STUDIES ON TUBERCLE BACILLUS-MONOCYTE RELATIONSHIP*

I. Quantitative Analysis of Effect of Serum of Animals Vaccinated with BCG upon Bacterium-Monocyte System

By JACOB FONG, Ph.D., PATRICIA SCHNEIDER, and SANFORD S. ELBERG, Ph.D.

(*From the Department of Bacteriology, University of California, Berkeley*)

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Studies of the mechanism of immunity in tuberculosis have focused attention upon a number of interesting but rather controversial issues. One of these is concerned with the relative importance of humoral and cellular factors in immunity.

The role of mononuclear phagocytes in the destruction of human tubercle bacilli in the natively resistant animal has been reported by Lewis and Sanderson (1). Histological and cultural studies by Lurie (2) of the fate of tubercle bacilli contained in normal and immune phagocytes cultivated in the anterior chambers of the eyes of normal rabbits have suggested a greater capacity of the mononuclear phagocytes of the tuberculous animal to inhibit multiplication of tubercle bacilli. The recent experiments by Suter (3) appear to confirm Lurie's conclusions that the mononuclear cells of immune animals are peculiarly and specially endowed with the capacity for handling virulent tubercle bacilli. Investigations by Mackaness (4), on the other hand, revealed no significant differences in the behavior of normal and immune monocytes toward virulent strains of *Mycobacterium tuberculosis*.

Although various reasons may account for these diametrically opposed findings, it seemed likely that a major contributing factor was the absence of any quantitative enumeration of the monocyte population used in the experiments. Since the conclusions are based on the numbers of intracellular bacilli per total monocyte population, it is axiomatic that either a decrease or an increase in cell population would markedly distort the final results.

The present investigation is an attempt at a more precise quantitative analysis of the problem. The survival of cell populations when cultivated *in vitro* under various conditions in the Mackaness type of chamber is described. Data indicating a degenerative effect of virulent tubercle bacilli upon normal and immune cells cultivated *in vitro* and an inhibitory or delaying effect of sera of animals vaccinated with BCG upon this cell degeneration are also presented.

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Materials and Methods

Monocytes.—An adult normal or BCG-vaccinated rabbit was used as a source of normal or immune monocytes. Each rabbit was inoculated intraperitoneally with 50 cc. of oil (klearol) 5 days prior to collection of monocytes. At the end of this period, the animal was sacrificed and the peritoneal cavity washed with 300 to 400 ml. of chilled Tyrode's solution. The wash fluid was collected by suction into a separatory funnel and the oil allowed to separate. The cell suspension in the lower portion of the container was filtered through a thin layer of gauze into a large centrifuge bottle and the cells sedimented by centrifugation at 250 *g* for 3 minutes. Following removal of the supernatant fluid, the deposited cells were redispersed in 6 to 8 ml. of freshly prepared 0.25 per cent trypsin in Tyrode's solution. This trypsin cell suspension was kept at room temperature (24 to 26°C.) for the desired period of time (25 minutes in most experiments) after which the trypsinized cells were washed twice with large volumes (200 ml.) of chilled Tyrode's solution. Subsequent to the second washing the packed cells were resuspended in a small volume of chilled Tyrode's solution and equal amounts of this distributed into small centrifuge tubes. Each of these cell suspensions was washed once again with Tyrode's solution and the cells sedimented by centrifugation at 250 *g* for 3 minutes. The packed cells were thoroughly dispersed in 3 to 5 cc. of freshly collected normal or immune rabbit serum (BCG-vaccinated animals) and the number of cells determined in a hemocytometer. These cells were then parasitized in the manner described below.

In experiments which involved comparison of trypsinized and nontrypsinized monocytes, the procedure for preparation of the latter was identical with the method described above except for the addition of trypsin. When various nutrients and balanced salt solutions other than Tyrode's solution were studied, the appropriate substitutions of reagents were made in the above procedure.

Cell suspensions obtained 5 days after administration of oil (arbitrarily designated as monocyte suspensions) generally contained 85 per cent or more large mononuclear cells although other cell types such as leucocytes, small mononuclear cells, and a few fibroblasts were also present.

Bacteria.—An H37Rv strain of *Mycobacterium tuberculosis* originally obtained from the Phipps Institute, Philadelphia, was used in these studies. This strain, which was recently passaged in guinea pigs and reisolated from infected tissues, has been maintained on Trudeau medium. For use in parasitization of monocytes, transplants of the stock culture were made in tween-albumin liquid medium. A week old culture of H37Rv in tween-albumin was centrifuged at 850 *g* for 30 minutes to sediment the bacteria. The packed bacilli were redispersed and washed twice with volumes of fresh medium equivalent each time to the original volume of fluid in the culture. Following the second washing and centrifugation at 850 *g* for 30 minutes, the sediment was resuspended in a small volume of medium and centrifuged at 250 *g* for 3 minutes to remove larger aggregates. The supernatant fluid, which largely consisted of bacilli occurring singly, was used for parasitization of monocytes after enumeration of the bacterial content in a Petroff-Hausser chamber under darkground illumination.

The BCG strain of *Mycobacterium tuberculosis* used for immunization of rabbits was also obtained from the Phipps Institute. A stock culture of this was maintained in Calmette's potato medium. For immunization the culture on potato medium was ground with steel balls and suspended in physiological saline. The number of bacteria was determined and the bacillary suspension was standardized to contain 2.5 to 5.0 $\times$ 10^8 bacteria per ml.

Parasitization of Monocytes.—The H37Rv strain of tubercle bacilli and normal or immune monocytes suspended in fresh normal or immune rabbit serum were mixed in the desired ratios (approximately 10 bacilli per monocyte in most experiments); 0.5 ml. of the mixture was placed in paraffin-lined bottles, centrifuged at 850 *g* for 5 minutes and refrigerated at 4°C. for 1 hour. At the end of this time the supernatant fluid was removed as completely as possible and the

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sediment resuspended in a small volume of the appropriate nutrient medium. The number of monocytes was determined in a hemocytometer and the infected monocyte suspension diluted with appropriate amounts of the proper nutrient medium to yield approximately 15 monocytes per cmm. This diluted suspension of infected monocytes was used for *in vitro* culture in the manner described below.

Uninfected control suspensions of monocytes were handled identically except that a volume of tween-albumin medium, equivalent to the volume of bacterial suspension used in parasitization, was added in place of the bacillary suspension.

Nutrient Media.—The standard nutrient medium in these studies consisted of 40 per cent aged, Selas-filtered rabbit serum (normal or immune) in Tyrode's solution. The pH of the medium was adjusted to 7.4 with 5 per cent CO_2 in air. The amount of serum was varied in some experiments to establish the effect of serum concentration upon survival of monocytes.

In experiments involving studies of other types of balanced salt solutions and nutrients on the maintenance of monocytes cultivated *in vitro*, appropriate substitutions of reagents were made. The following reagents were analyzed in this respect: Hanks's solution, lactalbumin–yeast extract medium, TC No. 199 medium (Difco) and TACPI medium (Tyrode–amino acids–cocarboxylase-*p*-aminobenzoic acid–insulin) as described by Trowell (5) for cultivation of lymph node cells.

Normal and Immune Sera.—Normal serum consisted of pooled sera of adult tuberculin-negative rabbits.

Immune serum was obtained from adult rabbits immunized with the BCG strain of tubercle bacilli prepared in the manner previously described. This represented pooled sera derived from rabbits given 1 to 3 injections of BCG (0.2 to 0.3 ml. intradermally in first dose and similar amounts intravenously in subsequent doses with about 30 days intervening between injections) and bled 4 to 5 weeks after the last injection. Most of the animals used as sources of immune serum had received more than one injection of BCG. All animals were tested for tuberculin sensitivity (0.2 ml. of 1:100 O.T. intradermally) and only positive reactors were used.

Normal and Immune Monocytes.—The same animals which were used as sources of normal and immune sera served as donors of normal and immune monocytes.

Cultivation of Monocytes.—The culture chamber used in these experiments has been described in detail by Mackaness (6). In brief, unplasticized "perspex" was cut in the shape of a slide with a central aperture and a counterbore on each side of the aperture to accommodate standard ⅞ inch coverslips. The space enclosed by a coverslip on the bottom side of the aperture and another on the top side of the aperture represented the culture chamber proper. The coverslips were sealed into position with paraffin. Before insertion of the bottom coverslip a small "perspex" ring (6 mm. internal bore) was affixed with paraffin to the coverslip. Two small lateral drill holes in the slide provided a means for introducing materials into and removing materials from the culture chamber when the coverslips were in position. When not in use the lateral drill holes were closed by two stainless steel pins set in a "perspex" plug.

The procedure for setting up the monocyte culture was somewhat different from that described by Mackaness (6). The bottom coverslip of the chamber was sealed into position and approximately 0.05 ml. of a monocyte suspension containing 15 cells per cmm. was introduced into the space enclosed by the small "perspex" ring. This yielded a total of about 500 to 1000 monocytes in the center well of the chamber. The top coverslip was inserted and the chamber left at room temperature for 10 to 15 minutes to allow settling and adherence of cells to the bottom coverslip. Sufficient nutrient medium to fill approximately two-thirds of the culture chamber was introduced *via* one of the lateral drill holes. The entire chamber was sealed by insertion of the stainless steel pins and incubated at 37°C.

Examination of Cultures.—The number of cells in the central well of the culture chamber was determined at the start of an experiment and at certain intervals thereafter. Counts were

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made with a phase contrast microscope and a 10 × objective. Enumeration of cells was facilitated by a special square constructed in the ocular of the microscope. This square was subdivided into nine smaller squares by cross hairs. The large square was correlated with horizontal and vertical line markings on a specially constructed mechanical stage whereby each marking on the stage corresponded to the area encompassed by the large square in the ocular. The entire area in which the monocytes were confined was covered in the following manner: The small "perspex" ring was brought into the field of vision of the objective, and the outer margins of the ring at its widest points in the horizontal and vertical axes were located. This served to delineate the area of examination as an imaginary square within which was located the "perspex" ring. By starting at one corner of this imaginary square and moving the stage one marking at a time in a horizontal direction, the monocytes along the entire width of the imaginary square were counted. The stage was then moved one marking in a vertical direction and the horizontal movements repeated. These successive movements were continued until the entire area of the imaginary square was included.

Morphologically intact cells only were counted, and no attempt was made to distinguish different cell types.

The reproducibility of cells counts by this method has been determined; counts made by different but experienced individuals did not vary more than 5 per cent, and replicate counts by the same worker seldom varied by more than 2 per cent.

Staining of Preparation on Coverslips.—When it was desired to establish the proportion of infected monocytes following parasitization, a coverslip was prepared as described under cultivation of monocytes and the parasitized cell suspension introduced into the space confined by the "perspex" ring. Following adherence of cells to the coverslip, the fluid was removed with a capillary pipette and the specimen allowed to air-dry. The specimen was fixed with heat, passed through successive changes of xylol and alcohol, and stained by the Ziehl-Neelsen method.

Proportion of Infected Cells.—The proportion of infected cells after parasitization was determined by counting a total of 200 stained cells.

EXPERIMENTAL

Effect of Trypsinization upon Cell Survival.—Earlier attempts at *in vitro* cultivation of untreated cell suspensions derived from a cellular exudate induced by oil in normal rabbit peritoneum consistently yielded cultures which exhibited varying degrees of degeneration after 24 hours of incubation. In order to minimize this initial loss of cells, it was deemed necessary to select only the sturdier cells for use in cultivation. Of the various procedures tested, exposure of cells to 0.25 per cent trypsin in Tyrode's solution at room temperature proved most satisfactory. The survival of trypsinized and non-trypsinized cells cultivated *in vitro* in the presence of 40 per cent normal rabbit serum in Tyrode's solution is set forth in Table I.

It is evident from the results shown in this table that non-trypsinized cells possessed less survival potential than similar cells preliminarily subjected to varying periods of exposure to proteolytic enzyme. The different capacities of these two categories of cells to survive during *in vitro* cultivation, as reflected in the average per cent degeneration of cells, were most manifest in the first 24 hours and somewhat less apparent after 48 and 72 hours of incubation. The period of trypsinization of cells influenced their ultimate survival. Thus,

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trypsinization of cells for 20 to 35 minutes at room temperature permitted better maintenance of cells than that afforded by a 5 minute period of exposure to enzyme; this difference in survival of the two groups of trypsinized cells was apparent at both the 24 and 48 hour intervals.

While the results of Table I definitely indicate a better level of cell maintenance after treatment with trypsin, the data do not fully depict the advantages of preliminary trypsinization of cells. This is better demonstrated in Table II where it may be seen that trypsinized cells cultivated in 40% normal rabbit serum in Tyrode's solution exhibited only minor changes in cell population over a period of approximately 72 hours. This situation is more truly representative of all later experiments, the results of which are presented in Tables II, III and IV. The reasons for the apparent differences in survival of

TABLE I

Relationship between Trypsinization and Cell Survival

Time of trypsinization*	No. of monocyte donors‡	Average per cent degeneration (hrs. after incubation)§		
		24	48	72
min.				
0	4	23.5	48.1	73.4
5	3	14.7	31.5	41.8
20	4	8.5	19.0	40.7
35	3	6.0	12.0	47.3

* Trypsinization was carried out at room temperature.

‡ Indicates number of rabbits used as sources of monocytes; the monocytes of each rabbit were cultivated separately and in duplicate or triplicate.

§ Refers to average per cent degeneration (from initial count) of all cultures in a given series; thus, if 4 rabbits were used and each cell suspension were prepared in duplicate or triplicate, the figures would represent the average per cent degeneration of 8 to 12 cultures.

trypsinized cells shown in Tables I and II were not investigated, but it should be pointed out that slight modifications in procedure were made in all experiments subsequent to those shown in Table I. Thus, in later experiments, cellular exudates were harvested in 5 instead of 4 days following injection of oil, and the period of exposure of cells to trypsin was standardized at 25 minutes.

Survival of Cells in Different Media.—Since non-trypsinized cells cultivated in 40 per cent normal rabbit serum in Tyrode's solution exhibited gross degeneration during the first 24 hours of incubation, the possibility existed that the nutrient medium used might have been inadequate; moreover, since both Mackaness (4) and Suter (3) had employed Hanks's solution in their studies, it seemed advisable to compare the relative survival of cells grown in rabbit serum in the presence of these two balanced salt solutions. In addition, three other nutrient media, TC No. 199, lactalbumin-yeast, and the TACPI medium of Trowell (5) were studied.

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In the experiments shown in Table II, cells obtained from normal rabbits 5 days after injection of oil and trypsinized 25 minutes at room temperature were cultivated in various media. As measured by the average per cent degeneration of cells after 24 hours of incubation, TC, lactalbumin-yeast, and TACPI media failed to yield adequate maintenance of cells. The amount of degeneration in these media, as opposed to that occurring in 40 per cent normal rabbit serum in Tyrode's solution, was approximately 12 to 18 times greater. In view of the poor results with these media, no counts were made after 24 hours

TABLE II

Survival of Monocytes in Different Media

Composition of Medium*	Average per cent degeneration (hrs. after incubation)			Ratio of degeneration in each medium/degeneration in 40 per cent RS-Tyrode (hrs. after incubation)		
	24	48	72	24	48	72
40 per cent RS-Hanks	15.3 (12)‡	18.7 (6)	6.3§ (3)	6.9:1	3.9:1	2.5:1
40 per cent RS-TC(199)	25.7 (3)	—	—	11.7:1	—	—
40 per cent RS-LY	27.7 (6)	43.5 (2)	—	12.6:1	9.1:1	—
35 per cent RS-TACPI	40.8 (8)	—	—	18.5:1	—	—
40 per cent RS-Tyrode	2.2 (67)	4.8 (37)	2.5§ (10)	1:1	1:1	1:1
20 per cent "	35.0 (3)	—	—	15.9:1	—	—
5 per cent "	45.0 (3)	—	—	20.4:1	—	—

— Indicates samples not counted.

* RS refers to normal rabbit serum; LY indicates lactalbumin-yeast medium; TACPI (see reference 5 of bibliography).

‡ The numbers in parentheses indicate the total number of culture chambers counted in the course of several experiments.

§ A number of the cultures counted showed an increase in cell population at 72 hours; these chambers were arbitrarily recorded as 0 per cent degeneration in calculating the average per cent degeneration.

except for one experiment with lactalbumin-yeast medium in which a slight further degeneration of cells was noted after incubation for 48 hours.

Comparison of the results obtained in 40 per cent normal rabbit serum in Hanks's solution with that in Tyrode's solution indicated a better survival of cells in the latter. The advantage of Tyrode's solution over Hanks's solution was most noticeable at 24 hours but a significant difference was still apparent at the 48 hour interval. The average per cent degeneration of cells in Tyrode's solution throughout the experimental period of 72 hours was within the range of the limits of error of the method of enumeration; hence, the degree of degeneration recorded may not necessarily represent loss of cells during this period. It may be noted that cellular multiplication occurred in some though not all of the cultures, as evidenced by a decrease in the average per cent

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degeneration of cells at 72 hours; this was true for cells cultivated in serum-Hanks medium as well as those grown in serum-Tyrode medium. Subsequent experiments (not shown) have indicated that although proliferation of cells may commence even after 24 hours of incubation, it more generally occurred somewhere between 40 and 72 hours. Studies on the influence of serum concentration upon cell survival, carried out in a limited number of chambers, indicated a more beneficial effect of high serum concentration. The amount of cellular degeneration in 5 to 20 per cent serum was 15 to 20 times greater than that with 40 per cent serum.

Degenerative Action of Tubercle Bacilli upon Cells.—Although cultivation of uninfected, trypsinized cells in 40 per cent normal rabbit serum in Tyrode's solution provided a consistent procedure for maintenance of cells *in vitro*, cells which had been parasitized with the virulent H37Rv strain of *Mycobacterium tuberculosis* and cultivated under similar conditions exhibited various degrees of degeneration. This degeneration was reflected in a disappearance of monocytes and some accumulation of granular debris. Actual lysis of cells was not seen since monocyte cultures were not under continuous observation; however, it was observed that "shadow" forms (non-refractile cells with indefinite boundaries and absence of internal structures) invariably preceded a decreased total monocyte count.

The degenerative effect of virulent tubercle bacilli on monocytes is shown in Table III. The experiments in this table were made with the trypsinized cells of normal rabbits and rabbits rendered tuberculin-positive by 1 to 3 injections of BCG. It may be seen that uninfected normal or immune cells (those obtained from rabbits injected with BCG) cultivated in the presence of normal rabbit serum showed little change in cell population during the experimental period. Very marked degeneration of both normal and immune cells occurred, however, upon parasitization of cells with virulent tubercle bacilli. The average per cent degeneration of normal or immune cells after exposure to H37Rv was about 26 to 35 per cent after 24 hours of incubation. Apparently immune cells derived from animals receiving three injections of BCG were no more resistant to the degenerative action of tubercle bacilli than were cells from animals given one or two injections of BCG. A further slight increase in cell degeneration in infected cultures was recorded after 48 hours, but its significance may be questioned.

It is of interest that the average per cent degeneration of parasitized cells was less at 72 hours than at either of the two preceding intervals. One inference which may be drawn is that some degree of cell proliferation occurred despite the presence of virulent tubercle bacilli. The number of experiments shown in Table III, in which parasitized cells exhibited an increased cell count, is small, but various other experiments (not shown) have tended to confirm this observation.

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Inhibition of H37Rv-Induced Cellular Degeneration by Serum of BCG-vaccinated Rabbits.—The fact that none of the usual manifestations of antibody

TABLE III

Survival of Cells after Infection with Tubercle Bacilli

Treatment of cells*	Average per cent degeneration (hrs. after incubation)		
	24	48	72
Uninfected normal cells	1.4 (13)‡	5.1 (11)	5.5 (2)
Infected normal cells	26.4 (15)	35.2 (13)	12.5 (2)
Uninfected immune cells§	2.4 (10)	2.0 (9)	0 (2)
Infected immune cells§	28.8 (13)	36.8 (13)	10.3 (3)
Uninfected immune cells‖	0 (4)	0 (2)	—
Infected immune cells‖	34.8 (6)	39.0 (3)	—

The proportion of infected monocytes in the parasitized cell suspensions of Tables III and IV varied in different experiments, but was generally between 20 and 30 per cent.

* All cultures were made in 40 per cent normal rabbit serum in Tyrode's solution.

‡ Numbers in parentheses refer to total number of cultures counted.

§ Refers to cells from rabbits given one or two injections of BCG.

‖ Refers to cells from rabbits given three injections of BCG.

TABLE IV

Effect of Serum of BCG-Vaccinated Animals upon Survival of Infected Cell Cultures

Nature of sample tested*	Average per cent degeneration (hrs. after incubation)			Ratio of degeneration in each sample/degeneration of immune cells in immune serum‡ (hrs. after incubation)		
	24	48	72	24	48	72
Infected normal cells in normal serum	28.6 (10)	34.1 (9)	12.5 (2)	9.9:1	6.2:1	4.1:1
Infected normal cells in immune serum	4.3 (11)	26.4 (11)	5.5 (2)	1.5:1	4.8:1	1.8:1
Infected immune cells in normal serum	27.4 (14)	33.2 (14)	10.3 (3)	9.4:1	6.0:1	3.4:1
Infected immune cells in immune serum	2.9 (13)	5.5 (13)	3.0 (2)	1:1	1:1	1:1

* Control (uninfected) cultures not shown since results on controls resembled data given in Tables II and III.

‡ The terms immune cells and immune serum are used qualifiedly (to designate a difference between normal and BCG-vaccinated animals).

action has been so far related to immunity in tuberculosis made it advisable to investigate the action of immune serum upon the degenerative effect of tubercle bacilli on cells. The results of these studies are given in Table IV.

It is evident that the serum of BCG-vaccinated rabbits exerted a noticeably

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favorable effect upon survival of cells following their parasitization with the virulent H37Rv strain. Whereas infected normal cells cultivated in normal serum exhibited marked degrees of cell degeneration in 24 hours, similar cells maintained in immune serum showed no change in cell population (the average per cent degeneration of 4.3 approximates limits of error inherent in method of counting) during the same interval. The protection of normal cells by immune serum, however, seemed to be merely a delaying action, for the degree of cell degeneration in immune serum after 48 hours approximated that of normal cells in normal serum.

A more persistent inhibition of the cellular degeneration induced by virulent tubercle bacilli was observed when immune cells were cultivated in immune serum. This prolonged protection of immune cells by immune serum is of particular interest; while the results of Table III, when taken alone, indicated no significant difference in the behavior of normal or immune cells, the results of Table IV would seem to imply a basic dissimilarity in immune and normal cells.

DISCUSSION

It is quite apparent that the role of cells in immunity against tuberculosis can be assessed only under properly controlled conditions of experimentation. Previous studies in this direction (3, 4) have used the numbers of intracellular bacteria as indication of suppression or non-suppression of bacterial multiplication; to be meaningful, such studies must be limited to a period during which there is neither an increase nor a decrease in cell population. The present paper has analyzed some of the requisite conditions for maintenance of constant cell populations *in vitro*. Preliminary trypsinization of cells is of prime importance in this connection to prevent a loss in the number of cells during the early hours of cultivation. The type of nutrient medium used for cultivation of cells may also influence the final results, and the present studies indicate the superiority of 40 per cent rabbit serum in Tyrode's solution over other types of nutrient media tested. A third factor to be considered may be the concentration of serum, for reduction in serum concentration resulted in a lessened survival potential of cells. The influence of environmental factors is not surprising and has been reported in one sense or another, as, for example, the effect of horse serum upon phagocytosis of tubercle bacilli by cells cultivated *in vitro* (7).

Various investigators (8–11) have demonstrated the relationship between tuberculin hypersensitivity and white blood cells types, and Favour (12) has described the cytotoxic effect of tuberculin on fluid suspensions of white blood cells of tuberculous animals. The present studies have indicated that a certain proportion of cells living in close proximity to virulent tubercle bacilli eventually succumbed, either to the organisms themselves or to products elaborated by them. The cytotoxic effect of tuberculin upon sensitized cells could hardly

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provide an adequate explanation for the results reported herein, since normal unsensitized cells proved as susceptible to the degenerative effect induced by virulent tubercle bacilli as sensitized cells. Further studies in this connection and in relation to the underlying mechanism in induction of cell degeneration are in progress.

The conclusion that proliferation of cells occurred in parasitized cultures seemed warranted in view of repeated increases in cell counts after 40 or more hours of cultivation. Whether this proliferation involved only uninfected cells or infected cells as well was not established in these experiments.

Various references in the literature (13–17) may be interpreted as indicative of an apparently insignificant role of humoral factors in immunity against tuberculosis; such studies have been concerned on the whole with the usual manifestations of antibody action. The degenerative action of virulent tubercle bacilli upon cells suggested a possible role for antibody in immunity to tuberculosis. The observation that sera of animals vaccinated with BCG suppressed or delayed destruction of parasitized cells would appear to support this concept if it should subsequently be demonstrated that this activity of the serum is related to antibody. Studies are in progress to establish the nature and specificity of this serum activity.

A further point of interest which emerged from these studies was the greater capacity of immune cells to resist the necrotizing effect of virulent tubercle bacilli; this situation was valid only for immune cells cultivated in immune serum and not for similar cells maintained in normal serum.

SUMMARY

Studies of the conditions necessary for maintenance of constant cell populations *in vitro* in the Mackaness type of culture chamber have indicated the importance of preliminary trypsinization of cells and the beneficial effect of 40 per cent rabbit serum in Tyrode's solution. Under these optimal conditions, uninfected suspensions of monocytes exhibited little change in cell numbers over a period of 40 to 72 hours.

Infection of monocytes with the virulent H37Rv strain of tubercle bacillus resulted in an early degeneration of a certain proportion of the cells cultivated in the presence of normal rabbit serum. This degeneration was apparent not only for cells of tuberculin-negative animals but also for those derived from tuberculin-positive animals.

The serum of animals vaccinated with BCG exerted a favorable effect upon the survival of monocytes infected with virulent tubercle bacilli. Treatment with this serum caused a delay in degeneration of infected normal cells (cells of tuberculin-negative rabbits) and a complete inhibition of degeneration of infected immune cells (cells of rabbits vaccinated with BCG).

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