6. Borst, P., Slater, E. C., Biochem. Biophys. Acta, 1961, v48, 362.
7. Crane, R. K., Lipmann, F., J. Biol. Chem., 1953, v201, 235.
8. Lardy, H. A., Johnson, D., McMurry, W. E., Arch. Biochem. Biophys., 1958, v78, 578.
9. Huijing, F., Slater, E. C., J. Biol. Chem., 1961, v49, 493.
10. Youngs, J. N., Cornatzer, W. E., Proc. Soc. Exp. Biol. and Med., 1963, v112, 308.
11. Vitale, J. J., Nakamura, M., Hegsted, D. M., J. Biol. Chem., 1957, v228, 573.
12. Buahene, K., Cornatzer, W. E., Arch. Internat. de Physiol. et de Biochem., 1963, v71, 195.
13. Nelson, D. R., Cornatzer, W. E., Fed. Proc., 1963, v22, 359; Endocrinology, 1965, v77, 37.
14. Artom, C., J. Biol. Chem., 1941, v139, 953.
15. Fiske, C. H., Subbarow, Y., ibid., 1925, v66, 375.
16. Hogeboom, C. H., Schneider, W. C., Pallade, G. E., ibid., 1948, v172, 619; Schneider, W. C., Hogeboom, G. H., ibid., 1950, v183, 123.
17. Swanson, M. S., S. P. Colowick, N. D. Kaplan, eds., Methods in Enzymology, II, Academic Press, New York, 1955, p541.
18. Hurlbert, R. B., Schmitz, H., Brumm, A. F., Potter, V. R., J. Biol. Chem., 1954, v209, 23.
19. Hevesy, G., Enzymologia, 1958, v5, 138.
20. Zilversmit, D. B., Enternman, C., Fishler, M. C., J. Gen. Physiol., 1943, v26, 325.
21. Cornatzer, W. E., Sarosi, G. A., Newland, J. R., Proc. Soc. Exp. Biol. and Med., 1961, v107, 463.
22. Miller, J. E., Cornatzer, W. E., ibid., 1965, v118, 948.
23. Chambers, E. G., Statistical Calculation for Beginners, Cambride Univ. Press, N. Y., 1952, 2nd edit.; Dixon, W. J., Massey, F. J., Introduction to Statistical Analysis, McGraw-Hill, 1957, 2nd edit.
24. Kennedy, E. P., J. Biol. Chem., 1953, v201, 399.
25. Garbus, J., DeLuca, H. F., Loomans, M. E., Strong, F. M., ibid., 1963, v238, 59.

Received February 26, 1965. P.S.E.B.M., 1965, v120.

Hydrolases of Rabbit Macrophages. III. Effect of BCG Vaccination, Tissue Culture, and Ingested Tubercle Bacilli.* (30513)

KIMIFUSA MIZUNOE† AND ARTHUR M. DANNENBERG, JR.
Division of Radiobiology, Department of Radiological Sciences, School of Hygiene and Public Health, and Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, Md.

Rabbit macrophages contain active proteases, esterases, lipase, lysozyme and acid phosphatase(1,2,3). To provide information on the *rate* at which immune and normal MN‡ might increase these hydrolases in the presence of specific antigen, the effects of (a) BCG vaccination, (b) a 2-day residence in cell culture, and (c) ingestion of heat-killed tubercle bacilli were investigated. The hydrolase levels of peritoneal and alveolar macrophages were also compared. These studies represent an effort to gain further insight into the mechanisms of cellular immunity.

Materials and methods. Thirty-two New Zealand white rabbits weighing about 2 kg were vaccinated with 2.0 ml of live BCG (Phipps) (1 mg per ml of Sauton's medium) by means of 20 intradermal injections. Six unvaccinated rabbits served as controls. Of the 32 rabbits, 12 received a booster injection of 1 ml of live BCG subcutaneously into *each* flank 7 weeks after their initial vaccina-

* This study was begun at Henry Phipps Inst., University of Pennsylvania and completed at Johns Hopkins University. Supported by grant AI-06261 from Nat. Inst. Health, USPHS, by contract Nonr-4010(15) with Office of Naval Research, and by a contract between Fort Detrick, Frederick, Md. and Johns Hopkins University.

† On leave of absence from Kitasato Inst. for Infectious Dis., Tokyo, Japan.

‡ Abbreviations used: MN: mononuclear(s) or specifically oil-induced mononuclear peritoneal exudate cells; AM: pulmonary alveolar macrophages; PMN: polymorphonuclears; BPN: N-benzoyl-D,L-phenylalanine-β-naphthol ester; BPNase: the enzyme hydrolyzing BPN; BCG: Bacillus of Calmette and Guérin, an attenuated strain of *Mycobacterium tuberculosis* employed world-wide as a vaccine.

tion (*cf.* 4). Four rabbits were sacrificed weekly for the first 6 weeks after the initial vaccination and also weekly for the first 3 weeks after revaccination, to obtain MN at these periods.

The rabbits were tested intradermally with a 1:10 dilution of Old Tuberculin approximately one week before sacrifice. All vaccinated rabbits showed moderately strong tuberculin reactions.

MN were harvested in Hanks' solution§ containing heparin (1:10,000) under sterile conditions 5 days after intraperitoneal injection of mineral oil (USP)(1). AM‡ were washed out of their lungs according to procedures developed by Myrvik(5,6).

Only the MN were cultured(7). Thirty million MN were washed once in sterile Hanks' solution and resuspended in a medium consisting of 0.3 ml of Hanks' solution, 2.3 ml of autologous serum, 0.1 ml of penicillin-streptomycin solution (10,000 units of each per ml) and 0.3 ml of either water (controls) or washed, heat-killed BCG (Phipps) (1 mg representing 100 million bacilli per ml of water, stored at 5°C). The cell suspensions were incubated for 2 days at 37°C with constant agitation by a magnetic stirrer.

The cells were collected by centrifugation, washed once in 0.4% citrate in physiological saline, resuspended in citrate-saline, counted, and frozen at −25°C for 3 or more days before they were assayed for enzymes. Cells at 0-hour were prepared similarly.

Enzyme assays were performed for the MN proteinase which hydrolyzes urea-denatured hemoglobin at pH 4(1), the MN protease which hydrolyzes N-benzoyl-D,L-phenylalanine-β-naphthol ester (BPNase)(1), the MN esterase which hydrolyzes β-naphthyl acetate (1), the MN lipase which hydrolyzes coconut oil(1), the MN lysozyme which hydrolyzes *Sarcina lutea*(8) and the MN acid phosphatase which hydrolyzes β-naphthyl acid phosphate‖(9). No allowance was made for the 2 to 8% PMN‡ which contaminated the MN and AM preparations, (*cf.* 1), for differential cell counts showed little variation among the rabbits.

The details of most of these assays, including pH curves and "straight-line" curves relating enzyme activity with cell number, have been presented(1,8). Our technique for acid phosphatase, adapted from that of Seligman *et al*(9) was: In 16 × 150 mm test tubes 2.0 million MN (or 0.5 million AM) were incubated at 38° for 30 minutes in 6 ml of 0.1 M acetate buffer (pH 5.1) containing 0.3 mg of β-naphthyl phosphate‖ and 2 mg of $MnCl_2$. After 1.0 ml was removed to measure the pH, the reaction was stopped by chilling the tubes in an ice bath and adding 4.5 ml of 0.05 M Veronal buffer (pH 8.5) which brought the pH of the reaction mixture to about 7.4 for diazocoupling. This was performed by adding 1.0 ml of Naphthanil Diazo Blue B‖ reagent (80 mg in 20 ml of cold water prepared just before use). The diazo reaction was stopped by addition of 1.0 ml of 40% trichloroacetic acid and the color extracted with 15 ml of ethyl acetate. The latter was cleared by a brief centrifugation and its optical density read immediately thereafter at 540 mμ. Controls without MN were essentially negative. A straight line relationship existed between the free naphthol liberated and the number of MN up to an optical density of 0.45. The pH optimum of the reaction was between 4.9 and 5.3.

Granulomatous lungs were produced in rabbits by methods similar to those described by Myrvik(10) and Cohn(2). A single intravenous injection of 0.1 mg of lyophilized heat-killed tubercle bacilli in 0.1 ml of Bayol F¶ and Falba** (2:1)(10), or 2 intravenous injections of 12 mg of lyophilized heat-killed BCG in 1.0 ml of saline containing 0.01% Tween 80 on 2 consecutive days (*cf.* 2) proved satisfactory. AM were washed from the lungs 2 to 4 weeks later.

Results. Peritoneal macrophages. Vaccination of rabbits with BCG seemed to have more effect on certain MN hydrolases than

§ 25 ml of Hanks' 10 X balanced solution (Baltimore Biological Laboratory, Baltimore, Md.), 225 ml of water, and 1.2 ml of 1.4% $NaHCO_3$.

‖ Dajac Laboratories, Borden Co., Chem. Div., Phila., Pa.

¶ Bayol F, Esso Humble Oil & Refining Co., Phila., Pa.

** Falba adsorption base, Pfaltz & Bauer, Inc., New York.

others. The BPNase activity showed the most increase (Fig. 1), as did, to lesser degrees, esterase, proteinase, lipase (Fig. 2) and lysozyme respectively. Only the increase in BPNase activity was statistically significant ($P = 0.01$), but this finding could not con-

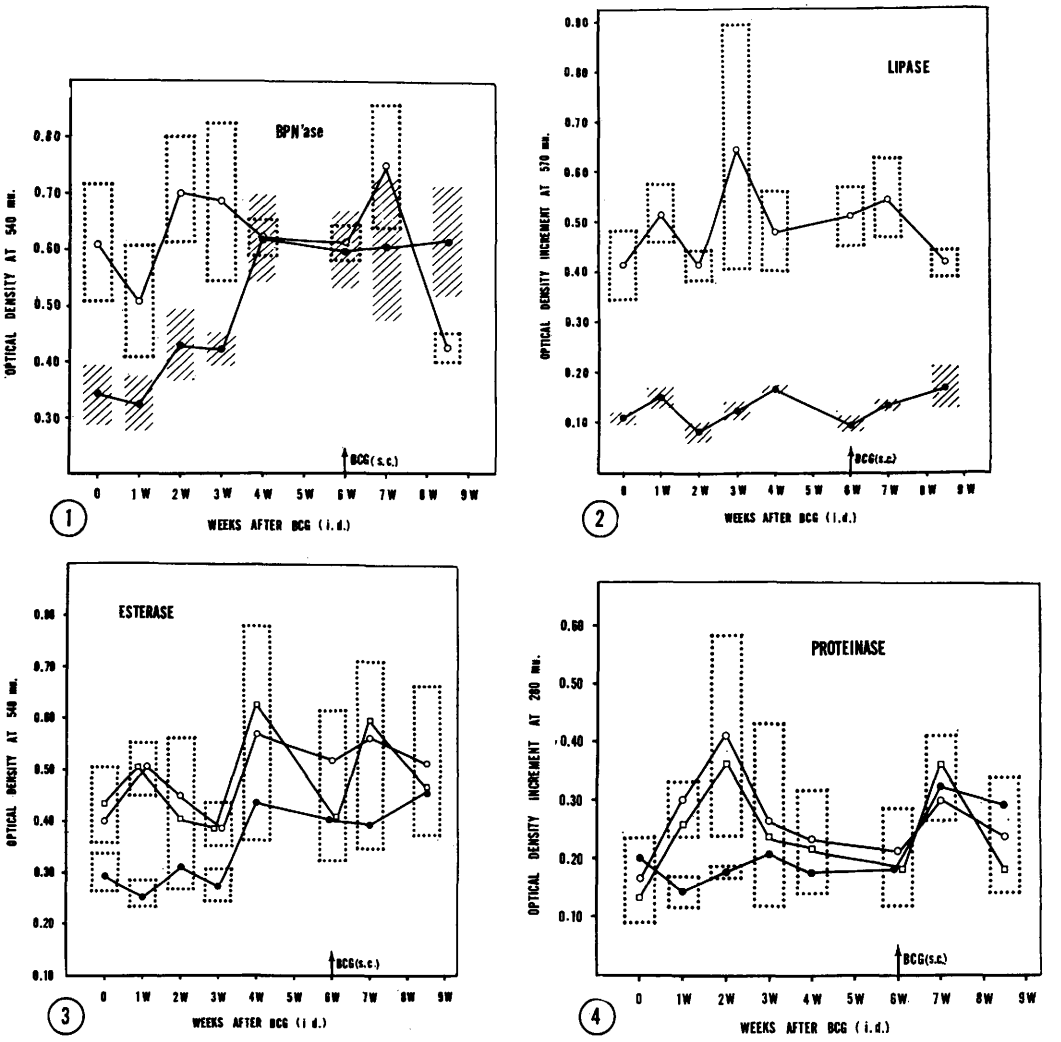

FIG. 1. BPNase activity (substrate: N-benzoyl-D,L-phenylalanine-β-naphthol ester) of 0.7 million MN (●) and 0.35 × 2 million AM (○) collected from rabbits at various times following intradermal BCG vaccination and subcutaneous revaccination. Rabbits killed at 6 weeks were not revaccinated. Each point represents average of 4 rabbits and standard errors of means are indicated. Esterase curves were similar to BPNase curves depicted here.

FIG. 2. Lipase activity (substrate: coconut oil) of 1.0 million MN (●) and 0.5 × 2 million AM (○) from rabbits in experiment described in Fig. 1. Proteinase curves were similar to lipase curves depicted here.

FIG. 3. Esterase activity (substrate: β-naphthyl acetate) of 1.0 million MN assayed at 0-hour (●) and after 48 hr in tissue culture in presence (□) and absence (○) of heat-killed tubercle bacilli. MN were collected at various times following BCG vaccination and revaccination. Rabbits killed at 6 weeks were not revaccinated. Each point represents average of 4 rabbits and standard errors of means are indicated. BPNase curves were similar to esterase curves depicted here.

FIG. 4. Proteinase activity (substrate: hemoglobin) of MN (2.0 million per ml) assayed at 0-hour (●) and after 48 hr in tissue culture in presence (□) and absence (○) of heat-killed tubercle bacilli. See Fig. 3 for details. Lipase curves were similar to proteinase curves depicted here.

sistently be reproduced in additional experiments. Our results with acid phosphatase are discussed below.

A "negative phase" in host resistance to tuberculosis was reported to follow revaccination with BCG(4,11,12). This was accompanied by a decrease in the activities of certain MN dehydrogenases(4,12), but no such decrease in MN hydrolase levels was found in our studies (Fig. 1). The differences in type of enzyme assayed or the differences in time of collecting MN (5 days after revaccination(4) *vs* 7 days (this report) may explain these discrepancies.

Peritoneal macrophages in cell culture. After 48 hours in cell culture, MN from control rabbits and rabbits with primary BCG vaccinations showed identical elevations of esterase (Fig. 3), BPNase, proteinase (Fig. 4) and lipase, each to lesser degrees. Only the elevations of esterase and BPNase were statistically significant ($P = 0.01$ and 0.03). In MN from both vaccinated and control animals, the lysozyme levels were decreased about 50% ($P = 0.01$). Leakage of lysozyme is indicative of cell injury(13). After revaccination no significant elevation of any of these MN enzymes occurred in cell culture (Fig. 3 and 4). With both vaccinated and control rabbits, the presence of intracellular, heat-killed tubercle bacilli did not change the numbers of MN in cell culture or alter their enzyme levels (Fig. 3 and 4).

It is possible that during the 2-day period in cell culture MN could absorb or pinocytose serum(14) which contains appreciable amounts of BPNase†† and esterase††. In an experiment employing 10, 30, 60, and 80% serum in the culture medium, the BPNase and esterase levels of washed MN increased after 48 hours roughly in proportion to the concentration of serum present. MN did not, however, absorb any detectable BPNase or esterase when incubated for 2 hours in 80% serum. The high concentrations of serum could help maintain the viability of MN and initiate the production of additional enzymes (see *Discussion*).

Ingestion and digestion of heat-killed tubercle bacilli. After 48 hours in culture, MN ingested an average of 1.6 bacilli per cell. This figure was independent of the state of immunity of the host. The per cent of MN that ingested bacteria, also independent of the immune state, averaged 48%. Calculations indicated that all the bacteria presented to the MN were ingested. Therefore, if immunity had any effect on phagocytosis, these experimental conditions were not appropriate for detecting it.

A study was made of the state of the bacilli in MN which had resided 2 days in cell culture. The relative percentages of whole bacilli, fractionated bacilli and faintly staining bacillary particles were tabulated. MN from the vaccinated and control rabbits showed no differences in number of faintly stained bacillary particles, but the vaccinated group showed somewhat fewer whole bacilli and more broken pieces after the 6th week. This result, however, might be due to the aging of the bacterial suspension in the refrigerator during this period, as the controls for this experiment were evaluated before any rabbits were vaccinated (*cf.* Fig. 3 and 4).

Acid phosphatase. The contribution of MN acid phosphatase to the intracellular digestion of tubercle bacilli seems less direct than lipase, proteinase or lysozyme, as the bacterial component it hydrolyzes, if any, is probably of low molecular weight. For this reason acid phosphatase was not included in our original series of enzyme assays, but was assayed at a later date as an incomplete series, since some of the MN samples had been depleted.

In confirmation of other investigators(4,15-20), it was found that MN from BCG-vaccinated rabbits had elevated acid phosphatase levels (2.2 times that of controls, $P=0.02$). Again the results were not consistently repro-

†† With BPN 0.1 ml of serum gave optical densities of 0.30 to 0.60, and with naphthyl acetate 0.002 ml of serum gave optical densities about 0.25 under our experimental conditions. These values correspond to the enzyme activity of 0.7 million and 1.0 million MN respectively. Since about 500 million MN have a packed volume of 1.0 ml, a given MN would have to absorb 70 times its volume of serum to obtain all of its BPNase from serum and only its own volume to obtain all of its esterase from serum. It is of interest that the titers of these serum enzymes did not increase following the BCG vaccinations herein described.

TABLE I. Hydrolase Levels of AM and MN 2 to 3 Weeks After Intravenous Injection of Heat-Killed Tubercle Bacilli.*

		AM‡	MN	*P* values:§ AM *vs* MN
Total cells in exudate or perfusate	E	332.0 ± 124	394 ± 90	
	C	34.0 ± 3.7	349 ± 87	
P values: E *vs* C		.013	.364	
Proteinase:				
Hydrolysis of urea hemoglobin by 2 million cells per ml pH 3.9: Optical density units at 280 mμ†	E	675 ± 52	250 ± 27	.000
	C	655 ± 39	220 ± 21	.000
P values: E *vs* C		.384	.189	
BPNase:				
Hydrolysis of N-benzoyl-D,L-phenylalanine β-naphthol ester by 0.7 million cells at pH 5.4: Optical density units at 540 mμ†	E	780 ± 90	425 ± 68	.002
	C	570 ± 56	315 ± 46	.001
P values: E *vs* C		.030	.088	
Esterase:				
Hydrolysis of β-naphthyl acetate by 1 million cells at pH 7.4: Optical density units at 540 mμ†	E	635 ± 140	295 ± 36	.014
	C	555 ± 50	230 ± 21	.000
P values: E *vs* C		.311	.063	
Lipase:				
Hydrolysis of coconut oil by 1 million cells. Optical density units at 570 mμ†	E	300 ± 50	80 ± 14	.000
	C	445 ± 57	90 ± 23	.000
P values: E *vs* C		−.036	−.347	
Lysozyme:				
Hydrolysis of *Sarcina lutea* by 0.25 million cells. Decrease in optical density units at 600 mμ†	E	780 ± 21	225 ± 27	.000
	C	640 ± 53	110 ± 30	.000
P values: E *vs* C		.017	.009	
Acid phosphatase:				
Hydrolysis of β-naphthyl acid phosphate by 2.0 million cells. Optical density units at 540 mμ†	E	1640 ± 156	225 ± 47	.002
	C	1140 ± 180	205 ± 29	.000
P values: E *vs* C		.040	.387	

* Cells from 14 experimental (E) and 14 control (C) rabbits were assayed for each enzyme except for lysozyme for which only 5 experimental and 5 controls were available, and acid phosphatase for which only 5 and 4, respectively, were available. Only rabbits with lungs showing a granulomatous response were included in the experimental series: Criterion for choosing these lungs was recovery of more than 50 million AM from them.

† Optical density of 0.100 corresponds to liberation of about 4 μg of β-naphthol from β-naphthyl acetate or from N-benzoyl-D,L-phenylalanine-β-naphthol ester or from β-naphthyl acid phosphate, and to liberation of about 1.0 μg of glycerol from coconut oil. It also represents the activity of about 8 μg of crystalline trypsin on hemoglobin and about 1.0 μg of crystallized egg white lysozyme on suspensions of *Sarcina lutea.*

‡ AM were assayed for the first 5 enzymes at half the concentration of MN and results multiplied by two. AM were assayed for acid phosphatase at one-fourth the concentration of MN and results multiplied by four.

§ Differences in enzyme content of AM and MN have been described(2,3,6,8).

ducible. The acid phosphatase levels of MN from these BCG-vaccinated rabbits did not show additional elevation after 2 days in cell culture. The presence of intracellular heat-killed tubercle bacilli also had no effect.

Pulmonary alveolar macrophages. AM showed higher proteinase, BPNase, esterase, lipase, lysozyme and acid phosphatase activities than MN from the same rabbit, (*cf.* Fig. 1 and 2, and Table I). Vaccination (intradermally and subcutaneously with viable BCG) had no effect on any of these AM enzymes‡‡ (*cf.* Fig. 1 and 2). In contrast, AM from lungs with innumerable, confluent granulomata, produced by intravenous heat-killed BCG, showed higher levels of acid phosphatase, lysozyme and BPNase, and lower levels of lipase than AM from normal lungs

‡‡ AM from rabbits vaccinated with viable BCG were not assayed for acid phosphatase.

(Table I). The hydrolase levels of peritoneal MN were essentially unchanged by this procedure, except for lysozyme which was increased (Table I).

Discussion. The present studies were performed to gain further insight into the mechanisms of cellular immunity, which is manifested by an enhanced ability of mononuclear phagocytes to destroy ingested microorganisms(21). This ability may involve mechanisms occurring at 4 levels: (a) increases in number of lysosomes (*cf.* 22) and in hydrolytic enzymes(4,15-20) and bactericidins(23, 24) which they may contain, (b) increases in rates of production of these factors in response to the antigenic stimulus (*cf.* 25), (c) increases in rates of discharge of lysosomal enzymes into phagocytic vacuoles which contain bacteria(26), and (d) increases in numbers of immune MN, the proliferation of which was stimulated by the presence of antigen(27-29). With some infections, antibodies also play a role in the intracellular destruction of bacteria(30).

Our work has been concerned with the first level, namely the digestive enzymes of macrophages. *Active* tuberculosis, which causes high levels of immunity against reinfection, did not result in increased levels of MN proteases, esterases or lipase(25). It did raise the levels of MN lysozyme 1.4 times(8) and BCG-vaccination raised their acid phosphatase levels(4,15-20 and this report). In mice BCG-vaccination also raised MN β-glucuronidase and cathepsin levels(19). Since *rates* of production of these enzymes in response to ingested tubercle bacilli might be greater in immunized animals (*cf.* 25), the *in vitro* experiments described here were performed.

MN from both BCG-vaccinated and control rabbits increased their hydrolase levels in cell culture at the *same* rate regardless of whether or not heat-killed tubercle bacilli were present. Also the rate of intracellular digestion (if any) of these bacilli seemed identical in these two groups. With peritoneal MN from normal(19,31-33) and BCG-vaccinated(19) mice, Cohn and Benson, and Saito and Suter also observed increases in certain hydrolase levels, which apparently followed ingestion (and digestion) of the serum proteins present in the culture medium(31-33). Our findings could likewise be the result of ingestion of serum, the effects of which could have obscured any effect caused by ingested tubercle bacilli. Of interest are the findings of Maeir and Forschirm(34) that the acid phosphatase content of cultured macrophages from tuberculin-sensitive guinea pigs increased within 12 to 24 hours after exposure to PPD *in vitro*.

Cohn(31-33) employed mouse MN, because rabbit MN which are cultured according to present-day techniques do not seem to be in a physiological environment. In our *in vitro* system, many MN degenerated in 2 days and the surviving cells were frequently vacuolated(7). These regressive changes may be additional reasons for their lack of response to ingested bacilli. It should be mentioned that the presence of low numbers of dead tubercle bacilli did not increase the amount of degeneration found in cultured MN from either control or vaccinated animals.

In vivo, however, macrophages showed more response to bacilli. In our laboratory and others(2,10), pulmonary alveolar macrophages (which *were* apparently digesting heat-killed bacilli) were found to have higher lysozyme and acid phosphatase than normal alveolar macrophages. They also had higher BPNase (this report) and "lipase" (naphthol laureate hydrolysis in presence of taurocholate)(2). Thus in response to tubercle bacilli, macrophages *in vivo* were able to increase their levels of certain hydrolases, and this increase, in all likelihood, was associated with high degrees of cellular immunity.

Summary. Rabbits were vaccinated intradermally with BCG and revaccinated 6 weeks later subcutaneously. Oil-induced macrophages (MN) were collected from the peritoneal cavities and alveolar macrophages were collected from the lungs. The MN were cultured for 2 days with and without heat-killed tubercle bacilli. Assays for proteases, lipase, esterase, lysozyme and acid phosphatase were performed.

Vaccination frequently increased the levels of MN acid phosphatase and BPNase, a protease hydrolyzing N-benzoyl-D,L-phenylala-

nine-β-naphthol ester. Vaccination did not increase the levels of the other MN hydrolases to a significant degree and had no effect on the hydrolases of alveolar macrophages.

After a 2-day residence in tissue culture, MN from both control and vaccinated rabbits increased their levels of BPNase and esterase to a similar degree. Proteinase and lipase were unchanged and lysozyme was decreased. The presence of heat-killed tubercle bacilli in the cultured MN had no effect on any of these hydrolases.

Peritoneal macrophages had lower hydrolase levels than alveolar macrophages (AM). Normal AM had lower BPNase, acid phosphatase and lysozyme levels than AM from lungs with confluent granulomata produced by injecting rabbits intravenously with heat-killed BCG. MN from these same rabbits had higher lysozyme levels than normal MN. These results suggest that BPNase, lysozyme and acid phosphatase contribute more to the cellular immunity of MN than the other "lysosomal" hydrolases evaluated.

We are indebted to Mildred L. Pennick, Dolores I. Ryer, Bruce S. Schoenberg, and Jeremy Altman for assistance during various portions of this investigation. We also wish to acknowledge the help of Dr. Frank A. Kapral, Philadelphia General Hospital, with the cell cultures.

1. Dannenberg, A. M., Jr., Bennett, W. E., J. Cell Biol., 1964, v21, 1.
2. Cohn, Z. A., Wiener, E., J. Exp. Med., 1963, v118, 991.
3. Leake, E. S., Gonzalez-Ojeda, D., Myrvik, Q. N., Exp. Cell Res., 1964, v33, 553.
4. Allison, M. J., Zappasodi, P., Lurie, M. B., Am. Rev. Resp. Dis., 1962, v85, 364.
5. Myrvik, Q. N., Leake, E. S., Fariss, B., J. Immunol., 1961, v86, 128.
6. Dannenberg, A. M., Jr., Burstone, M. S., Walter, P. C., Kinsley, J. W., J. Cell Biol., 1963, v17, 465.
7. Dannenberg, A. M., Jr., Walter, P. C., Kapral, F. A., J. Immunol., 1963, v90, 448.
8. Carson, M. E., Dannenberg, A. M., Jr., ibid., 1965, v94, 99.
9. Seligman, A. M., Chauncey, H. H., Nachlas, M. M., Manheimer, L. H., Ravin, H. A., J. Biol. Chem., 1951, v190, 7.
10. Myrvik, Q. N., Leake, E. S., Oshima, S., J. Immunol., 1962, v89, 745.
11. Schaedler, R. W., Dubos, R. J., J. Exp. Med., 1957, v106, 719.
12. Allison, M. J., Zappasodi, P., Lurie, M. B., ibid., 1962, v115, 881.
13. Kerby, G. P., Proc. Soc. Exp. Biol. and Med., 1952, v81, 129.
14. Hamburger, R. N., Pious, D. A., Mills, S. E., Immunol., 1963, v6, 439.
15. Grogg, E., Pearse, A. G. E., Brit. J. Exp. Path., 1952, v33, 567.
16. Suter, E., Hulliger, L., Ann. N. Y. Acad. Sci., 1960, v88, 1237.
17. Thorbecke, G. J., Old, L. J., Benacerraf, B., Clarke, D. A., J. Histochem. Cytochem., 1961, v9, 392.
18. Colwell, C. A., Hess, A. R., Tavaststjerna, M., Am. Rev. Resp. Dis., 1963, v88, 37.
19. Saito, K., Suter, E., J. Exp. Med., 1965, v121, 727.
20. Paunesco, E., Hadirca, E., Rev. Roumaine Biochim., 1964, v1, 183.
21. Lurie, M. B., Am. J. Med., 1950, v9, 591.
22. Yamori, T., Acta Path. Japon., 1964, v14, 1.
23. Ramseier, H., Suter, E., J. Immunol., 1964, v93, 511.
24. ———, ibid., 1964, v93, 518.
25. Dannenberg, A. M., Jr., Bennett, W. E., Arch. Path., 1963, v76, 581.
26. North, R. J., Mackaness, G. B., Brit. J. Exp. Path., 1963, v44, 608.
27. Mackaness, G. B., J. Exp. Med., 1962, v116, 381.
28. ———, ibid., 1964, v120, 105.
29. Khoo, K. K., Mackaness, G. B., Aust. J. Exp. Biol. Med. Sci., 1964, v42, 707.
30. Jenkin, C. R., Rowley, D., Bact. Rev., 1963, v27, 391.
31. Cohn, Z. A., Benson, B., J. Exp. Med., 1965, v121, 153.
32. ———, ibid., 1965, v121, 279.
33. ———, ibid., 1965, v121, 835.
34. Maeir, D. M., Forschirm, R., Fed. Proc., 1963, v22, 197.

Received March 10, 1965. P.S.E.B.M., 1965, v120.