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HOW MACROPHAGES KILL TUBERCLE BACILLI

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Despite over 30 years of intermittent investigations, there is no definitive evidence that macrophages kill *Mycobacterium tuberculosis*. This is largely because all attempts to demonstrate such killing by macrophages *in vitro* have produced only rather unconvincing results. How then can I discuss how macrophages kill tubercle bacilli when it is not proven that they do? The answer is that just as there is a large and growing body of indirect evidence that makes it highly likely that macrophages can kill tubercle bacilli, there is also a body of evidence that makes it likely that they do so by producing hydrogen peroxide.

I will spend most of this lecture summarising the more recent body of evidence that macrophages kill tubercle bacilli by producing hydrogen peroxide but I will also refer briefly to the question whether macrophage lysosomes have any direct activity against tubercle bacilli, for it is unlikely that macrophages are dependent solely on one antimycobacterial product. Different macrophage products may be of crucial importance under different circumstances.

The peroxide story, in relation to host defence against *M. tuberculosis*, begins around 1953. After the chemotherapeutic drug isoniazid had been in use for a few years, reports began to appear of the isolation of isoniazid-resistant mutants of *M. tuberculosis* that had low virulence in the guinea-pig, were catalase-negative and were susceptible to killing by hydrogen peroxide (Barnett, Bushby and Mitchison, 1953; Cohn *et al.*, 1954; Mitchison, 1954; Morse *et al.*, 1954; Peizer and Widelock, 1955). At that time hydrogen peroxide production by mammalian tissues had not even been demonstrated but the implication that peroxide might reach tuberculocidal concentrations inside leucocytes was not missed by Coleman and Middlebrook (1956). This idea was strengthened considerably in the early 1960s by reports that a substantial proportion of isolates of *M. tuberculosis* from patients in the Indian subcontinent were of low virulence in the guinea-pig and peroxide-susceptible even though they had a normal catalase content and isoniazid susceptibility (Subbaiah, Mitchison and Selkon,

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1960; Mitchison, Selkon and Lloyd, 1963). While this correlation between virulence and peroxide resistance in natural isolates was being established, the first demonstration of hydrogen peroxide production by polymorphonuclear leucocytes was reported (Iyer, Islam and Quastel, 1961) but we had to wait ten years before it was certain that mononuclear phagocytes also had this property (Gee *et al.*, 1970; Karnovsky *et al.*, 1970; Paul *et al.*, 1970). Now it is established that both mononuclear and polymorphonuclear leucocytes can use the peroxide released in response to phagocytosis to kill various microorganisms (Klebanoff and Hamon, 1975; Klebanoff and Clark, 1978) and that immunologically-activated macrophages can release increased amounts of peroxide (Johnston, 1981); the relevance of this to *M. tuberculosis* has been explored further.

Perhaps the most telling evidence for a role of hydrogen peroxide in the killing of tubercle bacilli by macrophages came from recent studies of *M. microti* in mouse peritoneal macrophages *in vitro* (Walker and Lowrie, 1981). *M. microti* causes a disease resembling tuberculosis in field voles and is pathogenic for mice. When monolayers of macrophages from normal mice were infected *in vitro* they exhibited little ability to kill *M. microti*; but if, in contrast, they had been maintained first in the presence of supernates from immunologically-activated spleen cells they killed >90% of the phagocytosed bacilli within 24 h (fig. 1).

This finding was notable because it was the first time that such a substantial killing of a pathogenic mycobacterium by macrophages had been demonstrated *in vitro* in the absence of antibiotics. This result can only encourage the view that under appropriate circumstances macrophages might do the same to *M. tuberculosis*. The absence of antibiotics was important because the assumption that antibiotics do not interfere with

FIG. 1.—Killing of intracellular *M. microti* by lymphokine-activated mouse peritoneal macrophages and the protective effect of catalase. ▲ = Lymphokine-activated control; □ = catalase present; ■ = heat-denatured catalase present.

tests of intracellular killing because they do not penetrate phagocytes adequately has been shown to be erroneous (Cole and Brostoff, 1975; Tulkens and Trouet, 1978; Lowrie, Aber and Carrol, 1979; Lowrie, Peters and Scoging, 1982).

The second notable point about the killing of *M. microti* by the activated macrophages was that the killing was due to macrophage hydrogen peroxide. This was indicated by the protective effect of exogenous catalase (fig. 1) and the finding that phagocytosis of *M. microti* evoked substantial release of hydrogen peroxide from the immunologically-activated macrophages in parallel with the uptake of the bacilli (fig. 2). With normal macrophages the peroxide response was barely detectable.

We have recognised for a long time that peroxide susceptibility cannot be the only cause of low virulence in tubercle bacilli. For example, there are the strains such as BCG and H37Ra that have undergone a marked attenuation of virulence as a

FIG. 2.—Release of hydrogen peroxide from lymphokine-activated macrophages. ○ = Without stimulation; △ = in response to phorbol myristate acetate; ■ = during phagocytosis of *M. microti*.

consequence of maintenance in the laboratory and yet they have retained peroxide resistance in full. Furthermore the correlation between peroxide susceptibility and low virulence in other groups of strains cannot be considered proof of a causative relationship, if only because peroxide susceptibility might itself be merely a reflection of some other underlying defect of greater significance in the macrophage-tubercle bacillus interaction. Therefore, Jackett, Aber and Lowrie (1978*a* and *b*; 1980) studied strains of *M. tuberculosis* representing three groups in which low virulence was associated with (i) laboratory attenuation, (ii) loss of catalase, and (iii) peroxide susceptibility without loss of catalase, together with their virulent counterparts. They compared these strains for susceptibility to killing by agents which studies of leucocyte microbicidal biochemistry had suggested might be relevant.

Because much of the hydrogen peroxide produced by leucocytes probably arises *via* superoxide—a reactive free radical with some microbicidal activity which is also released during phagocytosis (Rossi *et al.*, 1979)—susceptibility to an enzymic

superoxide-generating system was tested (Jackett *et al.*, 1978a). Only the peroxide-susceptible strains of tubercle bacilli were killed by products of this system (fig. 3) but there was no correlation between killing and bacterial content of superoxide dismutase, which converts superoxide to peroxide, and protection was afforded by adding catalase to the system and not by adding superoxide dismutase. Thus there was no indication that superoxide, or the potentially toxic reactive molecules that can arise through interaction of superoxide and hydrogen peroxide (hydroxyl radical and singlet oxygen), would have any direct role in killing tubercle bacilli in macrophages.

FIG. 3.—Selective killing of peroxide-susceptible strains of *M. tuberculosis* by an enzymatic (xanthine oxidase) superoxide-generating system and the protective effect of catalase. □ = Superoxide-generating system only; ▨ = plus superoxide dismutase; ▤ = plus catalase; ■ = plus superoxide dismutase and catalase.

The toxicity of peroxide was enhanced at the low *pH* values that probably arise within macrophages (*c. pH* 4.5) and a low *pH* was itself toxic but these effects showed no correlation with virulence.

Leucocyte peroxidase enhances the toxicity of peroxide for microorganisms, particularly in the presence of a halide (Klebanoff, 1975) and such peroxidative systems were shown to be effective against tubercle bacilli in experiments with lactoperoxidase (Jackett *et al.*, 1978b). The halide cofactor could be either iodide or, less potently, chloride. Catalase could substitute for peroxidase by acting peroxidatively under appropriate conditions (Jackett *et al.*, 1980). However, there was no correlation between low virulence and susceptibility to any of these systems; the availability of

peroxidase, catalase and iodide within macrophage phagocytic vacuoles, where they would be needed to have effect, is uncertain. Hence, although these studies indicated that low pH and peroxidative enzymatic activity might contribute to macrophage tuberculocidal action, they suggested no alternative to challenge the importance of the toxicity of peroxide implicit in correlations between virulence and peroxide resistance.

Jackett *et al.* (1981*a* and *b*) tested whether the inferred killing of tubercle bacilli *in vivo* by peroxide might be attributed predominantly to immunologically-activated macrophages. They compared the fates of parent strains and isoniazid-resistant peroxide-susceptible mutants in the organs of normal and BCG-vaccinated guinea-pigs and examined in parallel the capacity of macrophages obtained from the infected lungs to release peroxide. The mutants survived consistently less well than the parent bacilli (fig. 4). In the normal animals the effect of the bacterial mutation to peroxide susceptibility was the same during days 1–3 after infection as during days 4–6, which suggested that peroxide availability was the same in the two periods. In contrast, in the vaccinated animals the effect of peroxide susceptibility was greater in the second period, which suggested an increasing availability of peroxide. During the first period the effect of peroxide susceptibility was expressed equally in normal and vaccinated animals and this suggested an equal initial availability of peroxide in normal and vaccinated animals.

These implications about peroxide availability depended upon the assumption that the only significant consequence of the mutation to isoniazid resistance was acquisition of peroxide susceptibility and this was not necessarily true. Nevertheless, the

FIG. 4.—Effect of peroxide susceptibility of *M. tuberculosis* on the course of intravenous infection in the lungs of normal and BCG-vaccinated guinea-pigs. ○, ● = Parent *M. tuberculosis* strain H37Rv; △, ▲ = peroxide-susceptible (isoniazid-resistant) mutant of H37Rv; solid symbols = normal animals; open symbols = vaccinated animals.

implications were borne out by measurements of peroxide release from macrophages obtained from the infected lungs (fig. 5). Vaccination did not affect the release of peroxide from macrophages that were removed from the lungs immediately after infection of the animal and tested *in vitro* with or without phagocytic stimulation. However, macrophages that were removed from vaccinated animals on the third and sixth days after infection released progressively more peroxide than macrophages that were removed immediately after infection. This was not seen with macrophages from the infected normal animals. Phagocytosis of tubercle bacilli enhanced peroxide

FIG. 5.—Release of hydrogen peroxide by alveolar macrophages from normal and vaccinated guinea-pigs 3 h, 3 days and 6 days after intravenous infection. ○, ● = Cells unstimulated *in vitro*; □, ■ = cells phagocytosing *M. tuberculosis* *in vitro*. Solid symbols = cells from normal animals; open symbols = cells from vaccinated animals.

release and the increment per bacillus taken up by the cells increased with time since infection of the animal (fig. 6). This increase in responsiveness occurred faster in the vaccinated than in the normal animal, presumably as a consequence of lymphokine generation. Surprisingly, the increase in responsiveness was selective in that there was no difference with time or between normal and vaccinated animals in the amount of peroxide released from macrophages in response to an alternative stimulus—phorbol myristate acetate (Jackett *et al.*, 1981*b*). Thus a selective increase in the efficiency of linkage of phagocytic stimulation to peroxide response may have contributed to enhanced peroxide-mediated killing with the development of acquired immunity. The nature of the peroxide generating system, the manner of its linkage to phagocytic stimulation and hence the manner of its priming by lymphokines are at present unclear (Reiss and Roos, 1978; Andrew *et al.*, 1980; Bellavite *et al.*, 1981).

FIG. 6.—Increase in alveolar macrophage responsiveness to phagocytosis of tubercle bacilli during the 6 days after intravenous infection. ● = cells from normal animals; ○ = cells from vaccinated animals.

Whilst it is true that the relevance of these findings to *M. tuberculosis* in man remains to be established, there are several pointers. For example, there are indications that isoniazid-resistant tubercle bacilli may be of reduced virulence for man (Oestreicher *et al.*, 1955) and that disseminated BCG infection might follow routine vaccination when macrophages are peroxide-deficient in patients with chronic granulomatous disease (Mackay *et al.*, 1980; Urban *et al.*, 1980). Human alveolar macrophages can probably use hydrogen peroxide to kill other phagocytosed bacteria and they release more peroxide after priming with lymphokines (Greening, Rees and Lowrie, 1981*a* and *b*). Definitive evidence awaits the availability of a tuberculocidal human macrophage for in-vitro experiments.

I do not wish to give the impression that hydrogen peroxide can now be expected to provide a comprehensive explanation of immunity in tuberculosis. It is doubtful that an antimicrobial system as oxygen-dependent as hydrogen peroxide production can operate effectively in the densely packed granulomas that develop later in tuberculosis. Indeed, development of infection is impaired under conditions where oxygen availability is restricted (Dubos, 1955; Chandler *et al.*, 1965) and it is unlikely that this can be accounted for entirely by the oxygen dependence of tubercle-bacillus metabolism.

Attention has been focused on macrophage lysosomes as an alternative to peroxide since the demonstration by Armstrong and Hart (1971) that living tubercle bacilli have the ability to inhibit (or fail to elicit) phagosome-lysosome fusion whereas dead ones do not. This property is shared by *M. microti* (fig. 7). Much effort has been directed

FIG. 7a.—Electron micrograph of ultrathin section of mouse peritoneal macrophage showing fusion of lysosomes (L) with phagosomes containing heat-killed *M. microti* (HKM). The contents of the lysosomes were labelled with electron-opaque gold particles.

towards understanding the mechanisms underlying this intriguing biological phenomenon in the reasonable expectation that the bacillus benefits from avoiding contact with lysosomal contents. Three distinct but possibly complementary mechanisms have been proposed: bacterial release of polyanionic cell wall components (Goren *et al.*, 1976), release of ammonia (Gordon, Hart and Young, 1980) and either release of cyclic AMP or stimulation of macrophage synthesis of cyclic AMP (Lowrie, Jackett and Ratcliffe, 1975; Lowrie, Aber and Jackett, 1979; Lowrie *et al.*, 1980).

Some other successful intracellular parasites also have been found to inhibit phagosome-lysosome fusion (Jones and Hirsch, 1972; Friis, 1972; Weidner, 1975) and, with *Toxoplasma gondi*, promotion of fusion by coating the parasites with antibody before phagocytosis resulted in death of the parasites (Jones, 1975). With *M. tuberculosis* and *M. microti*, however, the results were less clear; promotion of fusion consequent upon antibody coating typically had no effect on bacterial survival or growth, but occasionally it caused bacteriostasis of *M. microti* in normal mouse peritoneal macrophages (fig. 8). Interpretation of studies of fusion by light and electron microscopy has not become any easier with the demonstration by biochemical analysis of subcellular fractions that macrophages contain multiple populations of lysosomes, each with distinctive enzyme content (Lowrie, Andrew and Peters, 1979)

FIG. 7b.—Electron micrograph of ultrathin section of mouse peritoneal macrophage showing absence of lysosome fusion with phagosomes containing live *M. microti* (LM). The contents of lysosomes were labelled with electron-opaque gold particles.

and with different tendency to fuse with phagosomes containing tubercle bacilli. However, it will be of great interest to see whether tubercle bacilli are able to inhibit fusion in immunologically activated macrophages and, if so, what the effect of fusion will be. Because antibody coating has no effect on the triggering of superoxide or hydrogen peroxide release during phagocytosis of tubercle bacilli (Jackett, Andrew and Lowrie, 1982), the effects of lysosome fusion should be readily distinguished from those of peroxide release.

It seems likely that both lysosomes and peroxide play a significant antitubercular role in macrophages but the relative importance of each must vary as granulomas develop. In particular we can anticipate that peroxide-dependent killing should be greatest in cells phagocytosing in a relatively aerobic environment, such as under pre-inflammation conditions or at the periphery of an immune granuloma, less in cells that are no longer phagocytosing or are in a relatively anaerobic environment, and least in cells that are in an anaerobic environment, as in the depths of a granuloma. Experimental analysis of the mechanisms of antimicrobial action in the latter situation, where peroxide-dependent killing does not operate, may prove more difficult but might be even more rewarding in the long term. This would be so particularly if the bacilli under these conditions are essentially dormant and contribute significantly to failures of chemotherapy and persistent states of infection.

FIG. 8.—Inhibition of multiplication of *M. microti* in mouse peritoneal macrophages by coating with antibody before phagocytosis. Monolayers were maintained for 1 week before infection with opsonised (●) or non-opsonised (○) bacilli. No antibiotics were present at any stage; extracellular bacilli were removed by frequent rinsing.

REFERENCES

- ARMSTRONG, J. A. AND HART, P. D'A. 1971. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *Journal of Experimental Medicine*, **134**, 713–740.
- ANDREW, P. W., LOWRIE, D. B., JACKETT, P. S. AND PETERS, T. J. 1980. Analytical subcellular fractionation of rabbit alveolar macrophages with particular reference to the subcellular localisation of pyridine nucleotide-dependent superoxide-generating systems and superoxide dismutase. *Biochimica et Biophysica Acta*, **611**, 61–71.
- BARNETT, M., BUSHBY, S. R. M. AND MITCHISON, D. A. 1953. Tubercle bacilli resistant to isoniazid: virulence and response to treatment with isoniazid in guinea-pigs and mice. *British Journal of Experimental Pathology*, **34**, 568–581.
- BELLAVITE, P., BERTON, G., DRI, P. AND SORANZO, M. R. 1981. Enzymatic basis of the respiratory burst of guinea-pig resident peritoneal macrophages. *Journal of the Reticuloendothelial Society*, **29**, 47–60.
- CHANDLER, P. J., ALLISON, M. J., MARGOLIS, G. AND GERSZTEN, E. 1965. The effects of intermittent hyperbaric oxygen therapy on the development of tuberculosis in the rabbit. *American Review of Respiratory Disease*, **91**, 855–860.
- COHN, M. L., KOVITZ, C., ODA, U. AND MIDDLEBROOK, G. 1954. Studies on isoniazid and tubercle bacilli. II. The growth requirements, catalase activities, and pathogenic properties of isoniazid-resistant mutants. *American Review of Tuberculosis*, **70**, 641–664.
- COLE, P. AND BROSTOFF, J. 1975. Intracellular killing of *Listeria monocytogenes* by activated macrophages (Mackness system) is due to antibiotic. *Nature*, **256**, 515–517.
- COLEMAN, C. M. AND MIDDLEBROOK, G. 1956. The effects of some sulphhydryl compounds on growth of catalase-positive and catalase-negative tubercle bacilli. *American Review of Tuberculosis*, **74**, 42–49.
- DUBOS, R. J. 1955. Properties and structures of tubercle bacilli concerned in their pathogenicity. *Symposium of the Society for General Microbiology*, **5**, 103–125.
- FRIIS, R. R. 1972. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. *Journal of Bacteriology*, **110**, 706–721.
- GEE, J. B. L., VASSALLO, C. L., BELL, P., KASKIN, J., BASFORD, R. E. AND FIELD, J. B. 1970. Catalase-dependent peroxidative metabolism in the alveolar macrophage during phagocytosis. *Journal of Clinical Investigation*, **49**, 1280–1287.

- GORDON, A. H., HART, P. D'A. AND YOUNG, M. R. 1980. Ammonia inhibits phagosome-lysosome fusion in macrophages. *Nature*, **286**, 79-80.
- GOREN, M. B., HART, P. D'A., YOUNG, M. R. AND ARMSTRONG, J. A. 1976. Prevention of phagosome-lysosome fusion in cultured macrophages by sulfatides of *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences*, **73**, 2510-2514.
- GREENING, A. P., REES, A. D. M. AND LOWRIE, D. B. 1981a. Extracellular release of hydrogen peroxide by human alveolar macrophages. *American Review of Respiratory Disease*, **123s**, 48.
- GREENING, A. P., REES, A. D. M. AND LOWRIE, D. B. 1981b. Enhancement of human alveolar macrophage function by lymphokines. *Thorax*, **36**, 717-718.
- IYER, G. Y. M., ISLAM, M. F. AND QUASTEL, J. H. 1961. Biochemical aspects of phagocytosis. *Nature*, **192**, 535-541.
- JACKETT, P. S., ABER, V. R. AND LOWRIE, D. B. 1978a. Virulence and resistance to superoxide, low pH and hydrogen peroxide among strains of *Mycobacterium tuberculosis*. *Journal of General Microbiology*, **104**, 37-45.
- JACKETT, P. S., ABER, V. R. AND LOWRIE, D. B. 1978b. Virulence of *Mycobacterium tuberculosis* and susceptibility to peroxidative killing systems. *Journal of General Microbiology*, **107**, 273-278.
- JACKETT, P. S., ABER, V. R. AND LOWRIE, D. B. 1980. The susceptibility of strains of *Mycobacterium tuberculosis* to catalase-mediated peroxidative killing. *Journal of General Microbiology*, **121**, 381-386.
- JACKETT, P. S., ABER, V. R., MITCHISON, D. A. AND LOWRIE, D. B. 1981a. The contribution of hydrogen peroxide resistance to virulence of *Mycobacterium tuberculosis* during the first six days after intravenous infection of normal and BCG-vaccinated guinea-pigs. *British Journal of Experimental Pathology*, **62**, 34-40.
- JACKETT, P. S., ANDREW, P. W., ABER, V. R. AND LOWRIE, D. B. 1981b. Hydrogen peroxide and superoxide release by alveolar macrophages from normal and BCG-vaccinated guinea-pigs after intravenous challenge with *Mycobacterium tuberculosis*. *British Journal of Experimental Pathology*, **62**, 419-428.
- JACKETT, P. S., ANDREW, P. W. AND LOWRIE, D. B. 1982. Release of superoxide and hydrogen peroxide from guinea-pig alveolar macrophages during phagocytosis of *Mycobacterium bovis* BCG. In *Macrophages and NK cell regulation and function*, edited by S. Normann and E. Sorkin. Plenum: New York (in press).
- JOHNSTON, R. B. 1981. Enhancement of phagocytosis-associated oxidative metabolism as a manifestation of macrophage activation. In *Lymphokines*, vol. 3, edited by E. Pick. Academic Press: New York, pp. 33-56.
- JONES, T. C. 1975. Phagosome-lysosome interaction with *Toxoplasma*. In *Mononuclear phagocytes in immunity, infection and pathology*, edited by R. van Furth. Blackwell, Oxford, pp. 595-605.
- JONES, T. C. AND HIRSCH, J. G. 1972. The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. *Journal of Experimental Medicine*, **136**, 1173-1194.
- KARNOVSKY, M. L., SIMMONS, S., GLASS, E. A., SHAFER, A. W. AND HART, P. D'A. 1970. Metabolism of macrophages. In *Mononuclear phagocytes*, edited by R. van Furth. Blackwell, Oxford, pp. 103-120.
- KLEBANOFF, S. J. 1975. Antimicrobial mechanisms in neutrophilic polymorphonuclear leukocytes. *Seminars in Hematology*, **12**, 117-142.
- KLEBANOFF, S. J. AND CLARK, R. A. 1978. The Neutrophil: Function and Clinical Disorders. Elsevier/North-Holland, Amsterdam, pp. 409-426.
- KLEBANOFF, S. J. AND HAMON, C. B. 1975. Antimicrobial systems of mononuclear phagocytes. In *Mononuclear phagocytes in immunity, infection and pathology*, edited by R. van Furth. Blackwell, Oxford, pp. 507-529.
- LOWRIE, D. B., ABER, V. R. AND CARROL, M. E. W. 1979. Division and death rates of *Salmonella typhimurium* inside macrophages: use of penicillin as a probe. *Journal of General Microbiology*, **110**, 409-419.
- LOWRIE, D. B., ABER, V. R. AND JACKETT, P. S. 1979. Phagosome-lysosome fusion and cyclic

- adenosine 3':5'-monophosphate in macrophages infected with *Mycobacterium microti*, *Mycobacterium bovis* BCG or *Mycobacterium lepraemurium*. *Journal of General Microbiology*, **110**, 431-441.
- LOWRIE, D. B., ANDREW, P. W. AND PETERS, T. J. 1979. Analytical subcellular fractionation of alveolar macrophages from normal and BCG-vaccinated rabbits with particular reference to heterogeneity of hydrolase-containing granules. *Biochemical Journal*, **178**, 761-767.
- LOWRIE, D. B., JACKETT, P. S., ABER, V. R. AND CARROL, M. E. W. 1980. Cyclic nucleotides and phagosome-lysosome fusion in mouse peritoneal macrophages. In *Mononuclear phagocytes, Functional aspects*, vol. 2, edited by R. van Furth. Martinus Nijhoff, The Hague, Boston, London, pp. 1057-1075.
- LOWRIE, D. B., JACKETT, P. S. AND RATCLIFFE, N. A. 1975. *Mycobacterium microti* may protect itself from intracellular destruction by releasing cyclic AMP into phagosomes. *Nature*, **254**, 600-602.
- LOWRIE, D. B., PETERS, T. J. AND SCOGING, A. 1982. Benzylpenicillin transport and subcellular distribution in mouse peritoneal macrophage monolayers. *Biochemical Pharmacology*, **31**, 423-432.
- MACKAY, A., AND 7 OTHERS. 1980. Fatal disseminated BCG infection in an 18-year-old boy. *Lancet*, **2**, 1332-1334.
- MITCHISON, D. A. 1954. Tubercle bacilli resistant to isoniazid. Virulence and response to treatment with isoniazid in guinea-pigs. *British Medical Journal*, **1**, 128-130.
- MITCHISON, D. A., SELKON, J. B. AND LLOYD, J. 1963. Virulence in the guinea-pig, susceptibility to hydrogen peroxide, and catalase activity of isoniazid-sensitive tubercle bacilli from South Indian and British patients. *Journal of Pathology and Bacteriology*, **86**, 377-386.
- MORSE, W. C., WEISER, O. L., KUHN, D. M., FUSILLO, M., DAIL, M. C. AND EVANS, J. R. 1954. Study of the virulence of isoniazid-resistant tubercle bacilli in guinea-pigs and mice. *American Review of Tuberculosis*, **69**, 464-468.
- OESTREICHER, R., DRESSLER, S. H., RUSSELL, W. F., GROW, J. B. AND MIDDLEBROOK, G. 1955. Observations on the pathogenicity of isoniazid-resistant mutants of tubercle bacilli for tuberculous patients. *American Review of Tuberculosis and Pulmonary Disease*, **71**, 390-405.
- PAUL, B. B., STRAUSS, R. R., JACOBS, A. A. AND SBARRA, A. J. 1970. Function of H₂O₂, myeloperoxidase and hexosemonophosphate shunt enzymes in phagocytizing cells from different species. *Infection and Immunity*, **1**, 338-344.
- PEIZER, R. AND WIDELock, D. 1955. The correlation of rate of catalase activity, guinea-pig virulence, and isoniazid resistance of tubercle bacilli from specimens of patients under isoniazid therapy. *American Review of Tuberculosis*, **72**, 246-251.
- REISS, M. AND ROOS, D. 1978. Differences in oxygen metabolism of phagocytosing monocytes and neutrophils. *Journal of Clinical Investigation*, **61**, 480-488.
- ROSSI, F., DRI, P., BELLAVITE, P., ZABUCCHI, G. AND BERTON, G. 1979. Oxidative metabolism of inflammatory cells. In *Advances in inflammation research*, vol. 1, edited by G. Weissmann. Raven Press, New York, pp. 139-155.
- SUBBAIAH, T. V., MITCHISON, D. A. AND SELKON, J. B. 1960. The susceptibility to hydrogen peroxide of Indian and British isoniazid-sensitive and isoniazid-resistant tubercle bacilli. *Tubercle*, **41**, 323-333.
- TULKENS, P. AND TROUET, A. 1978. The uptake and intracellular accumulation of aminoglycoside antibiotics in lysosomes of cultured rat fibroblasts. *Biochemical Pharmacology*, **27**, 415-424.
- URBAN, C., BECKER, H., MUTZ, I. AND FRITSCH, G. 1980. BCG-impfkomplikation bei septischer granulomatose. *Klinische Paediatric*, **192**, 13-18.
- WALKER, L. AND LOWRIE, D. B. 1981. Killing of *Mycobacterium microti* by immunologically activated macrophages. *Nature*, **293**, 69-70.
- WEIDNER, E. 1975. Interactions between *Encephalitozoon cuniculi* and macrophages. Parasitophorous vacuole growth and the absence of lysosomal fusion. *Zeitschrift für Parasitenkunde*, **47**, 1-9.