived from an actively tuberculous and normal animal respectively in the presence of the former's serum.

SUMMARY AND DISCUSSION

An endeavor has been made to elucidate the mechanism of the more rapid mobilization of mononuclear phagocytes that characterizes the response to reinfection of the tuberculous or allergic animal, as distinguished from the reaction to a primary inoculation. It has been found that actively tuberculous or vaccinated rabbits and guinea pigs react with an accelerated appearance of mononuclears in response to non-specific inflammatory irritants also. Furthermore the entire succession of cells that characterizes inflammation in general is accelerated in the allergic animal in reaction to irritants, such as aleuronat-starch or mineral oil; *i.e.* in an allergic animal the replacement of the first mobilized polymorphonuclears by mononuclears and the latter by lymphocytes takes place more rapidly than in a normal animal.

This more rapid mobilization of mononuclears is not accounted for by the more acid reaction which frequently develops at the site of introduction of these irritants in a tuberculous as compared with that in a normal animal. This result was not expected for, as considered above, in view of the pH range for the optimum activity of some of the proteases of the polymorphonuclear and mononuclear cells respec-

tively, one might reasonably anticipate such a relationship. That the higher hydrogen ion concentration often found at the site of inflammation in the allergic animal may be more inimical to the survival of polymorphonuclears than of mononuclears is considered likely, but their mobilization seems to be controlled by other factors. For in rabbits there is no significant difference in the pH level at the site of a non-specific inflammation between the normal and the sensitized animals; yet the mononuclears are present in larger numbers in the latter. Again, even in the guinea pig, while there is a consistently greater acidity at the site of inflammation in the allergic animal, there is no consistent correlation between the leukocytic formula of the exudate and its pH level. Nor does the greater local tissue acidosis that develops at the site of a specific reinfection, as compared with that of a primary inoculation, and as observed with the agar focus technique, account for the inhibition of growth of tubercle in the former. For the pH difference, an average of 0.2, seems insufficient for the effects noted on the tubercle bacillus, the pH growth range of which is so wide. It is significant that a similar slightly greater acidity was found within collodion-impregnated silk bags containing tubercle bacilli placed in the peritoneal cavity of tuberculous, as compared with the contents of similar bags situated in normal rabbits (11). In this instance also the observed inhibition of growth in the body fluids of the immune animal seems unexplained by the slight pH difference. No explanation for this phenomenon has thus far been found.

However, definite data have been obtained which seem to explain the more accelerated inflammatory response that characterizes the immunized animal. In the first place the very fact that non-specific irritants also bring about an acceleration of the succession of cells that characterize inflammation in general in the tuberculous animal suggests that this may be based on a heightened physiological activity on the part of the sensitized animal. Again the mononuclears at the site of a non-specific inflammation in the tuberculous animal are larger, have more abundant cytoplasm, and in supravital smears, frequently contain more numerous and prominent neutral red stained vacuoles, which are often of a yellower tint than those of mononuclears mobilized

in the normal animal. Their pseudopodia also are in many cases more prominent. These differences all suggest an increased physiological activity on the part of the immune phagocytes. Furthermore the rate of both mitotic and amitotic division of mononuclears is conspicuously greater in the exudate of immunized rabbits and guinea pigs than in that of normal animals. It would seem that it is this increased rate of division that is a significant factor in their more rapid mobilization in the immunized animal in response to both specific and non-specific irritants.

Evidence was obtained suggesting an increase in the phagocytic capacity of the immune cells for carbon particles *in vivo*. This, however, was not conclusive. But *in vitro*, unequivocal and statistically significant observations indicate that, in the presence of normal or immune serum, mononuclears derived from tuberculous or vaccinated guinea pigs ingest more carbon particles than mononuclears obtained from normal animals. Mononuclears originating in tuberculous rabbits ingest more staphylococci than do these phagocytes when obtained from normal animals. A similar, though statistically less significant increment in the phagocytic capacity of mononuclears for collodion particles was observed in cells derived from vaccinated rabbits. Thus, the physiological phagocytic activity of the mononuclears of tuberculous rabbits and guinea pigs is enhanced nonspecifically in relation to substances that have no bearing on the tuberculous process.

Furthermore mononuclears derived from tuberculous rabbits and guinea pigs exhibit an enhancement in their phagocytic capacity for virulent tubercle bacilli in the presence of both normal and immune serum which is of fairly good statistical significance.

It is clear, therefore, that what has been inferred from the studies on the correlation between the fate of the bacilli and the host responses *in vivo*, namely that immunity to tuberculosis rests upon an increased physiological capacity of the mononuclear phagocytes to destroy or inhibit the growth of tubercle bacilli, has found a suggestive confirmation in these *in vitro* studies. For it is plain that tuberculosis confers upon the mononuclears an increased capacity to divide in response

to irritation, and a capacity to ingest a greater amount of particles available for phagocytosis, whether these be non-specific substances, such as carbon particles, staphylococci or collodion, or specific tubercle bacilli.

It is significant in this relation that the enhancement of the phagocytic capacity for tubercle bacilli afforded mononuclears by a virulent active tuberculosis is greater than that conferred upon them by vaccination with avirulent organisms. Indeed the increment noted in the latter is hardly of any statistical significance. This parallels the well known fact that active tuberculosis confers a greater relative immunity to reinfection than vaccination with an organism of low virulence.

The limited observations of a more acid pH within the cytoplasm of immune mononuclear phagocytes suggest that, just as their propensity to divide and to phagocyte is increased by the presence of tuberculosis, so may it be with their digestive capacities. This would lead to the more rapid destruction or more effective inhibition of the growth of tubercle bacilli within their cytoplasm, as has been previously concluded.

It is interesting in this connection that Metchnikoff, the founder of the theory of phagocytosis, originally held that the increased phagocytosis observed on reinoculation of an animal that had recovered from an acute infectious disease was due to an enhancement of the ingesting capacity of the phagocytes (20). It was soon shown, however, that in these conditions, the increased phagocytosis was the result of specific bacteriotropins present in the serum of the immunized animal and could be elicited with phagocytes obtained from a normal animal. It is clear from this study that in tuberculosis, beside the phagocytosis-promoting antibodies that accumulate in the blood, there also develops a generalized increased physiological activity on the part of these cells, one of which is an increased phagocytic capacity for specific as well as for non-specific substances.

These observations throw light on a number of obscure phenomena. Lewis and Loomis (21) have observed that tuberculous guinea pigs generate antibodies more rapidly than normal animals similarly

stimulated. Dienes (22) discovered that the presence of tuberculous lesions greatly intensifies the pre-anaphylactic sensitizing capacity of antigens. It has been claimed by some observers (23) that tuberculous animals resist other infections with greater efficiency than non-tuberculous individuals.

It is possible that these observations are expressions of the enhanced physiological activity on the part of the mesenchymal cells as demonstrated in this study. For in the production of antibodies and in resistance to infections, the rôle of these cells is certainly significant.

CONCLUSIONS

1. Tuberculous and vaccinated rabbits and guinea pigs mobilize mononuclear phagocytes at the site of a non-specific inflammation with greater rapidity than do normal animals, just as they respond to tubercle bacilli.

2. The succession of cells that characterizes inflammation in general is accelerated in allergic rabbits and guinea pigs in response to non-specific irritants.

3. The pH at the site of reinfection with tubercle bacilli in immunized rabbits and guinea pigs and at the site of a non-specific inflammation in the latter is slightly lower than in a similar site in a normal animal.

4. No constant relation was found between the mobilization of mononuclears and the hydrogen ion concentration at the site of inflammation.

5. The rate of mitotic and amitotic division of mononuclears in allergic rabbits and guinea pigs in response to non-specific irritants is greater than in normal animals.

6. Mononuclears derived from actively tuberculous or vaccinated guinea pigs exhibit greater *in vitro* phagocytic capacity for carbon particles than mononuclears obtained from normal animals.

7. Mononuclears of tuberculous rabbits ingest more staphylococci than the phagocytes of the same type originating from normal animals.

8. Mononuclears originating from actively tuberculous rabbits and guinea pigs exhibit greater *in vitro* phagocytic capacity for tubercle bacilli than mononuclears obtained from normal animals.

9. The enhancement of the phagocytic capacity for tubercle bacilli afforded mononuclears by vaccination with a bacillus of low virulence is lower, and of questionable significance.

10. The increased phagocytic activity of mononuclears derived from tuberculous or vaccinated rabbits and guinea pigs for tubercle bacilli and for non-specific particulate matter occurs in media containing sera derived from normal and from tuberculous individuals.

11. The more rapid mobilization of mononuclears by immunized animals in response to specific as well as non-specific irritants is associated with their increased physiological activity.

The significance of this enhanced activity conferred by the tuberculous process on the mesenchyme cells is discussed in relation to the mechanism of immunity to tuberculosis and other phenomena.

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EXPLANATION OF PLATES

Smears depicted in Figs. 1 to 7 inclusive were prepared with Wright's stain. Those depicted in Figs. 8 to 13 inclusive were stained with the Ziehl-Neelsen procedure. The magnifications are about × 1400.

PLATE 35

FIG. 1. *In vivo* phagocytosis of carbon particles by mononuclears of tuberculous guinea pig 30.

FIG. 2. *In vivo* phagocytosis of carbon particles by mononuclears of normal guinea pig 73.

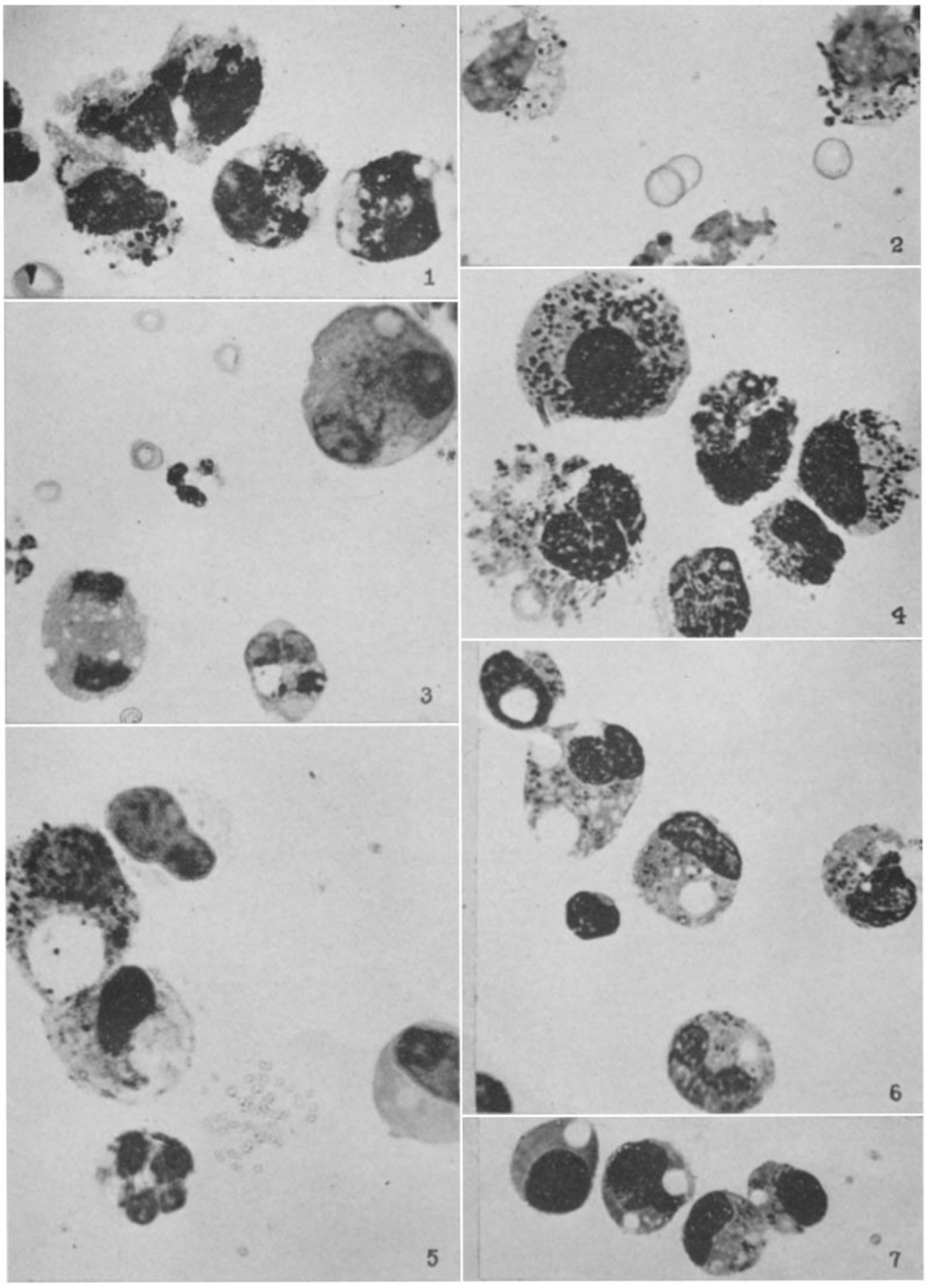
FIG. 3. Mitotic and amitotic division of mononuclears in exudate of tuberculous guinea pig 93.

FIG. 4. *In vitro* phagocytosis of carbon particles by mononuclears of tuberculous guinea pig 36-1 in the presence of its own serum.

FIG. 5. *In vitro* phagocytosis of carbon particles by mononuclears of normal guinea pig 41-1 in the presence of the serum of tuberculous guinea pig 36-1, the cells of which are shown in Fig. 4.

FIG. 6. *In vitro* phagocytosis of staphylococci by mononuclears of tuberculous rabbit 20-7 in the presence of serum of normal rabbit 10-6, the cells of which are shown in Fig. 7.

FIG. 7. *In vitro* phagocytosis of staphylococci by mononuclears of normal rabbit 10-6 in the presence of its own serum.



(Lurie: Mechanism of immunity in tuberculosis)

PLATE 36

FIG. 8. *In vitro* phagocytosis of collodion particles by mononuclears of vaccinated rabbit 33-7 in the presence of its own serum.

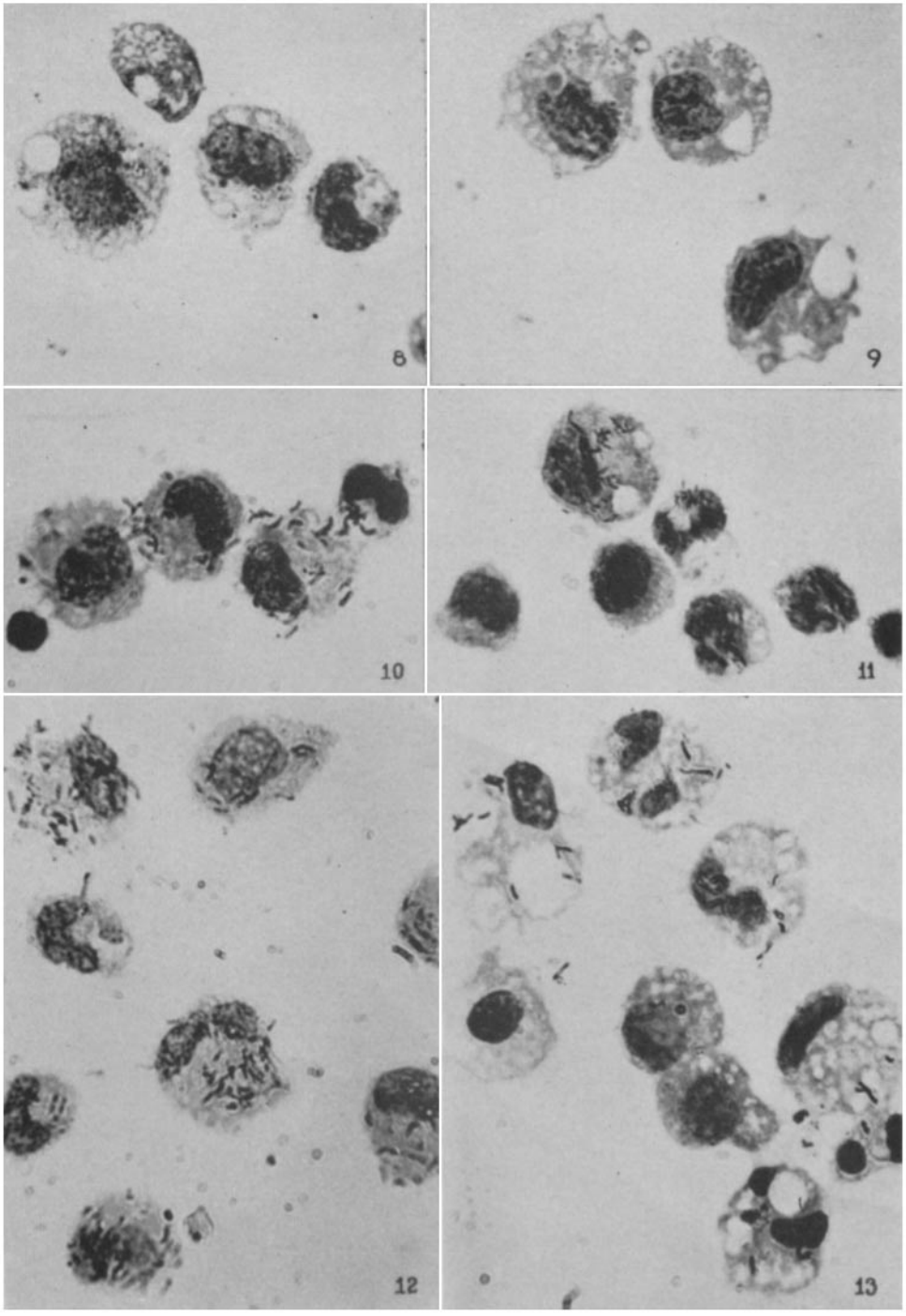
FIG. 9. *In vitro* phagocytosis of collodion particles by mononuclears of normal rabbit 33-6 in the presence of serum of vaccinated rabbit 33-7, the cells of which are shown in Fig. 8.

FIG. 10. *In vitro* phagocytosis of tubercle bacilli by mononuclears of tuberculous guinea pig 94 in the presence of its own serum.

FIG. 11. *In vitro* phagocytosis of tubercle bacilli by mononuclears of normal guinea pig 30-1 in the presence of serum of tuberculous guinea pig 94, the cells of which are shown in Fig. 10.

FIG. 12. *In vitro* phagocytosis of tubercle bacilli by mononuclears of tuberculous rabbit 16 in the presence of its own serum.

FIG. 13. *In vitro* phagocytosis of tubercle bacilli by mononuclears of normal rabbit 31-8 in the presence of serum of tuberculous rabbit 16, the cells of which are shown in Fig. 12.



(Lurie: Mechanism of immunity in tuberculosis)