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Opportunities and challenges in the design of implantable biodegradable polymeric systems for the delivery of antimicrobial agents and vaccines

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Abbreviations: PLGA, poly(lactic/glycolic) acid; FCA, Freund's Complete Adjuvant; LL, lepromatous leprosy; DSS, diaminodiphenylsulfone.

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Summary

An increasing number of implantable biodegradable polymeric drug systems are now in clinical use or undergoing experimental and clinical investigations for diverse therapeutic applications. This article summarizes the use of copolymers of lactic and glycolic acid as carrier materials for the controlled release of antigens for vaccination and of chemotherapeutic agents for the therapy of chronic microbial diseases. These approaches illustrate some of the important conceptual and technical issues that must be considered in the future development of implantable biodegradable polymeric systems. These systems offer considerable promise for achieving controlled release of the new classes of biological mediators produced by recombinant technology, particularly those vaccines intended for use in the Third World. The future development of these systems will almost certainly require the use of specialized approaches to particular delivery problems but, overall, the development is viewed with considerable optimism.

I. Introduction

Biodegradable polymeric drug-delivery systems have been the subject of applied research and development for approximately two decades (for reviews, see Refs. 1–3). This paper provides a brief review of the future opportunities and challenges for implantable, biodegradable drug carriers. Particular emphasis will be given to the use of copolymers of lactic and glycolic acids in a matrix type formulation to illustrate the potential merits and shortcomings of such systems [4–18].

It is considered that the future application of these delivery systems will embrace two broad areas of therapeutic utility. The first is in the delivery of the emerging new classes of biological mediators, most notably peptides and proteins produced by increasingly powerful synthetic methods and by recombinant DNA technology. One particularly intriguing application concerns the potential utility of polymeric systems in engineering novel controlled release profiles to augment the immunogenicity of molecules in new vaccination strategies. This contrasts with the historical emphasis to polymeric systems which have focused primarily on their ability to achieve longer-term zero-order release profiles. Biodegradable polymers also offer important opportunities for the 'protection' of labile macromolecules as an additional property, coupled to the advantages of controlled release. The conformational subleties of complex macromolecular biological mediators can be preserved in polymers in much the way a delicate rose is preserved for display in plastic. Of course, the requirement for mediator stability must be coupled with the design and synthesis of a protective polymer that fulfills the desired biodegradability plus reproducible release kinetics.

The second major area of future application of biodegradable polymeric drug-delivery systems is in improved patient compliance. Several examples will be discussed as to how this can be exploited to therapeutic advantage in chronic diseases, affecting populations in which socio-economic and cultural factors often impose significant obstacles to sustained therapy with conventional dosage forms.

This brief review provides an outline of some of the emerging applications of biodegradable polymeric systems in developing novel approaches to vaccine technology, the use of controlled release technology in improved therapy of microbial infections and also in promoting patient compliance.

II. Background

The significant research interest in the development of subcutaneous implantation devices for long-term maintenance of therapeutic drug levels coincides with the increased medical and public acceptance of such drug-delivery systems. This represents an area of investigation in which significant progress can be expected, and a diverse array of drugs and support materials are presently under investigation. These systems take the form of either encapsulation or a formed matrix, or a combination of the two systems.

These two types of polymeric delivery system, in turn, can be further divided into two subgroups: those in which no residual material remains at the implant site at the end of the delivery period and those in which some non-degradable or non-absorbable material remains. In the former, the biodegradable structural material is either a natural substance, such as cellulose or protein, or a synthetic polymer which degrades to natural products, such as small acids found in metabolic pathways, e.g., copolymers of lactic and glycolic acids. In contrast, in the second category of non-degradable polymers the implant must be tolerated long-term and not evoke any adverse responses, or it must be removed surgically. Not surprisingly, biodegradable polymers are preferred because follow-up surgical procedures pose various risk levels (depending on the site of implantation), and significant invasive procedures are both costly and worrying to the patient.

Polylactic acid, polyglycolic acid and the poly(lactic/glycolic) acid copolymers (PLGA) have been used for many years as surgical sutures because they are biodegradable, biocompatible and exhibit moderate strength in tension, compression and bending. These properties make PLGA copolymers desirable for use in other medical applications. Over the past two decades, PLGA copolymers have been investigated extensively for the delivery and controlled release of compounds such as proteins and various pharmaceutical products.

The physical advantages of PLGA copolymers include strength, hydrophobicity and pliability. The polymer is water insoluble but is degraded by hydrolysis to the monomers, lactic acid and glycolic acid, which are water soluble. PLGA is miscible with a wide variety of biologically active compounds. Solid formulations of polymer with a biologically active compound can therefore be prepared and designed to respond to (an) aqueous environment(s) by slowly releasing both the compound and lactic and glycolic acids.

A variety of drug/PLGA systems are currently under development as candidate systems for the long-term maintenance of therapeutic drug levels. Systems have been devised successfully for the delivery of a range of chemical classes and to achieve delivery periods that vary from days to years. Typically, these systems are fabricated in forms in which the drug is either encapsulated or incorporated into

the matrix, or a combination of these two forms. Only matrix-type systems will be discussed in this article. The final formulation must be characterized not only in terms of its chemical composition but also with respect to physical features such as size, shape, density, and porosity which determine the drug/polymer release profile.

Materials are released from PLGA-compressed matrices via a combination of diffusion and erosion. As drug particles which are solvated diffuse out of the matrix, the exposed polymer is hydrolyzed and released as monomers. New drug/matrix surface is thus exposed and the process of diffusion and erosion continues. The mechanism of release is complex. Despite extensive mathematical modeling of release profiles, no single model is able to correlate the impact of polymer formulation, drug properties, drug loading profile and dosage form on the release rate(s). Empirically, however, sufficient data are available to permit the design of PLGA systems with desired release characteristics.

PLGA polymers can be prepared in any molar ratio of lactic to glycolic acids. The proportion chosen is important in determining the in vivo degradation rate [19]. Polymers prepared in a 50:50 proportion are hydrolyzed much faster than those which have a higher proportion of either monomer. For use in drug delivery systems, lactic acid is usually selected as the predominant species because it is more hydrophobic than glycolic acid.

Both the molecular weight and the molecular-weight distribution of the polymer affect the lifetime of the device. The dependence of release rate on polymer molecular weight was demonstrated in work on the sustained release of sulfadiazine from polylactic acid (Fig. 1). The release rate was reduced as the molecular weight of the polymer increased up to 100000. Beyond this size no differences were observed. It has also been shown that lowering the polymer dispersity from 2.0 to

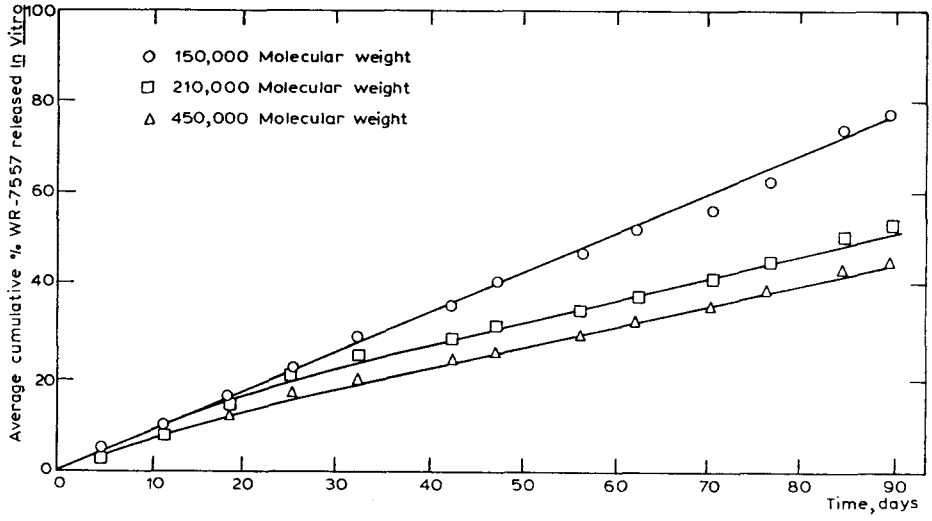

Fig. 1. Effect of molecular weight of 50dl/50L(+)-lactic acid copolymers on the cumulative percent WR-7557 released in vitro from 1.5 mm diameter beads containing 33.3% WR-7557.

1.4 produced a system for the release of pyrimethamine, which had a reduced initial level of release and a constant release rate for a longer period [2].

The rate of release of a particular drug from a specific PLGA matrix can be controlled by manipulating the percentage drug content in the drug/polymer composite. This is illustrated by the release of the narcotic antagonist, naltrexone from PLGA matrix beads [7]. Four different drug-loading percentages, ranging from 50 to 80%, were prepared in a 75L/25G PLGA copolymer prepared as rods 1.6 mm in diameter. Analysis of drug release in vitro showed that release rates increased as loading increased (Fig. 2). The time taken to achieve 80% release varied from 8 to 45 days.

Very soluble active macromolecules, such as proteins, are released relatively rapidly from PLGA systems. Long-term delivery of protein such as antigens for single-dose immunization systems or peptide hormones in various therapeutic settings will require PLGA matrix systems with very low drug contents.

The gross physical features of the dosage form (size and shape) are of less sig-

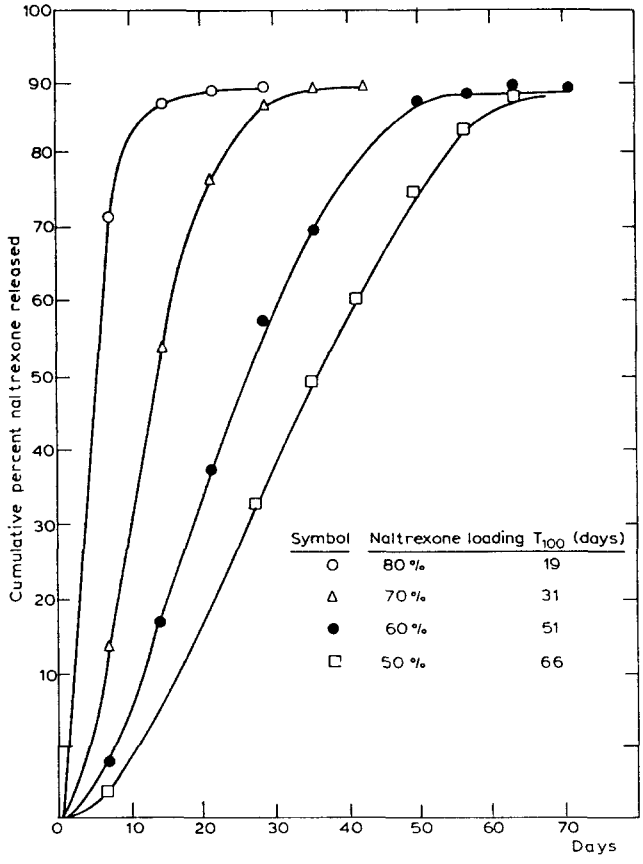

Fig. 2. The effect of naltrexone loading on drug release rate and duration from 1.6 mm diameter rods (75L/25G PLGA) showing the dependence of release rate on drug loading.

nificance in determining the release rate. The choice is made primarily on the basis of the specific delivery requirements. Oral systems may be coated beads or powders. Bolus devices are often monolithic cylinders 1.9 cm in diameter. Subcutaneous or intramuscular implantation can be constructed as rods, beads or suspended powders. Often the ability to retrieve implanted devices after a period of use is desirable. This is accomplished most easily with rods, but is impossible with powders, and difficult for beads that are small enough to be easily implanted through a trocar.

The prepared materials can be sterilized by gamma radiation. The cobalt-60 process, at 2.5 Mrad dosage, is effective and has been shown to have minimal impact on release rate [2].

III. Pulsed release

One of the most attractive features of polymeric drug-delivery systems is that the release of materials can be engineered to come within precise and well-controlled time periods thus enabling 'pulses' of drug release to be created. This is particularly attractive in situations where multiple drug combinations are required.

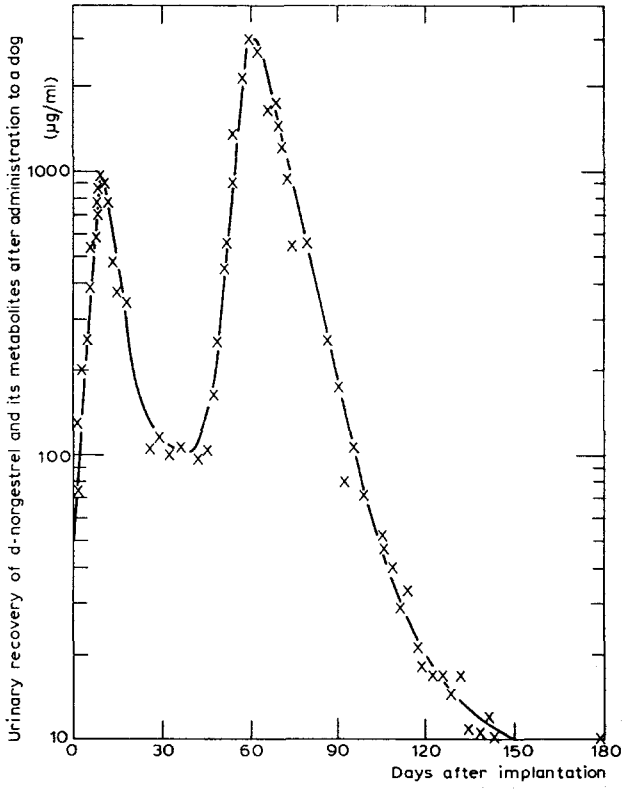

Fig. 3. Biphasic-pulsed release of d-norgestrel from PLGA polymer (90% dl-lactide/10% glycolide).

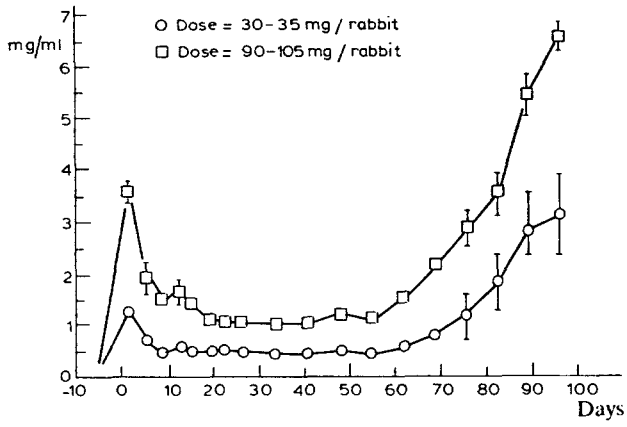

Fig. 4. Biphasic dual-pulsed release of norgestrel from 50% levonorgestrel/50% PLGA matrices (75% L-lactic/25% glycolic acid). Note the different release profiles to Fig. 3.

The utility of pulsed release is illustrated by studies on the controlled release of the contraceptive steroid, d-norgestrel. PLGA matrices were prepared and extruded as cylindrical rods 1.5 mm in diameter. Some rods were coated with polymer of the same composition as the matrix. Fig. 3 shows the in vivo release of d-norgestrel from implants composed of 90% dl-lactic/10% glycolic copolymer with an average molecular mass of 145 kDa. These results reveal an initial burst of drug release lasting two weeks, followed by a second burst of greater duration occurring approximately two months after implantation. The coated rods (not shown in Fig. 3) did not show the first burst. Similar results were obtained with a lower molecular mass (40 kDa) PLGA (75% L-lactic/25% glycolic acids). In tests with rabbits an initial burst is seen in the first week, followed by a second burst peaking at about day 100 (Fig. 4).

IV. Potential applications of polymers in the controlled release of antigens for immunization

Implantable, biodegradable systems offer considerable promise for the sequential pulses of biological mediators such as peptide and proteins. Such molecules are now attracting considerable attention as therapeutic candidates because of recent advances in synthetic chemistry and genetic engineering which have made it possible to obtain and characterize such molecules on a scale that could never be achieved by classical approaches of extraction from tissues. Biocompatible, biodegradable polymeric systems also offer several attractive features as immunological adjuvants in single-dose vaccines. Immunization schemes against a number of parasitic, viral and bacterial infections, as well as toxins of military interest, could benefit from these systems. For example, a vaccine preparation could be constructed to deliver two timed-release pulses of immunogen. The challenge is to engineer the presentation of antigen as two temporally distinct, controlled 'bursts', in a manner sufficient to stimulate lasting immunity. Success would be encourag-

ing because it would enable safe, single-dose mass vaccination of military personnel or others at risk of exposure to unusual agents, and would enhance the effectiveness of worldwide vaccination programs against conventional diseases, such as malaria and other diseases endemic in the Third World, in which logistical considerations are all too often a major obstacle to repeated vaccination of individuals living under poor socio-economic conditions and/or remote locations. Pulsed delivery of two immunizing doses from a single injection would eliminate the need for a second visit for 'booster' vaccination. Naturally, such systems are applicable only to those diseases in which lifelong, or at least prolonged, immunity can be evoked by exposure to antigen(s) for the time for which pulsed release can be achieved and sustained.

Vaccine preparations could be implanted subcutaneously as rod-shaped matrices of poly(lactic/glycolic) acid together with the immunizing antigen. In this novel system the rods would also act as adjuvants and release antigen slowly over a period of several days post-implant, following a second repeat inoculation via a pulse of antigen release after a period of weeks. The aim is to achieve a controlled release system designed expressly for immunization and not to seek to achieve the traditional zero-order controlled release kinetics for which polymeric systems have been used in the past.

In the future, biodegradable polymer matrices for biologicals could be developed for the treatment of many different diseases. The release pattern for these mediators will not necessarily follow traditional zero-order release kinetics, and will be designed to achieve release profiles that will elicit the optimal immunological or therapeutic response.

For the examples cited above, the need is to protect personnel from incapacitation caused by microbial infections or toxins. The threatening sources of exposure are both natural and unnatural (biological warfare agents). The latter must still be considered by military commands despite current treaties banning the use of such agents. In both the military and non-military situations, the number of people to be protected is large and presents major logistical problems. The method of immunization must be simple, safe, effective, economic, and it is highly advantageous if a single-dose immunization system can be used.

Vaccines of interest could include, for example, anthrax, cholera, plague, yellow fever and malaria. Multi-dose vaccines for these infections are becoming available, but the logistics of repeated dosing often render them impractical for military use and is certainly a hindrance to their rapid deployment. Similarly, in the more routine non-military indications, multiple-dose vaccination programs are difficult to implement in parts of the world where the need is often greatest, in which economic, cultural and/or geographic factors are major handicaps to obtaining repeated access to individuals at risk to disease.

A possible solution is to develop a biodegradable, implantable immunological synthetic adjuvant that has the timed-release profile required to provide long-lasting immunological protection. An implanted vaccine of this kind would present the immunogen(s) in such a manner as to enhance the response of the immune system. As in conventional vaccines, it is obligatory that the immunogen be properly

stabilized prior to release and that the epitope sites which evoke protective immunity, whether humoral or cell-mediated, are presented to the immune apparatus in an optimal manner.

To achieve effective protective immunity, it is likely that the delivery system will need to provide bi-modal or dual-pulsed timed release of antigen(s). An initial burst of antigen release over a period of several days after implantation will be required to induce the primary immune response. After a period of weeks, during which little, if any, antigen will be released, the system will then need to release spontaneously a second antigen pulse. The release profile will mimic the events obtained with two separate inoculations. An idealized release profile is shown in Fig. 5. We consider that multipulsed systems could be developed in the future that would be able to simulate three or more separate injections.

Current evidence suggests that a suitable dosage form will be as a rod-shaped matrix of poly(lactic/glycolic) acid (PLGA) plus the antigen(s). The rod-shaped dose form is easily implanted subcutaneously with a trocar [20]. The total dose will be small. Once implanted, the device should not be unusually noticeable. Mass inoculations could be given safely to all personnel at risk. For solid delivery forms, surgical removal is possible if this should become necessary. However, since the entire device is biodegradable, it would leave no final residue.

In classical immunization procedures, a single-dose of antigen is delivered in one injection. Antigen is present at a high level for a short time, but is soon lost from the inoculation site. Short-term immunity may be achieved in this way. With repeated treatments a secondary immune response is evoked which may impart lasting immunity. The length of time that free antigen from a single injection is present may be extended by delivering the antigen in an oil-based medium, as in Freund's Complete Adjuvant (FCA), which degrades and releases antigen slowly. However, even with the use of adjuvant, multiple doses are usually required. Also,

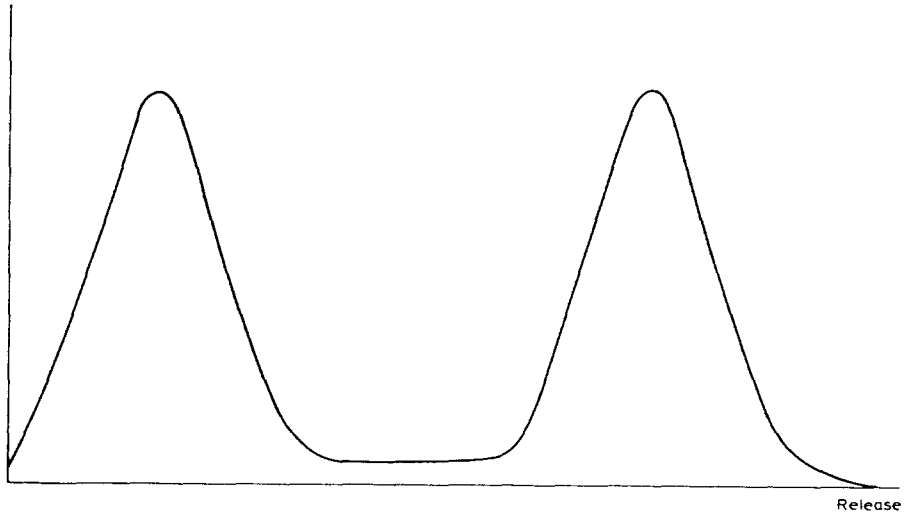

Fig. 5. Idealized modulated bi-modal dual-pulsed release profile.

the nature of the degradation products of FCA renders it unsuitable for human use. The issue of optimal antigenic 'mass' must also be considered. If the concentration is too low the immune tolerance may develop rather than protective immunity.

Long-term release of antigen from a non-degradable polymer matrix has been demonstrated by Langer [21]. This method was as effective in inducing antibodies in mice as conventional administration of two doses of the antigen in FCA. A controlled release system that would allow a second burst of antigen to be delivered should elicit a secondary immune response and thus provide lasting immunity (at least for those immunogens which elicit long-term immunological memory).

A second example of the application-controlled release systems for vaccination is in poliovirus immunization. Polio remains a serious public-health threat in many areas of the world. Although vaccination programs have been effective in reducing the incidence of disease in western countries, efforts to provide worldwide protection have been less successful. The success of poliovirus immunization schemes in the Third World has been compromised by the problem of intercurrent disease, particularly enteric disease, and the stability of the vaccine. In addition, the logistical problems involved in the administration of two or more vaccine doses which require repeated visits are substantial. Many populations may be difficult to revisit because of remote location, nomadic lifestyle or various economic or political problems.

It has been shown in some countries that poliovirus immunization programs, although wide-reaching, produce only limited levels of herd immunity. Although the reasons for failure are undoubtedly complex, the lack of potency of oral polio vaccines in areas where diarrhea is endemic is viewed as a major problem. It is not uncommon for more than three sequential doses to be required to evoke protective immunity under these conditions. Even when an extended immunization can be achieved the frequency of successful immunization may still be inadequate.

Viral vaccines have had a major beneficial impact on world health. The success of these products has, however, been greatest in the more developed countries in which the widespread availability of primary health care, strong public health schemes and the availability of refrigerated facilities for the transport and storage of vaccines each facilitate efficient immunization. Unfortunately, in the Third World such services are not always available and the transfer of vaccine from the manufacturer to the village, can be dislocated by many factors. The lack of refrigeration facilities is a particular problem and a breakdown in the 'cold-chain' from manufacturer to patient results in loss of vacine activity.

There is, therefore, an urgent need for a poliovirus vaccine that could offer two advantages over current vaccines. Firstly, improved heat stability would reduce storage needs and increase the reliability of immunization in diverse climates. Secondly, a single-dose vaccine would eliminate the need for repeated follow-up contacts with every individual receiving vaccination.

Two different types of poliovirus vaccine are presently available. An injectable dose form, consisting of killed polio viruses, is utilized to create an immunological memory of the polio antigen. When an adequate dose is given, life-long memory

is induced. Upon subsequent exposure to the disease, neutralizing antibodies are induced rapidly and prevent the onset of clinical disease. This type of vaccine is sold in the U.S.A. by Squibb/Connaught. The vaccine must be refrigerated until used, and has a shelf-life of 12 months; multiple doses are given to ensure that adequate immunological memory is achieved.

The oral vaccine contains a live attenuated strain of poliovirus. This vaccine has achieved significant popularity in vaccination programs in western countries. The live virus propagates in the gut and induces seroconversion and long-term immunologic memory in analogous fashion to natural infection.

Significant advances in both biology and engineering have occurred since the introduction of the present poliovirus vaccines. It is now feasible instead to consider the use of a heat-stable, killed poliovirus vaccine which could be given as a single dose. This goal can be accomplished by using recent advances in biotechnology. These technologies include:

- high-yield virus-production methods which reduce the cost and the number of batches needed for system verification;
- new methods for drying killed virus to increase its stability;
- improved knowledge of the causes of protein destabilization;
- new methods for the stabilization of macromolecules and viruses;
- new knowledge of the structure of poliovirus;
- the use of genetic engineering for large-scale production of heat-stable antigens and/or antigenic variants produced by site-specific mutagenesis.

In summary, stabilization of the poliovirus by entrapment in a polymeric matrix, in conjunction with these technical advances, could offer two substantial advantages: (1) elimination of the need for refrigeration and (2) immunization with a single inoculation.

V. Applications of controlled-release systems to enhance patient compliance in anti-infectives therapy and vaccination

As noted earlier, a major area for future use of controlled-release technologies is in addressing problems of patient compliance, this approach of new formulation for traditional pharmaceutical products as well as the newer product classes being generated by biotechnology. Potential applications in the therapy of mycobacterial diseases will be discussed as representative examples.

VA. Drug-delivery systems in the treatment of chronic microbial diseases tuberculosis

The development of chemotherapeutic agents for infections caused by *Mycobacterium tuberculosis* has changed dramatically the importance and threat of this disease in western countries. Although current drug regimens are therapeutically effective and well tolerated, problems remain. Some patients may not have access to therapy because of their location in Third World nations. For these individuals

therapy may be too expensive or unavailable. Even when these obstacles do not exist, therapy is burdensome and extends over long periods. For a vast number of cases, the ultimate problem in effective treatment is compliance.

Compliance problems can be approached by the use of controlled-release drug delivery systems. Recognizing that effective chemotherapy of tuberculosis involves the administration of multiple drugs, a possible delivery system would need to be one in which several drugs are released at rates appropriate to each other. In addition, since dosing regimens change during the course of chemotherapy, it may be advantageous to develop a system with biphase release kinetics in which an initial two-month release profile is followed by a four-month cycle of release of a second drug combination. The development problems inherent in considering this type of delivery system for drug combinations are complex. However, discussion of such systems in the context of the therapy of a prevalent and well-known disease serves to illustrate the exciting prospects for the future of controlled-release drug-delivery systems.

For a disease considered curable and preventable, tuberculosis has continued its assault on the United States' and world's populations. In 1983, over 23000 new cases of tuberculosis were reported in the United States alone and approximately 2000 deaths from tuberculosis have been recorded by the National Center for Health Statistics in each of recent years. Also, in the growing epidemic of AIDS, mycobacterial infections are emerging as important opportunistic pathogens in this particular immunocompromised population. Internationally, approximately 10 million new cases occur each year with 2 million annual deaths. In addition, millions more of the world's population are believe to be infected by the tubercle bacilli, many of whom will develop clinical disease. Tuberculosis thus warrants renewed attention, with full eradication of the disease as the objective.

The primary reason for the failure to achieve clinical cure in tuberculosis despite the availability of effective chemotherapy, is not lack of expertise on the part of the practising physician in prescribing therapy, but reflects the inability of many patients to fulfill the complete treatment cycle. Patient compliance is a major obstacle to total disease eradication, and compliance problems are particularly prevalent in Third World countries.

It is unfortunate that tuberculosis is not like syphillis, which can be cured with a single injection, or like pneumococcal pneumonia, which can be treated effectively with 5–7 days therapy with a single oral agent. The current state-of-the-art therapy for tuberculosis requires, at a minimum, 6 months of multi-drug therapy to achieve a high likelihood of cure. Herein lies the problem of total disease eradication. The socio-economic status and geographic locations of the many stricken populations are not conducive to compliance with such complex treatment regimens.

Treatment for tuberculosis has undergone several major changes over the past few decades [23]. One of the first effective modes of treatment to be used was the use of enforced rest and exercise in isolated sanatoria. Lung collapse procedures and resections were also probably beneficial in selected cases, but their benefit was never proved. The development of specific chemotherapeutic agents represented

a watershed, and is perhaps the only true revolution in tuberculosis treatment. Although the morbidity and mortality attributable to tuberculosis were decreasing under older regimens, chemotherapy markedly accelerated the decline of the disease in western countries.

Truly effective chemotherapy for tuberculosis started in 1952 with the introduction of isoniazid (Hopewell, P.C., personal communication; in part). Although streptomycin had been introduced 6 years earlier, the lack of a suitable companion agent to inhibit the emergence of resistant organisms had limited its value [24]. Shortly after streptomycin was introduced it became apparent that patients who improved initially after drug treatment worsened subsequently [25]. It was soon determined that the organisms isolated from patients who had relapsed were no longer susceptible to streptomycin, thereby defining the important principle that bacterial populations are not uniform in their susceptibility to these agents. The concept of multi-drug chemotherapy in tuberculosis was first validated in a British Medical Council study in which streptomycin was supplemented by para-aminosalicylic acid [26]. Treatment with more than one drug has now become standard in the management of tuberculosis.

With identification of the need for a multi-drug regimen of isoniazid, plus streptomycin and/or *para*-aminosalicylic acid, subsequent efforts focused on defining the optimum combinations of these agents and the duration of therapy. In 1962, the Medical Research Council reported that for inpatients with cavitary pulmonary tuberculosis the relapse rate was significantly less after 24 months of therapy than after 12 months; relapse rates of 4% vs. 22%, respectively [27]. This same study also demonstrated that an initial 6 weeks of therapy with streptomycin to supplement isoniazid and *para*-aminosalicylic acid reduced the incidence of treatment failures, but did not alter relapse frequency.

Based on these results, it came to be generally accepted that the optimum duration of treatment was 24 months. Treatment with isoniazid and *para*-aminosalicylic acid was regarded as sufficient for patients with limited disease, but streptomycin was commonly given for an initial 30–90 days in patients with more severe disease.

In addition to improvements in patient survival, a second important benefit of intensive chemotherapy was that fewer survivors served as potential sources of new infections due to the reduction in tubercle bacilli in their sputum. As in the case of improved survival, the reduction in the prevalence of chronically positive patients reached its maximum in 1956.

In the 10–15 years after an understanding of the basic elements of effective drug therapy was developed, a number of important modifications have been introduced in programs by which tuberculosis patients have been treated. Hospitalization, which had been regarded as an essential component of care for all, or part, of the treatment period, was shown to confer no benefit compared to treatment at home [28]. Moreover, it was demonstrated that chemotherapy was very effective in reducing or eliminating the infectivity of patients, thereby decreasing the need for their isolation [29]. Other long-held notions concerning effective treatment were also eliminated. Diet and rest were shown not to be important factors in deter-

mining the outcome of disease [30,31]. Surgical procedures became of less and less importance in patient management [32]. Finally, it was recognized that anti-tuberculosis drugs could be given in single daily doses or even twice weekly without decreasing their effectiveness [33,34].

In 1967, the effectiveness of ethambutol as a substitute for *para*-aminosalicyclic acid was reported [35]. Ethambutol was readily accepted as a much more tolerable and less toxic companion drug for isoniazid. However, in terms of potency, ethambutol (at least in the doses studied; 15–25 mg/kg per day) did not represent a significant improvement over *para*-aminosalicylic acid. Thus, by 1970 the standard treatment for patients with pulmonary tuberculosis in the United States, Canada and the United Kingdom consisted of treatment with isoniazid and ethambutol for 18–24 months, often supplemented by an initial 1–3 months of streptomycin [36]. Therapy was administered as single daily doses, largely on an outpatient basis, with little emphasis on diet or rest. Isolation was less commonly used, although still employed in certain centers.

The next major advance in chemotherapy for pulmonary tuberculosis resulted from the discovery of the antimycobacterial effect of rifampin, and the demonstration that using the combination of isoniazid and rifampin, generally with ethambutol, streptomycin or pyrazinamide, duration of treatment could be shortened dramatically [37,38]. The impetus for attempting to decrease the duration of treatment owed much to the observation that the available regimens were very much less satisfactory under usual operational circumstances [39]. Compliance continues to be a major problem in the United States as well as in developing countries [40,41]. It was assumed that, by shortening the length of treatment, efforts could be focused on compliance. In addition, a treatment program of less than 12 months would not pose a daunting prospect for the new patient, thereby fostering compliance.

A large number of the clinical trials of drug regimens for pulmonary tuberculosis involved different durations of therapy. Interpretation and comparison of the outcome of one study vs. another is complicated, since the inclusion and exclusion criteria are often poorly defined. It is difficult to determine if selection bias influenced the composition of the groups studied. In many reports appropriate statistical analysis is lacking, and insufficient information is provided to allow statistical significance to be assessed. The treatment periods, and the definitions of treatment failure, relapse and adverse reactions also vary in different reports. Often, one cannot determine whether patients were managed in a routine manner or received special care. This last factor is particularly important in assessing the potential regimen for use under field conditions.

In spite of these shortcomings, the data accumulated in these studies allow several important generalizations to be made. Firstly, although there are reports of good clinical results obtained with regimens lasting less than 6 months, relapse rates with regimens of this duration are high. Secondly, in regimens of less than 9 months, and probably in any regimen of less than 12 months, rifampin is an essential component, at least for the initial 1–2 months. In addition, the outcome of regimens of less than 8 months duration is probably better if rifampin is used throughout.

Thirdly, pyrazinamide given in the initial phase of treatment improves the efficacy of regimens of less than 9 months duration. It does not appear, however, that continuing pyrazinamide beyond the initial two months improves the outcome of treatment. Fourthly, at least in the doses usually given, ethambutol detracts from efficacy when substituted for pyrazinamide. There is suggestive evidence, however, that ethambutol may be a satisfactorily substituted for streptomycin. Fifthly, it also appears that continuation of streptomycin beyond the initial 2 months does not improve outcome. Finally, there is good evidence that intermittent administration of appropriately chosen drugs after an initial daily phase, and perhaps from the outset of treatment, achieves results equal to those of daily administration.

In summary, it seems safe to state that the minimum acceptable duration of treatment is 6 months. The initial phase of a 6-month regimen should consist of isoniazid, rifampin, pyrazinamide and either streptomycin or ethambutol given daily for 2 months. The second phase should consist of a 4-month regimen of isoniazid and rifampin given daily, 3 times weekly or 2 times weekly. With treatment durations of 9 months, the need for three or four drugs in the initial phase is not clear. Moreover, isoniazid and rifampin may be given twice weekly after an initial 1 month of daily treatment, or perhaps even from the outset.

There are several caveats that apply to these recommendations. Firstly, the organisms must be susceptible to the drug(s) used. Secondly, patients must take all, or nearly all, of the prescribed treatment. Thirdly, the sputum should be negative for tubercle bacilli by culture at the end of 3 months of treatment. If the sputum still contains *M. tuberculosis* after 3 months of therapy, the patient should be reassessed carefully to determine whether a change in treatment regimen is indicated.

VB. Rationale for a controlled-release drug-delivery system for tuberculosis treatment

The development of effective chemotherapeutic agents for tuberculosis has done much to contribute towards the eradication of this important debilitating disease. It is encouraging to note that research on this disease has progressed to the point that 'cure' can be assured for almost all cases provided that long-term patient compliance is guaranteed. One of the major reasons for treatment failure is premature curtailment of therapy.

By definition, a 100% cure rate in all cases requires a vigilant approach to treatment. Recognizing, however, that one of the obstacles to total disease eradication lies in the treatment of the noncompliant patient, a case can be made for a controlled-release drug-delivery system for treatment of tuberculosis. A drug-delivery system for a combination of currently available drugs administered as as single injection would make it possible to contemplate a global disease eradication program with the aim of achieving the more modest, yet realistic, goal of a cure rate of greater than 50%.

The dosing schedule for a possible drug delivery system of this kind is shown in Fig. 6. This scheme seeks to achieve a 6-month regimen in which an initial 2-month treatment cycle with one drug combination is followed by a 4-month course with

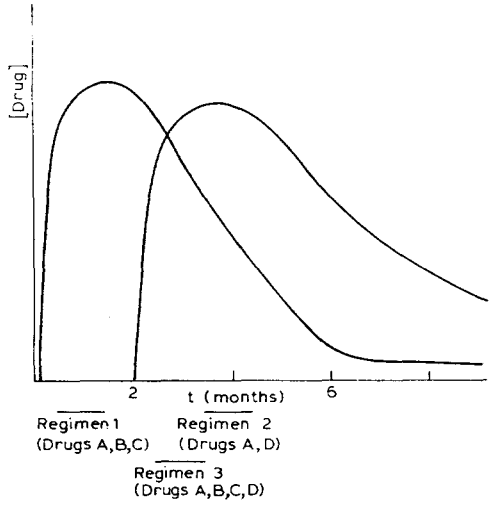

Fig. 6. Dose profile for proposed controlled release delivery of multiple drug combinations for the therapy of tuberculosis.

a second combination. The objective is to deliver one two-component system (regimen 3) at the time of the initial diagnosis. One component (regimen 1) of the system contains drugs of first-course chemotherapy (shown as A, B and C, e.g., rifampin, pyrazinamide, ethanbutol), which is projected for the first 2 months. The second component (regimen 2) of the system contains the drugs of second-course chemotherapy (shown as A and D, e.g., rifampin and isoniazid). The advantage of such a system lies in its design. By incorporating a judicious choice of drugs, it may be possible to elicit a 'cure' in a majority of potentially non-compliant patients with minimally toxic side effects. Thus, with a reasonable degree of confidence, the physician may be able to treat patients that are likely to present compliance problems.

It is possible that the objectives of a multi-drug delivery system can be accomplished via the development of an implantable 'rod' containing the appropriate drugs in combination with a biocompatible biodegradable polymer, such as PLGA (polylactic/glycolic acid). This is a type of controlled-release drug-delivery system which has met with success for a number of years.

Its formulation for use in tuberculosis is viewed as realistic. Immediate efforts would focus on the fabrication of a multi-drug-delivery system that is capable of achieving the projected drug-release profiles, while longer term goals would address the overall efficacy of the system from the perspective of treatment of populations with a high level of non-compliance caused by challenging economic and/or logistical considerations.

Although the overall goal would be the formulation of a 'single dose', 6-month controlled-release system, interim research goals could be met by the successful development of two separate formulations: one multi-drug controlled-release system for use in the initial 2-month cycle, and another for a subsequent 4-month

treatment cycle. Thus, depending on physician judgement and the level of patient compliance, a 'double dose' treatment schedule could be used if the 'single-dose' system had not been developed.

VC. Leprosy

A second example of an area for future application of controlled-release technology in which the problem of patient compliance is also severe involves another mycobacterial infection: leprosy.

The term 'leprosy' is frequently avoided because of its unfortunate historical and social connotations. It is often referred to as Hansen's disease and is caused by the microorganism *Mycobacterium leprae*. Like tuberculosis, leprosy has long plagued mankind and is described in texts dating from 600 B.C. [42–45]. The majority of cases today occur primarily in Africa and Asia, where leprosy is endemic. The disease is also prevalent in South America with an estimated 200000 cases. It is estimated that there are 10.6 million leprosy patients world-wide, but this may be a conservative figure [46,47]. Of these, only 3 million are registered. Leprosy is not a major problem in the United States, which has approximately 5000 cases, with 300 new cases diagnosed each year.

Lepromatous leprosy (LL) or nodular leprosy is the more severe form of the disease causing incapacitating deformity of the hands and feet. Progressive physical disability carries with it social rejection, resulting in segregation of infected individuals from the rest of the community. After infection is established, the ensuing disease is a benign process, that may take years to reach a terminal stage. The other form of leprosy, nerve leprosy, is associated with atrophy of the muscles and nerves, and disease progression takes longer than nodular leprosy.

Current problems in the treatment of leprosy are attributed to the growing resistance of *M. leprae* to the chemotherapeutic agent dapsone [49,51] and to the lack of patient compliance [52]. For almost 40 years, diaminodiphenylsulfone (DDS, Dapsone) has been used widely as the drug of choice in leprosy control programs. Dapsone is cheap, safe and effective, usually achieving rapid clinical but slower bacteriological improvement. There is, however, some tendency to relapse when drug therapy is discontinued.

Presently, a major problem in dapsone monotherapy is the increasing resistance of *M. Laprae* to this drug. A study by the World Health Organization (WHO) reports that primary resistance (i.e. confirmed before treatment) to dapsone is widespread and increasing [46]. It has been reported that secondary resistance (i.e., drug resistance during the course of treatment) is also increasing in treated or relapsed patients [53]. Resistant cases pose an obvious risk as a source of new infections.

Multi-drug therapy has been recommended for all leprosy cases as a countermeasure to drug resistance. The principal drug combination involves intermittent administration of rifampin with dapsone [54]. In the U.S.A. the prescribed dose is 100 mg dapsone daily and 600 mg rifampin weekly [55]. In other parts of the world, a combination of dapsone, rifampin and clofazimine has been recommended by W.H.O. Even with this regimen there is evidence of rifampin resist-

ance occurring in relapsed patients. Reports of the apparent absence of clofazimine resistance has enhanced its use in therapy [56]. Unfortunately, few studies have examined the question of clofazimine resistance in detail. In addition, this compound produces an objectionable reddish-brown pigmentation of the skin.

The limited number of anti-leprosy drugs currently available and the growing problem of resistance make the development of anti-leprosy drugs or a vaccine a difficult problem. A further problem is the lack of rapid in vitro methods for cultivating *M. leprae*. Numerous attempts to cultivate *M. leprae* on artificial media have been unsuccessful and the supply of purified *M. leprae* is obtained from infected Armadillo tissues [46]. Present screening methods employ the mouse foot pad for the study of drugs for activity against *M. leprae* [59,60]. While there are many advantages to using the mouse foot pad in terms of statistical requirements and convenience, the toxicity and the pharmokinetics of agents in this species are often unfavorable and not necessarily predictive of the human response.

As in the case of tuberculosis, the problem of patient compliance in leprosy is due primarily to the long duration of therapy. A typical drug regimen includes self-administered daily dapsone with a once-per-week dose of rifampin. However, a once-per-month dose of rifampin has also been shown to be effective. A maximum period of therapy is 36 months for paucibacillary cases and a minimum of 24 monthly clinic visits and supervised doses for multi-bacillary cases [54]. One reason given for the patient non-compliance in following this regimen is the lack of confidence in modern treatment because the regimen and the illness are relatively enduring during initial treatment [61,62].

The magnitude of non-compliance in leprosy is high. A study by Collier [64] showed that at the end of the first year of treatment, the default rate was nearly 50%. According to another study, irregular attendance (clinical treatment visits less than 9 times out of a possible 13 yearly visits) was seen in 30–50% of patients each year [64]. A combination of factors are responsible for the high magnitude of non-compliance in Third World nations: population migrations and displacements, cost, health beliefs, lack of education and instruction, social rejection, and the lack of overall care for the patient's disabilities.

A leprosy vaccine is only in the early stages of development, and the search to improve the efficacy of existing drugs, as well as the development of new compounds, continues. As mentioned earlier, new drug development is hampered by the inability to cultivate *M. leprae* in vitro. Given that non-compliance is a major problem in leprosy control, it would be an advantage to have a drug-delivery system for anti-leprosy drugs that would alleviate the need for the individual to administer daily and monthly doses of dapsone and rifampin, respectively. The technology and development of a long-acting delivery system for these anti-leprosy drugs has yet to be accomplished because of some major obstacles. For the drugs currently used in leprosy chemotherapy, a system would have to be devised to deliver very large amounts of drugs (700 mg dapsone weekly). This is not considered feasible. Even if the dapsone dosage could be reduced, the long duration of the drug treatment still requires very large amounts of drug to be incorporated in a delivery system. If lower doses of existing drugs were efficacious or new drugs were

effective against *M. leprae* in lower-dose forms, the concept of a long-acting drug-delivery system would have considerable merit for use in this extremely widespread and debilitating disease of man.

The long duration of the drug treatment requires very large amounts of drug to be incorporated in a delivery system. The major question of how best to administer therapeutic drugs in large amounts, such as those used in leprosy chemotherapy, is an important focus of immediate and future work which is needed in the design and development of new long-acting drug-delivery system.

References

1 Wise, D.L. (ed.) (1984) Burn Wound Coverings, Vols. I and II, CRC Press, Boca Raton, FL
2 Wise, D.L. (ed.) (1984) Biopolymeric Controlled Release Systems, Vols. I and II, CRC Press, Boca Raton, FL
3 Wise, D.L. (with Prof. R.S. Langer, M.I.T.) (1984) Medical Application of Controlled Release, Vols. I and II, CRC Press, Boca Raton, FL
4 Wise, D.L., Reuning, R.H., Liao, S.H.T., Staubus, A.E., Ashcraft, S.B., Downs, D.A., Harrigan, S.E. and Wiley, J.N. (1983) Pharmaco-kinetic Quantitation of Naltrexone Controlled Release from a Copolymer Delivery System, J. Pharmacokin. Biopharm. 2
5 Wise, D.L., Carl Sharon, A., Willette, R.E. and Barnett, G., (eds.) (1980) Development of Drug Delivery Systems for Use in Treatment of Narcotic Addiction, National Institute on Drug Abuse, pp. 194–213
6 Wise, D.L., Gresser, J.D. and McCormick, G.J. (1979) Sustained Release of a Dual Antimalarial System, J. Pharm. Pharmacol., 31, 201–204
7 Wise, D.L., Fellman, T.D., Sanderson, J.E. and Wentworth, R.L. (1979) Lactic/Glycolic Acid Polymers, in Drug Carriers in Biology and Medicine, Academic Press, New York
8 Wise, D.L., Benagiano, G., Schmitt, G.E. and Goodman, M. (1979) Sustained Release Hormonal Preparations for the Delivery of Fertility-Regulating Agents, J. Polymer Sci., Polymer Symposia 66, 129–148
9 Wise, D.L., McCormick, G.J., Willet G.P., Anderson, L.C. and Howes, J.F. (1978) Sustained Release of Sulphadiazine, J. Pharm. Pharmac. 30
10 Wise, D.L., Schwope, A.D., Harringan, S., McCarthy, D.A. and Howes, J.F. (1977) Sustained Delivery of a Narcotic Antagonist from Lactic/Glycolic Acid Copolymer Implants, in Polymeric Delivery Systems, Midland Macromolecular Institute Monograph, Vol. 5, Gordon & Breach, New York
11 Wise, D.L. and Schwope, A.D. (1977) Evaluation of Wound-Covering Materials, J. Biomed. Mater. Res. 11, 489
12 Wise, D.L., Gregory, J.B., Newberne, P.M., Bartholow, L.C. and Stanburg, J. (1977) Results on Biodegradable Cylindrical Subdermal Implants for Fertility Control, in Polymeric Delivery Systems, Midland Macromolecular Institute Monograph, Vol. 5, Gordon & Breach, New York
13 Wise, D.L., McCormick, G.V. and Willet, G.P. (1976) Sustained Release of an Anti-Malarial Drug Using a Copolymer of Glycolic/Lactic Acid, Life Sciences, 19, 867
14 Wise, D.L., Schwope, A.D. and Howes, J.F. (1976) Development of Polylactic/Glycolic Acid Delivery Systems for Use in Treatment of Narcotic Addiction, National Institute on Drug Abuse, Research Monograph, No. 4
15 Wise, D.L., Anderson, L.C. and Howes, J.F., (1976) An Injectable Sustained Release Fertility Control System, Contraception 13, 375
16 Wise, D.L., Schwope, A.D. and Howes, J.F. (1975) Lactic/Glycolic Acid Polymers as Narcotic Antagonist Delivery Systems, Life Sciences 17, 1877
17 Wise, D.L., Schwope, A.D., Sell, K.W., Skornick, W.A. and Dressler, D.P. (1974) Development of a Synthetic Burn Covering, Trans Am. Soc. Artif. Intern. Organs 20, 103

18 Wise, D.L., Jackanics, T.M., Nash, H.A. and Gregory, J.B. (1973) Polylactic Acid as a Biodegradable Carrier for Contraceptive Steroids, Contraception 8, 227–234
19 Miller, R.A., Brady, J.M. and Cutright, D.E. (1977) J. Biomed. Mater. Res. 11, 711
20 Kitchell, J.P., Crooker, S.C., Wise, D.L. and Zaneveld, L.J.D. (1984) Long Acting Contraceptive Delivery Systems (Zatuchni, G.L., Goldsmith, A., Shelton, J.D. and Sciarra, J.J., eds.), Harper and Row, pp. 164–168
21 Langer, R.S., Rhine, W.D., Hsieh, D.S.T. and Bawa, R.S. (1980) Controlled Release of Bioactive Materials (Baker, R.W., ed.), pp. 83–98, Academic Press, New York
22 Langer, R.S. Methods Enzymol. 73, 57–75
23 Reference deleted
24 Robtizek, E.H. and Selikoff, I.J. (1952) Hydrazine Derivative of Isonicotinic Acid (Rimifon, Marsalid) in the Treatment of Acute Progressive Caseous-Pneumonic Tuberculosis. A Preliminary Report. Am. Rev. Tuberc. 65, 402
25 Hinshaw, H.C. and Feldman, W.H. (1945) Streptomycin in the Treatment of Clinical Tuberculosis. A Preliminary Report, Proc. Staff Meet. Mayo Clin. 20, 313
26 McDermott, W., Muschenheim, C., Haldey, S.J., Bunn, P.A. and Gorman, R.V. (1947) Streptomycin in the Treatment of Tuberculosis in Humans, I. Meningitis and Generalized Hematogenous Tuberculosis, Ann. Int. Med. 27, 769
27 Medical Research Council (1950) Treatment of Pulmonary Tuberculosis with Streptomycin and Para-Aminosalicylic Acid, Brit. Med. J. 2, 1073
28 Medical Research Council (1962) Long-Term Chemotherapy in the Treatment of Chronic Pulmonary Tuberculosis with Cavitation Tuberculosis 43, 201
29 Dawson, J.J.Y. et al. (1966) A 5-Year Study of Patients with Pulmonary Tuberculosis in a Concurrent Comparison of Home and Sanitorium Treatment for one Year with Isoniazid and PAS, Bull. Wld. Hlth. Org. 34, 533
30 Kamat, R.S., Dawson, J.J. and Devadatta, S. (1966) A Controlled Study of the Influence of Segregation of Tuberculous Patients for One Year on the Attack Rate of Tuberculosis in a 5-Year Period in Close Family Contacts in South India, Bull Wld. Hlth. Org. 34, 517
31 Ramakrishnan, C.V. (1961) The Role of Diet in the Treatment of Pulmonary Tuberculosis, An Evaluation in a Controlled Chemotherapy Study in Home and Sanitorium Patients in South India, Bull. Wld. Hlth. Org. 25, 339
32 Wier, J.A., Schless, J.M., O'Connor, L.E. and Weiser, O.L. (1961) Ambulatory Treatment of Tuberculosis, Results in 105 Tuberculosis Patients Treated with Chemotherapy and Active Physical Rehabilitation, Am. Rev. Resp. Dis. 84, 17
33 Gaensler, E.A. (1982) The Surgery for Pulmonary Tuberculosis, Am. Rev. Resp. Dis. 125, 73
34 Tuberculosis Chemotherapy Center, Madras (1960) A Concurrent Comparison of Isoniazid Plus PAS with Three Regimens of Isoniazid Alone in the Domiciliary Treatment of Pulmonary Tuberculosis in South India, Bull. Wld. Hlth. Org. 23, 535
35 Tuberculosis Chemotherapy Center, Madras (1964) A Concurrent Comparison of Intermittent (Twice Weekly) Isoniazid Plus Streptomycin and Daily Isoniazid plus PAS in the Domiciliary Treatment of Pulmonary Tuberculosis, Bull. Wld. Hlth. Org. 31, 247
36 Bobrowitz, I.D. and Robins, D.E. (1967) Ethambutol-Isoniazid Versus PAS-Isoniazid in Original Treatment of Pulmonary Tuberculosis, Am. Rev. Resp. Dis., 96, 428
37 Johnston, R.F. and Hopewell, P.C. (1969) Chemotherapy of Pulmonary Tuberculosis, Ann. Int. Med. 70, 359
38 Hobby, G.L. and Lenert, T.F. (1968) The Antimycobacterial Effect of Rifampin, Am. Rev. Resp.Dis. 97, 713
39 Fox, W. and Mitchison, D.L. (1975) Short-Course Chemotherapy for Tuberculosis, Am. Rev. Resp. Dis. 111, 325
40 Kent, P.W. and Fox, W., Miller, A.B., Nunn, A.J., Tall, R. and Mitchison, D.A. (1970) The Therapy of Pulmonary Tuberculosis in Kenya, A Comparison of the Results Achieved in Controlled Clinical Trials with Those Achieved by the Routine Treatment Services, Tuberculosis 51, 24
41 Addington, W.W. (1979) Patient Compliance, The Most Serious Remaining Problem in the Control of Tuberculosis in the United States, Chest 75(S), 741

42 Hopewell, P.C., Sánchez Hernández, M., Baron, R.B. and Ganter, B. (1984) Operational Evaluation of Treatment for Tuberculosis. Results of a 'Standard' 12-Month Regimen in Peru, Am. Rev. Resp. Dis. 129, 439
43 Cochrane, R.G. (ed.) (1964) Leprosy in Theory and Practice, John Wright and Sons, Bristol, 2nd Edn
44 Browne, S.G. (1970) Leprosy. Documenta Geigy, Acta Clinica, No. 11, Geigy, Basel, Switzerland
45 Skinones, O.K. (1964) Leprosy in Society, II, The Pattern of Concept and Reaction to Leprosy in Oriental Antiquity, Lepr. Rev. 35, 106–122
46 Browne, S.G. (1964) Tuberculosis and Leprosy, Tuberculosis 45, 56–57
47 Leprosy (1985) Tropical Disease Research, Program Report No. 7, World Health Organization, Geneva
48 Bechelli, L.M. and Dominquez, D.V. (1966) The Leprosy Problem in the World, Bull. W.H.O. 34, 811–826
49 Convit, J. et al. (1970) Therapy of Leprosy, Bull. W.H.O. 42, 667–672
50 Shepard, C.C. (1969) Chemotherapy of Leprosy, Ann. Rev. Pharmacol. 9, 37–50
51 Almeida, J.G. et al. (1983) The Significance of Dapsone (DDS)-Resistant *M. leprae* in Untreated Patients, Int. J. Leprosy 51(3), 374–377
52 Almeida, J.G. et al. (1983) DDS-Resistant Infection Among Leprosy Patients in the Population of Gudiyatham Taluk, South India, 3, Prevalence, Incidence, Risk Factors, and Interpretation of Mouse Foot Pad Test Results, Int. J. Leprosy 51(3), 366–373
53 Huikeshoven, H. (1985) Patient Compliance in Leprosy Control, A Necessity in Old and New Regimens, Int. J. Leprosy 53, 474–480
54 Ji, B.H. (1983) Secondary Dapsone-Resistant Leprosy in Shanghai Municipality, Leprosy Rev. 54, 197–202
55 World Health Organization (1982) Chemotherapy of Leprosy for Control Programmes, Tech. Rep. Ser. No. 675
56 Levis, W.R. (1984) Treatment of Leprosy in the United States, Bull. NY Acad. Med. 60, 696–711
57 Warndorff-Van Diepen, T. (1982) Clorfazimine-Resistant Leprosy – A Case Report, Int. J. Leprosy 50, 139–142
58 Hooper, M. and Kulkaini, S.N. (1982) The Search for New Anti-Tubercular and Anti-Leprotic Drugs, Brit. J. Clin. Pharmacol. 77, 574
59 Ji, L.B. et al. (1983) The Activity of Thirty-Five Drugs in Suppressing Experimental *M. leprae* Infection in Mice, J. Clin. Dermat. 12(5), 230–232
60 Rees, R.J.W. and Weddell, A.G.M. (1970) Transmission of Human Leprosy to the Mouse and its Clinical Implications, Trans. R. Soc. Trop. Med. Hyg. 64, 31–42
61 Shepard, C.C. (1960) The Experimental Disease that Follows the Injection of Human Leprosy Bacilli into Foot Pads of Mice, J. Exp. Med. 112, 445–454
62 Naik, S.S. (1978) Irregularity of Dapsone Intake in Infectious Leprosy Patients Attending an Urban Treatment Centre – Its Magnitude and Causes, Lepr. Endica 50, 45–53
63 Nigam, P. and Siddique (1979) Irregularity of Treatment in Leprosy Patients: Its Magnitude and Causes, Lepr. Indica, 521–532
64 Collier, P.J. (1983) A Study of Case-Holding in Leprosy Patients in Asia, Based on Duration of Treatment, 1976–1980, Lepr. Rev. 54, 89–94
65 Hertros, A.R. (1974) A Study of Some Factors Affecting the Attendance of Patients in a Leprosy Control Scheme, Int. J. Lepr. 42, 419–427
66 Bloom, B.R. (1983) Rationales for Vaccines Against Leprosy, Int. J. Leprosy 51, 505–509

reagents and in vitro immunisation show promise as an alternative strategy (Vaux, D. unpublished results). Association of organelles with the cytoskeleton may also play an important role in control of intracellular fusion by facilitating their movement within cytoplasm [77]. The roles of free Ca^{2+} and of intracellular metabolic signalling events have not been defined.

Delivery of endosome content to lysosomes is influenced by the nature of the receptors involved (cf. uptake of opsonised vs. living pathogens) and by their degree of cross-linking. As shown by Mellman and his colleagues, polyvalent FcR-ligand interactions result in degradation within lysosomes, unlike monovalent binding which results in FcR recycling from endosomes to the surface. The degree of acidification as endocytic organelles move into the cell and pH-sensitivity of receptor-ligand interactions determines whether and where a ligand (e.g., transferrin) or a constituent (e.g., Fe^{2+}) dissociates from its receptor.

At one time the properties of macrophage acid hydrolases seemed well defined, but in recent years studies in this area have lagged behind in relation to other aspects of vacuolar physiology. It is known that MØ contain relatively high levels of numerous hydrolytic activities capable of degrading all natural macromolecules, and that levels of some of these can be markedly induced by digestible and non-digestible endocytic stimuli. Recently there has been interest in catheptic activities in endosomes, i.e., pre-lysosomal organelles, because of their possible importance in 'processing' of protein antigens. The molecular biology of MØ hydrolases deserves further study because of their potential role in catabolism.

III.9. Secretion and extracellular interactions

Although these are major MØ responses regulated by plasma membrane receptors, a detailed consideration of these functions falls outside the scope of this review [78,79]. MØ are able to release a wide range of products (protein, lipid and low molecular weight products) by rapid mobilisation from cellular precursors (e.g., leukotrienes from arachidonate) or by vesicular secretion, following induced synthesis. Some products have only a short lifespan or range of action (e.g., O_2^-), others enter the circulation and influence targets locally and systemically (e.g., IL-1, cachectin/TNF). Targeting of MØ by agents could induce secretion via receptor ligation and therefore result in desirable and undesirable consequences for the host. The selectivity of responses (e.g., proteinase release vs. respiratory burst) is not understood and receptors such as CR_3 induce endocytosis without triggering a respiratory burst. For example, CR_3 and MFR contribute to uptake of unopsonised zymosan, a yeast wall product which is a potent stimulant of both a respiratory burst and arachidonate release, but the role of individual MØ receptors in mediating these secretory responses is still unclear.

Extracellular cytotoxicity, e.g., antibody-dependent lysis, is an important host defence mechanism involving mainly O_2-dependent killing. Since FcRs, for example, are excellent triggers of the respiratory burst oxidase complex, it is possible to target the destruction of cells or organisms by an appropriate antibody. In some murine experimental tumour models specific monoclonal antibodies of the IgG2a