tion in an ordinary gas. However, usually we can, by dimensional considerations, estimate the thermal conductivity in a similar case and then generalize it for a special case, determining the necessary coefficients empirically. In the case of plasma the process depends on many more parameters and the problem of determining the convectional thermal conductivity is more complicated than in an ordinary gas. But theoretically we may estimate which factors have most influence on the rate of convection. To sustain convection one must supply energy. In a gas this energy is drawn from the kinetic energy of the flow.

In a quiescent plasma there is no such source of energy. But in an ionized plasma there may be another source of energy that will excite convection. This source is connected with temperature gradients and some of the thermal energy flux could produce convection. Quantitatively this process is described by internal stresses and was first studied by Maxwell (*9*), who showed that internal stresses are proportional to the square of viscosity derivative and of the temperature gradient. In an ordinary gas the stresses are so small that up to now they have not yet been experimentally observed. This is because the viscosity, which is proportional to the mean free path at normal pressures, is equal to about 10^{-5} cm, and hence at low temperature gradients the stresses are small.

In the plasma the mean free path of electrons and ions is of the order of centimeters, and the temperature gradients are high. In this case the internal stresses following Maxwell's formula are ten orders of magnitude greater than in a gas, and we may expect both convection currents and turbulence. The presence of a magnetic field certainly can have an effect on this phenomenon and, with additional effect of an electric field on convection, it makes even a rough theoretical approach to estimating the magnitude of convection very unreliable. Therefore the only alternative is to study these processes experimentally and this is what we are now doing.

However, convectional thermal conductivity will lower the heating of ions and will lead to a greater critical cross section for the thermonuclear plasma cord. Correspondingly, the size of the reactors for useful energy production will be greater.

If this size will be out of our practical reach, then we should consider methods to decrease convectional heat transfer. This may be done by creating on the boundary of the plasma a layer without turbulence as happens in fluids where we have the Prandtl boundary layer. This possibility has been theoretically considered (*4*, p. 1002).

In conclusion we may say that the pulsed method used in Tokamaks can now be fully worked out theoretically, but the construction of a thermonuclear reactor, based on this method, leads to a large and complicated machine. In contrast, our thermonuclear reactor is simple in construction, but practical means of its realization and size depend on convection heat transfer processes that cannot be treated on a theoretical basis alone.

The main attraction of scientific work is that it leads to problems, the solutions of which cannot be foreseen, and that is why, to a scientist, research on the controlled thermonuclear reactions is so fascinating.

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Comparative Biochemistry and Drug Design for Infectious Disease

Proteins should be targets for chemotherapeutic agents.

Seymour S. Cohen

Some 70 years ago, Paul Ehrlich stated the aim of a specific chemotherapy to be the use of drugs to exterminate an invading organism without injury to the host (*1*, *2*). Occasionally, this aim has been achieved as a result of the empirical screening of selected drugs. For the most part, useful compounds were found without insight as to the essential role of the structure affected by the discriminating inhibitory agent. Such insights have usually developed long after the initial discovery of the compound and have occurred in the course of studies on the mode of action of the inhibitory antibiotic or synthetic substance. The knowledge gained from such studies is now among the major elements in our perceptions of the complexities and differences among organisms (*3*–*5*). These biochemical differences, which constitute the growing body of data of comparative biochemistry, are of two major kinds. One relates to the diversity of metabolic systems in various organisms and may be exemplified by differences in the biosynthesis of essential metabolites such as lysine and nicotinamide, or in other metabolic pathways. The second relates to the new data of molecular biology which affirm that differences in nucleic acid composition and sequence between organisms compel differences in the structures of essentially all of the proteins and enzymes of parasites and their hosts. The first type of difference can yield information as to the essentiality of a metabolic system for the multiplication or survival of both parasite and host. In order that such an essential sys-

The author is American Cancer Society Professor of Pharmacological Sciences at the State University of New York at Stony Brook 11794. This article is adapted from a talk given at a symposium of the Institute of Medicine on "Pharmaceuticals for Developing Countries" on 30 January 1979 in Washington, D.C.

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tem be exploited, we must define the unique structure of the protein of the parasite and design and prepare a selective reagent in a form that can penetrate and inhibit the essential reaction specifically in an intact infected host (*6*).

Chemotherapy and the Dissection of the Biological World

In 1904, Ehrlich had used trypan red to cure mice infected by certain trypanosomes (*1*). His observations had convinced him of the possibilities of a "chemotherapia specifica," a conviction that had fired the long search leading to the clinically useful arsphenamine or Salvarsan (*7*). Many years later, in the late 1930's, the discovery that prontosil and the derived sulfanilamide were effective in the therapy of many bacterial infections supported Ehrlich's hypothesis.

However, the demonstration that, in the bacteria, the sulfa drugs affected the incorporation of para-aminobenzoic acid into the vitamin folic acid, which animal tissues require and obtain intact, suggested once again that bacteria and man had many features in common. The difference seemed minor; man had lost early steps in the biosynthesis of the folates and many bacterial pathogens lack the carrier for the folates present in animal cells. Thus, a specific chemotherapy need not require so-called major evolutionary biochemical divergences. Although the inability of a pathogenic bacterium to use intact folic acid might be a serious matter to the bacterium and even to a patient, it was not considered a major evolutionary difference by most biochemists. However, the differences, both small and smaller, in folic acid metabolism among many organisms were exploited (*8*), and have led to the preparation of numerous substances that almost specifically inhibit aspects of the folic acid metabolism in bacteria, protozoa, and other cells.

Nevertheless, the paradigm of "biochemical unity," which might be construed to minimize the existence of diversity and its possible utility in the design of chemotherapeutic agents, was not seriously shaken until the discovery of antibiotics. The existence of antibiotics and the clarification of their modes of action in numerous instances—for example, penicillin and chloramphenicol—divided the biological world into prokaryotic and eukaryotic organisms (*5*). The specific therapeutic action of penicillin stems from an inhibition of an enzyme essential to the synthesis of the peptidoglycans of the prokaryotic bacteria, structures for which no homologs, that is, structural counterparts descended from a common ancestral form, exist in eukaryotes. The specific sensitivity of bacterial ribosomes to chloramphenicol and many other antibiotics has led us to believe that there are major qualitative biochemical differences between pathogenic prokaryotes and their eukaryotic hosts. The cells of eukaryotes do have walls and cytoplasmic ribosomes, which are therefore analogous to these structures in bacteria. However, these eukaryotic structures appear to have evolved independently of the prokaryotic structures.

Although we know that there are specifically prokaryotic substances and essential biosynthetic pathways characteristic of certain prokaryotic cells, very little work has been done to exploit such known differences systematically. The most obvious reasons for this deficiency are the continuing chemotherapeutic successes of the old method and its improvements. Major new antibacterial substances have been found, and creative synthetic modifications of the antibiotics have been introduced, such as penicillin-like derivatives to improve stability, distribution, and antibacterial spectrum. Because the compounds obtained in this way can control most bacterial infection, the problems of bacterial infection in the developed world have not yet compelled new types of search.

Summary. In the past two decades, biochemistry and molecular biology have demonstrated the existence of potentially exploitable biochemical differences between etiologic agents of disease and their hosts. Known differences between organism and host with respect to metabolism and polymer structure point to the detailed characterization of key proteins as the focus for the development of potential inhibitors. In the last decade, the methodology of the isolation, characterization, and inactivation of proteins and enzymes has been advanced. The present scientific and technological base suggests that new efforts toward the development of selective chemotherapeutic agents for infections caused by bacteria, viruses, protozoa, and higher eukaryotes should exploit the known differences in proteins or other specific biopolymers serving crucial structural or metabolic roles in the economy of the parasite.

The Search for Antiviral Agents

In contrast to successes with most bacterial infections, similar attempts to screen synthetic compounds and natural products for specific antiviral activity have not led to a significant number of clinically useful antiviral compounds. After more than three decades of much effort and growing sophistication, the search has probably expended several hundred million dollars and has yielded only about a dozen compounds of very limited clinical utility in controlling viral infection. Indeed, positive and potentially useful results have been so sparse that many pharmaceutical companies have drastically reduced or abandoned their search for antiviral compounds. Nor has this search been taken up by academia as a major activity.

Beginning in the late 1930's, the infectivity of viruses in mice and embryonated eggs was the basis of major test systems for animal viral development. However, work with these biological systems did not yield the biological and the biochemical knowledge required to develop a chemotherapy based on a knowledge of the mechanisms of viral development. The recognition that virus multiplication is primarily a cellular event led to the study of bacteriophage systems. The temporary detour in the 1940's to the study of model bacteriophage systems set the required conditions for the development of both adequate biological systems and chemical knowledge essential to the study of many animal viruses and, more recently, of plant viruses as well. In 1953, the use of animal tissue culture systems in the analysis of animal virus multiplication (*9*) was reported almost simultaneously with the discovery of the structure of DNA (*10*) and the discovery of virus-induced synthesis of new metabolic systems (*11*).

With the new biological systems, pharmaceutical companies (and others interested in drug development) could now (i) distinguish inhibitors for inactivating viral particles from those affecting viral multiplication, and (ii) determine whether uninfected cells capable of supporting virus multiplication were inhibited in their growth. Furthermore, new plating techniques on animal tissue cultures permitted the detection of antiviral activities with small amounts of synthetic or natural products. The companies set out enthusiastically to exploit the new knowledge, much of which appeared to center on the nucleic acids. Because the biosynthesis of nucleic acid mainly involves nucleotides or other phosphorylated com-

pounds, nonphosphorylated antimetabolites had to be converted to truly inhibitory compounds. On the other hand, phosphorylated analogs penetrated cells slowly. These circumstances limited the efficacy of the newly synthesized inhibitors. Nevertheless, some synthetic products were found to have antitumor activities, and the work persevered. Two decades and thousands of compounds later, it is clear that, with a few exceptions, most analogs of pyrimidines or purines or their nucleosides, whether discovered as a synthetic or natural product, were most often as toxic to an uninfected cell as to virus multiplication in that cell.

Because most classes of viruses genetically determine very few metabolic steps, none of which involve the synthesis of components comparable to the peptidoglycans of bacterial cell walls or utilize the equivalent of bacterial ribosomes, the viruses do not function with the range of prokaryotic metabolism specifically sensitive to the antibiotics or other synthetic inhibitors of, for example, bacteria. Because the synthesis of viruses requires low-molecular-weight intermediates identical to those used by their host cells, inhibitors that restrict the availability or utilization of these intermediates will most frequently prevent normal cell growth and virus multiplication similarly.

Antiviral Therapy and Virus-Induced Enzymes and Other Proteins

Nevertheless, a few substances that possess a considerable degree of selectivity in blocking virus multiplication have been detected. Two groups of such substances active against the multiplication of herpesvirus are presented in Fig. 1; the compounds of the A group are activated specifically by a virus-induced pyrimidine nucleoside kinase. The nucleotides generated in this way affect the synthesis of viral DNA, as does the triphosphate of arabinosyladenine (araA) which shows some selectivity in inhibiting the herpesvirus-induced DNA polymerase. Thus, the few compounds shown to have some selective antiviral action do so by taking advantage of the synthesis of enzymatic proteins determined by the parasitic genome, that is, herpesvirus DNA.

That virus infection leads to the synthesis of new metabolically active proteins essential to their multiplication was first detected 25 years ago (*11*). It was shown that T-even phage infection of a cell lacking thymidylate synthetase led to the synthesis of this enzyme (*12*), as well as another enzyme which synthesized a unique viral pyrimidine nucleotide. This result began the flood of discovery of other phage-induced enzymes and changed the thinking about the mechanisms used by viruses in the course of their multiplication. The virus-induced thymidylate synthetase was shown to have different physical (*13*), chemical, and serological (*14*) properties from that normally present in the bacterium.

In the subsequent two decades, the phenomenon has been shown to be of general import; that is, almost all viruses compel the synthesis of new enzymes essential to the multiplication of the virus. These new enzymes may make low-molecular-weight intermediates or polymerize nucleotides to viral nucleic acid, and the existence of these activities in infected cells should provide unique sites for inhibitor action. The tiny viroids are possibly exceptions to this rule in that no new proteins may be made at all.

In some virus infections, proteins may be elaborated whose catalytic roles have not yet been defined. In SV40 (simian virus 40) infection, catalytic functions have not yet been demonstrated for the T antigen, which is not present in the virion but is essential in virus multiplication and transformation (*15*). Among the picornaviruses, such as poliovirus (*16*) or foot and mouth disease virus (*17*), small proteins have been found covalently linked to the 5′-end of the viral RNA and these appear essential to viral RNA replication. For these systems, selective damage to these proteins by inhibitors can be assessed by examining the course of virus multiplication.

Nor should the coat proteins be neglected in these studies. In the last decade, it has become clear that major steps of virus assembly require the interaction of specific sites of protein molecules or subunits. Systematic efforts to take advantage of the existence of such sites in order to block assembly have not been described. Thus, recent efforts at antiviral chemotherapy have neglected numerous specific steps in viral biosynthesis and assembly.

Antiviral therapy and organizational shortcomings. The academic institutions and research institutes, which have established most of our current knowledge on virus multiplication, are not organized to set up the multidisciplinary laboratories necessary to explore the production, isolation, and characterization of a virus-induced protein in sufficient detail and to prepare a pharmacologically active specific inhibitor that can be demonstrated to inhibit this protein in the infected cell (*6*). This sequence of the development of information is an essential preliminary to the selective inhibition of virus multiplication in infected animals and eventually in man. The shortest time scale one can imagine for such an enterprise is 10 years. If it were to be undertaken by the usual methods in academia, involving cycles of publication and small, shared and repetitive advances in competing groups, it could not be done in less than 20 years.

I might exemplify the process with the systemically useful antiviral agent, araA. An antibacterial activity of araA was first detected in 1961, and in 1964 the compound was found to be cytocidal and to inhibit herpesvirus multiplication, as summarized in (*18*). The activity of araA against herpesvirus in man was detected some years later. As a result of the studies of a pharmaceutical company which undertook to develop araA for human use, the compound was approved in 1978 for treatment of herpes encephalitis in man. However, it has been known since 1961 that araA is very extensively deaminated and only a small fraction is converted intracellularly to the toxic compound ara-ATP (arabinosyladenine triphosphate). Inhibitors of the deamination were found in 1974 (*19*), but a test in man of the efficacy of araA in combination with an inhibitor of the deaminase is not yet under way. Nor is it clear that araA should be used in virus infections that are not life-threatening. In any case, the excessive duration of the time table has scarcely been exaggerated.

Although a multidisciplinary capability in this area is clearly within the potential of several industrial groups, with few exceptions most pharmaceutical companies do not have groups of investigators sufficiently experienced in protein chemistry to permit them to undertake the definition of a protein in the detail necessary for the design of specific inhibitors. This is so despite the extraordinary advances in techniques of protein chemistry of the past decade, advances that have markedly reduced the scale of operation and have simplified the isolation of proteins and characterization of their structure. If the skills relating to proteins and enzymes exist in academia and the organizational capabilities of integrating synthesis, animal trials, and pharmacological and pharmaceutical know-how exist in industry, it must be asked how one might best facilitate the working together of three major interested parties, including government, in this type of complex enterprise.

The following might exemplify the ad-

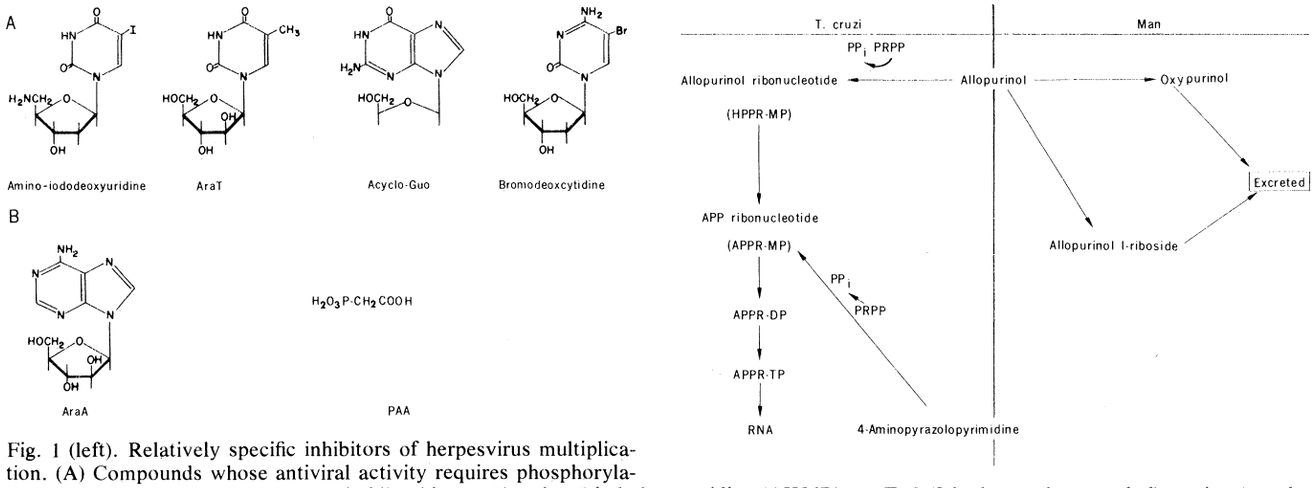

Fig. 1 (left). Relatively specific inhibitors of herpesvirus multiplication. (A) Compounds whose antiviral activity requires phosphorylation by the herpes-induced deoxypyrimidine kinase: 5′-amino-5-iododeoxyuridine (AIUdR); araT; 9-(2-hydroxyethoxymethyl)guanine (acyclo-Guo); and 5-bromodeoxycytidine. (B) Compounds that do not utilize the herpes-induced deoxypyrimidine kinase: araA and phosphonoacetic acid (PAA) (*61*). Fig. 2 (right). Metabolic transformation of allopurinol in *T. cruzi* and man. Human erythrocytes, when incubated with allopurinol, synthesize trace amounts of 1-allopurinol 5′-phosphate. Small amounts of oxypurinol 7-riboside are found in human urine. The major metabolic pathway of allopurinol in *T. cruzi* is that to allopurinol ribonucleotide (HPPR-MP), which is converted by the adenylosuccinate synthetase to the adenylate analog, APP ribonucleotide (APPR-MP), which is phosphorylated to the diphosphate (DP) and triphosphate (TP), and then incorporated into RNA (*52*).

vances in techniques of protein isolation and characterization:

1) A pure and homogenous glutamate dehydrogenase was isolated from a gram batch of *Trypanosoma cruzi* and shown to be markedly different in structure from the mammalian enzyme (*20*).

2) A complete sequence has recently been elucidated for β-galactosidase, which contains 1021 amino acids (*21*).

3) A sequence determination of the first 47 residues at the NH_2-terminus of sperm whale myoglobin was completed with 200 picomoles of unlabeled protein (*22*).

4) Computer techniques help to generate space-filling models of specific enzymes with and without reactive ligands (*23*).

Thus, it is no longer necessary to prepare tremendous quantities of organisms or of pure protein for the task that I have described. Furthermore, in the case of several virus-induced proteins, such as HSV (herpes simplex virus)-induced DNA polymerase, isolation procedures and some properties of the enzymes have already been described (*24*). It can be asked whether a compound designed from a knowledge of the structure of the virus-induced DNA polymerase will be more selectively inhibitory than substances such as araA or phosphonoacetate.

Molecular biology and protein specificity. Knowledge of the relation between base sequences in a nucleic acid and amino acid sequence in a protein permitted the calculation and experimental finding that small differences in DNA composition of bacteria lead to major differences in the amino acid composition of the bacterial proteins (*25*), a result supported by serological studies. Furthermore, in the study of nucleic acid interactions, improvements in hybridization methods have provided more sensitive tools to seek sequence homology. Such interactions are not found in the nucleic acids of organisms differing by several percent in the base compositions of their DNA's, in confirmation of the previous generalization suggested by the lack of serological cross-reactivities of bacterial, viral, and animal cell proteins.

Some proteins that lack sequence homology and that are obtained from very different types of organisms have significant similarities in their topologies; these proteins have been suggested to possess some common evolutionary ancestry (*26*). However, it appears that these configurational similarities arise in some instances via evolutionary convergence rather than divergence (*27*). The crucial fact is that the microstructure of any protein determined by a bacterium or virus is likely to be sufficiently different from that of any eukaryotic protein as to permit the design of potentially discriminating antagonists.

It may be argued that enzymes possessing comparable function are likely to have similar active sites which operate via similar mechanisms. This is true in some instances, accounting for some degree of cross-reactivities of inhibitors with various enzymes of analogous function. However, as seen in Table 1, dihydrofolate reductases derived from the prokaryotic *Escherichia coli*, the protozoans *Plasmodium berghei* and *Trypanosoma equiperdum*, and the cells of three different mammals have considerable differences in their sensitivities to three different inhibitors. These inhibitors, which distinguish the reductases of the malaria parasite, *E. coli*, and human cells, are in active clinical use. Thus, the active sites of analogous enzymes may be of sufficient chemical difference to permit the design of utilizably discriminatory inhibitors. Dihydrofolate reductase is known to be essential for the maintenance of the active coenzyme, tetrahydrofolate, which is required for the activity of thymidylate synthetase, another crucial enzyme. For this reason and the obvious promise of effective inhibitors of the former enzyme, Burroughs Wellcome and Merck have for the past several years undertaken to define the structure of the reductase from different organisms and are trying to synthesize effective inhibitors guided by considerations of protein structure. Dihydrofolate reductase represents only one of many proteins essential to the survival of parasites, and the Wellcome-Merck exercise should be applied to other essential enzymes of recalcitrant parasites.

On the Choice of an Essential Enzyme

Penicillin and erythromycin are clinically useful although the first is bacteriocidal and the second bacteriostatic. Host defenses can eliminate many infections if the defenses are not overwhelmed by rapid growth of the etiologic agent. Thus, a useful inhibitor need not be a lethal agent.

Table 1. Concentrations of antifolates needed for 50 percent inhibition of dihydrofolate reductase, isolated from six sources [from (*7*)].

Substance	Concentration ($10^{-8}M$)					
	Human liver	Rat liver	Mouse red cell	*P. berghei*	*T. equiperdum*	*E. coli*
Pyrimethamine*	180	70	100	0.05	20	2500
Trimethoprim†	30,000	26,000	100,000	7.0	100	0.5
Methotrexate‡	9	0.2		0.07	0.02	0.1

*Antimalarial. †Antibacterial. ‡Antineoplastic.

In model influenza virus infections, the inhibition of viral neuraminidase by trifluoroacetylneuraminic acid shows little effect on virus replication in a single cycle of infection. However, inhibition of virus multiplication does occur if the compound is present during successive cycles of infection initiated at a low multiplicity of infection, a result similar to that produced by antibody to viral neuraminidase which depresses replication over multiple cycles (*28*). Thus, compounds may be developed to restrain the most virulent phase of an infection and to permit an immunizing subclinical infection.

Of course we may feel more effective if we inhibit enzymes essential for an etiologic agent, as suggested by the temporary success of inhibitors such as those against dihydrofolate reductase. The functionally related enzyme, thymidylate synthetase, studied extensively as a target for antitumor agents, has scarcely been examined from the point of view of the chemotherapy of infectious disease, although a successful specific inhibition of this enzyme should lead to death of the parasite. This might be particularly true for the erythrocytic form of *Plasmodium*, since the mammalian erythrocyte cannot make thymidylate or DNA. The suggestion by Konigk (*29*) that circulating deoxycytidine is a major potential precursor for thymidylate in the malaria parasite via a salvage path warrants an investigation of the plasmodial enzymes for this pathway. Such information may permit the insertion of an inhibitory pyrimidine nucleoside analog specifically into the parasite DNA, in a manner comparable to the compounds presented in Fig. 1A in herpesvirus infections.

Because most parasites have reduced their metabolic capabilities to take advantage of the metabolism carried out by their hosts, the parasites have an unusual dependence for survival on a few limited paths. Thus, it is believed that during the parasitic stages of schistosomes, and in the intraerythrocytic plasmodia and trypanosomes, glycolysis is the sole mechanism for degrading glucose and generating ATP (*30, 31*). It can be supposed that particular enzymes in such paths in these organisms are appropriate specific targets of chemotherapy.

A greater knowledge of the enzyme systems lacking in or determined by the parasite might reveal numerous proteins potentially crucial for the survival of the parasite. For example, although the synthesis of orotate by *Plasmodium* is similar in many aspects to the synthesis in the mammal, the two reactions employ different ubiquinones, and the antimalarial naphthoquinone, menoctone, inhibits the *P. knowlesi* enzyme relatively specifically (*32*).

The observation that in its development *Plasmodium* has a special requirement for coenzyme A (coA) (*33*), which does not lie in the metabolic area of glycolysis, warrants a study of the nature of this requirement. The observations that cordycepin (3′-deoxyadenosine), tubercidin, and araA are inhibitory to the multiplication of the parasite (*29*) suggests an analysis, not only of the key sites of inhibitory action, and the possible exacerbating effects of inhibitors of adenosine deaminases for these compounds, but also of the possible incorporation of these analogs into a CoA-like molecule. For example, an inhibitory 3′-deoxy-3′-dephospho analog of CoA might conceivably be generated from cordycepin.

Many considerations could influence the choice of one or another enzyme in the development of a long-term program directed toward the development of specific and effective chemotherapeutic agents. Recent studies have pointed to relatively invariant proteins in some of the viruses, and it is possible that the relative invariance of an essential protein may be an important parameter in the choice of a protein to be inhibited. Investigators would wish to know not only something about the reaction catalyzed by the enzyme and its biological and biochemical role, but also some details of the availability, stability, molecular weight, and possible subunit structure of the protein in order to develop a strategic approach to the system. A sound decision concerning such a program requires the participation of representatives of all the major disciplines deemed essential to its development.

Some New Tools of Organic Chemistry

Many modern chemists believe that it should be possible to define the protein target adequately and to prepare an inhibitor sufficiently specific to complement an essential surface. In a recent analysis of the antigenic sites of lysozyme, defined by x-ray crystallography and "surface simulation," peptides complementary to two of these sites were synthesized and shown to bind specifically to lysozyme (*34*).

More than 10 years ago, Baker (*35*) developed the concept of "active-site-directed irreversible enzyme inhibitors," Baker and his colleagues were able to design inhibitors that reacted with amino acid residues near the substrate site; some of these compounds were able to distinguish analogous enzymes found in different organisms. More recently, similarly selective irreversible inhibitors have distinguished between tumor and thymus thymidylate synthetases (*36*) and between rabbit, pig, and carp adenylate kinases (*37*).

It is now recognized that there are at least several major approaches to the design of such inhibitors (*38*). Among these, the K_s inhibitors form a covalent bond with a reactive residue of the enzyme during recognition steps, whereas the K_{cat} inhibitors form such a bond as a result of the transformation of the substrate during the catalytic steps of the enzymatic process. K_{cat} inhibitors are necessarily more specific than most reversible inhibitors, which need not participate past early recognition steps. Such K_{cat} inhibitors may inhibit the target molecule after a single turnover and therefore may be able to reduce enzyme concentration effectively and rapidly as a function of the turnover number and the rate of inactivation of the enzyme, even if the apparent affinity of the inhibitor for the enzyme is relatively low. Site-specific inhibition may therefore help to minimize the nonspecific binding and toxicity observed with many substances, such as the marginally useful therapeutic agents suramin and ethidium (*30*). Another group of inhibitors comprises the transition state analogs, which bind very tightly even without forming a covalent bond.

The nucleic acids of some viruses and chromosomal genes are now being defined as sequences of thousands of nucleotides. Much is now known of the speci-

ficity and molecular basis of reactions of various proteins (for example, repressors, restriction enzymes, RNA polymerase) with the nucleic acids. Linking reactions (organic or photo) are being used to determine the contiguity of proteins and nucleic acids. Various cross-linking reactions have already provided much new evidence on the nature of the substrate-active site interactions.

Certainly the enormous differences between vertebrate DNA and the DNA's of many parasites should permit devising more tightly binding discriminating reagents. The preparation of base-specific intercalating agents spaced by defined polyamines may constitute an approach to such discrimination. Thus, a compound such as bis(methidium)spermine has a binding constant to DNA that is greater by a factor of 10^4 than that of methidium alone (*39*).

On Therapeutic Approaches to Tropical Disease

The Special Programme for Research and Training in Tropical Diseases of the World Health Organization has chosen to focus its attention on six diseases. These are three protozoan infections (malaria, trypanosomiasis, and leishmaniasis), two of the helminth afflictions [schistosomiasis (Platyhelminthes) and filariasis (Aschelminthes)], and a bacterial infection, namely, leprosy. I comment below on the applicability of comparative biochemistry to the therapy of an affliction of each group.

The concentration of the Special Programme on these diseases reflects numerous circumstances, for example, the large populations suffering, or at risk, from the disease, the considerable impact of these diseases on economic progress in developing countries, and the recognition of the need to overcome a relative inattention to tropical disease. Also, there has been a growing awareness of opportunities to apply recent advances in biomedical knowledge and organization to selected disease. The discoveries that the intraerythrocytic stages of the virulent *P. falciparum* (*40*) or the bloodstream forms of *T. brucei* can be cultivated in vitro (*41*) have raised the possibility of the availability of significant amounts of organisms approaching the infectious condition. A major opportunity for the possible development of a vaccine to these agents was perceived in the study of active antigens of these now more readily obtained organisms. However the increased availability of these organisms also raises the possibility of the study of their metabolic systems, and of the isolation and characterization of their structures, enzymes, and other proteins.

In the treatment of leishmaniasis until recently, a pentavalent antimonial was used mainly for treatment of the cutaneous disease whereas a diamidine was used for the visceral disease. Trivalent antimonials were thought to be dangerously toxic. However, by encasing these compounds, such as sodium antimony gluconate or potassium antimony tartrate, in liposomes that are selectively taken into the reticuloendothelial cells where the hemoflagellate protozoan multiplies, an increase of several hundredfold in effectiveness of treatment is obtained with marked reductions in toxicity (*42*). Thus, a knowledge of the major biological site of multiplication and damage by the parasite and the relatively specific uptake of liposomes has led to a striking advance in the therapy of this disease in experimental animals.

It is obvious that, in the absence of major new leads or evidence of therapeutic efficacy, it is necessary to continue to attempt to improve old drugs that may have a marginal activity. However, because it is now clear that these diseases are caused by infectious agents whose biochemistry is markedly different from that of the host, a major effort in chemotherapy should also be directed toward the exploitation of these differences between parasite and host and not only to the modification of old drugs or of drug delivery.

Remarks on Trypanosomiasis

Although effective compounds are known for some tropical diseases and are underutilized for many reasons, such as lack of primary health care and poor distribution of drug, the trypanosomiases are clearly diseases in which new and better drugs are needed.

The bloodstream trypanosomes of the African trypanosomiases have a number of distinctive structural and biochemical features that separate these from the trypanosomes of Chagas' disease, such as *T. cruzi*. The properties of the flagellum and the surface coat have been studied in relation to a possible immunological response of protective antibody. This work led to the surprising observation of extensive antigenic variation in the organisms, such as *T. brucei*, separate clones of which were found to contain markedly different surface proteins or glycoproteins (*43*). It is now postulated that the organisms may be able to express alternative surface glycoprotein genes in response to antibody and thereby minimize the effects of agglutinating antibody. This surprising capability may vitiate the possible effectiveness of a vaccine based on such proteins, in contrast to the promise of antimalarial vaccines.

The study of energy metabolism of bloodstream forms soon revealed the relative emphasis on a system that converted glucose to pyruvate in air and that did not form carbon dioxide, acetate, or citrate (Table 2). In the African trypanosomes these patterns are consistent with active anaerobic glycolysis in air, the absence of a tricarboxylic acid cycle, and the minimal use of an oxidative hexose monophosphate shunt (*30*). Nevertheless the formation of pyruvate and glycerol rather than lactate suggested the possible presence of unusual systems for the reoxidation of reduced nicotinamide adenine dinucleotide (NADH) needed to maintain glycolysis. This meant also that the usual function of mitochondria, namely, oxidative phosphorylation, would not exist in the bloodstream forms. This fact thereby accentuates the unusual structure of the trypanosome kinetoplast, which contains a large fraction of the protozoan DNA and is so important in the virulence of the protozoan.

An early chemotherapeutic agent, ethidium, was found to generate akineto-

Table 2. End products of aerobic glucose metabolism in bloodstream trypanosomes [from (*32*)]. Results are expressed as follows: −, none; ±, trace; +, small quantities; ++, substantial quantities.

Organism	CO_2	Glycerol	Ethanol	Lactate	Pyruvate	Acetate	Succinate	Citrate
T. rhodesiense	±	+	−	−	++	−	−	−
T. gambiense	−	+	−	−	++	−	−	−
T. brucei	−	+	−	−	++	−	−	−
T. evansi	−	+	−	−	++	−	−	−
T. equinum	−	+	−	−	++	−	−	−
T. equiperdum	−	+	−	−	++	−	−	−
T. vivax	+	+	−	±	+	+	−	−
T. congolense	+	+	−	−	−	+	+	−
T. cruzi	++	−	−	±	−	+	+	−
T. lewisi	++	−	−	±	−	+	+	−

plastic forms and to intercalate into the circular double-stranded DNA present in the kinetoplast and other mitochondria (*44*). In their turn, the study of kinetoplast DNA and the DNA-binding properties of ethidium have become active fields of research. Ethidium is toxic and is used almost exclusively in trypanosomal infections of cattle. Not all substances that induce dyskinetoplasty are as toxic as ethidium. Berenil, which is also used mainly in infections of cattle, is concentrated in all DNA-containing organelles of the protozoan, but it is also reported to have some value in treatment in man (*45*).

The study of hydrogen transport in the trypanosomes revealed the existence of two unusual enzymes which are active on intermediates of anaerobic glycolysis and reoxidize NADH to NAD and produce water (*30*). The first, termed L-α-glycerophosphate dehydrogenase, catalyzes the reversible reaction of dihydroxyacetone phosphate plus NADH to NAD plus L-glycerophosphate. The second enzyme, an L-α-glycerophosphate oxidase, which exists in the poorly developed mitochondria, oxidizes glycerophosphate to dihydroxyacetone phosphate and water. An active inhibitor of this enzyme is suramin, an aromatic polysulfonate whose antitrypanosomal action may relate to this plus other inhibitory activities, for example, on both dihydrofolate reductase and glycerophosphate dehydrogenase. The glycerophosphate oxidase requires iron and is inhibited by chelators, such as salicylhydroxamic acid (SHAM). Another route of reoxidizing NADH involves a reduction of triose to glycerol. The presence of glycerol, which also prevents this reduction, plus SHAM were recently found to be trypanocidal (*46*). Thus, our knowledge of the special energy metabolism of the trypanosomes, a metabolism which is used to regenerate essential NAD and ATP, can be exploited to kill the parasite and to improve survival of the infected animal.

The α-glycerophosphate dehydrogenase of *T. brucei*, which is concentrated in unusual microbodies (*47*), has been shown to be activated maximally by low concentrations of the polyamine spermidine (*48*) present in many species of trypanosomes (*49*). This enzyme is also reactive with ethidium (*50*), being inhibited by high concentration of the dye. Ethidium and spermidine may compete for various binding sites, as a result of the similar distributions of amino groups in the two molecules. Compounds that inhibit spermidine-synthesizing enzymes inhibit the growth of *T. brucei*; and most recently it has been found that the curative effects of bleomycin on *T. brucei* infections in animals are prevented by spermidine (*51*). Thus, a most unusual group of inhibitory effects has been detected, which may relate to the apparent role of a polyamine in the unique glycerophosphate dehydrogenase of the bloodstream trypanosomes. The enzymes noted above are obvious candidates for the chemotherapeutic strategy described earlier, which bases the development of specific inhibitors on the identification of essential proteins of the parasite.

As described earlier (Fig. 1A), the biosynthetic enzymes determined by a parasite may be sufficiently different from those of man as to permit the metabolism of analogs relatively inert in man into compounds selectively detrimental to the parasite. This situation has also been found in the case of the trypanosome of Chagas' disease (*T. cruzi*) as well as for several pathogenic leishmaniae. The compound, allopurinol, widely used in the treatment of gout in man, is converted by an enzyme of the parasite to the 4-hydroxypyrazolo-pyrimidine nucleotide, allopurinol mononucleotide, in the parasites. It is then transformed, only within the parasites, to the adenine analog, 4-aminopyrazolo-pyrimidine mononucleotide. This substance is converted to the triphosphate and enters the RNA of the parasite (*52*). This process (Fig. 3) is believed to be the basis of the selective toxicity of allopurinol to *T. cruzi* as compared to animal cells.

On schistosomiasis. The search for effective and safe antischistosomal drugs is far from complete, and it would be appropriate to explore the special structural and metabolic features of the organisms as a guide to drug development. The aim of an antischistosomal drug would be to kill or weaken the parasite and prevent egg deposition.

In the early 1950's, Bueding and his collaborators (*53*) had determined that several enzymes of the glycolytic system of *Schistosoma mansoni* were immunologically different from functionally analogous enzymes in mammals (*53*). It is relevant that in later years the DNA of the schistosome was found (*54*) to be high in adenine (A), and thymine (T) (66 percent), as is that of man (where the DNA is 60 percent AT). Nevertheless, this difference is sufficient to compel major changes in the amino acid composition and organization of analogous proteins. Differences were also found in various kinetic properties of the analogous enzymes, as well as in their responses to inhibitors. Organic antimonials inhibited the schistosomal phosphofructokinase at a concentration only 1 percent of that required to inhibit the mammalian enzyme similarly (*55*). Inhibition of the schistosomal enzyme selectively reduced carbohydrate metabolism of the parasite, a result subsequently obtained with the antimonial on the liver fluke (*56*). The toxicity of the antimonials and their chemotherapeutic failure relate to their inactivation of other systems apart from that of the parasite phosphofructokinase. By the 1960's, Bueding and others had identified several distinctive enzymes highly important to the survival of the parasite, whose more specific inactivation should now be sought. The ability of the schistosomal worm to synthesize purines is limited, and the selective lethality of some nucleosides, such as tubercidin, to the schistosomal worm has been observed (*57*).

Oxygen is important for survival of *S. mansoni* (*58*) and particularly for steps required for egg production. Since egg production in the infected human is the major cause of pathology, it is evident that selective lethal effects on male or female warrant great interest. The recently discovered drug oxamniquine selectively kills males and thereby is highly effective on *S. mansoni* infections. As a specific biochemical property possibly amenable to selective attack, numerous observers have pointed to the presence of chitin as a parasite-specific polymer in the cuticle of the worm egg. A search for inhibitors of chitin synthetase may warrant study in this respect.

Mycobacterium leprae as a Prokaryotic Organism

Little is known of the biochemistry of *Mycobacterium leprae*, whose growth is best achieved at present in the armadillo. In the following discussion it will be assumed that the organism possesses many biochemical features characteristic of other *Mycobacterium*. In concentrating on the cell wall polysaccharide, I shall consider a pentose sugar whose presence should be easily identifiable in the bacterial fractions obtainable from the infected armadillo.

It was noted earlier that the cell walls of prokaryotic organisms are very different from those of eukaryotes, and provide sites for selective chemotherapy. For example, these structures in prokaryotic organisms frequently contain polymers such as teichoic acid and peptidoglycan, as well as simple substances, such as muramic acid and diaminopimelic acid, which have not been found in the

walls of eukaryotic cells. Among the latter in many bacteria is also found 2-keto-deoxyoctonate (KDO), which is synthesized from another strictly prokaryotic metabolite, D-arabinose 5-phosphate (*18*). D-Arabinose 5-phosphate is produced from ribulose 5-phosphate by the action of phosphoarabinoisomerase, an enzyme which has not been detected in mouse fibroblasts or in yeast (*59*). Bacteria blocked specifically in the synthesis of KDO are killed during growth (*60*).

Mycobacterium, as well as *Corynebacterium* and *Nocardia*, lack KDO but synthesize a cell wall polysaccharide, about half of which consists of D-arabinose. This prokaryotic pentose is therefore not terminal but occurs within the chain, and, by analogy to known mechanisms of polysaccharide synthesis, is derived from a nucleoside diphospho-D-arabinose. The origin of such a compound probably requires the conversion of D-arabinose 5-phosphate to D-arabinose 1-phosphate, which after reaction with a nucleoside triphosphate will provide the immediate precursor for polysaccharide synthesis. Thus the synthesis of the cell wall polysaccharide of organisms of three important genera would appear to require four enzymes specific to these bacteria. If it is supposed that the cell wall polysaccharides of these organisms are important in the devleopment of their pathogenic properties, we might well have four proteins to consider as possible targets for a specific chemotherapy, if the hypotheses described above are confirmed.

Conclusions

It appears that biochemistry has identified unique metabolic steps essential to survival and multiplication of important parasites. The science and technology applicable to analysis of the origin and structure of essential proteins is ready for a major new direction of attack on the chemotherapy of all infectious disease. The proposed strategy is such that, contrary to current approaches by funding agencies, the research budget for tropical disease should be viewed as related also to the budgets for research on bacterial and viral disease.

The nature of the problem and the approach outlined in this article require an integrated multidisciplinary attack on parasite-determined proteins, scarcely being attempted at present. A concentration on inhibitors specifically reactive on proteins could avoid problems of mutagenicity and carcinogenicity of compounds designed to affect nucleic acid metabolism. The time required for such an effort should not be greater than the time usually needed for drug development in a pharmaceutical company. Although the nature of the approach will identify therapeutic leads relatively late in a development process that requires considerable study of the unique essential target protein, the search for specificity in the inhibitory process should minimize the waste arising from discovery of nonselective toxicity in animal or clinical studies. The funds to be expended could be much smaller than those spent in empirical approaches, as customary in the search for antiviral agents. Finally, a well-organized multidisciplinary effort would provide excellent opportunities for training international groups of investigators who must ultimately develop in their own countries the drugs to treat the multitude of bacterial, viral, fungal, protozoan, and worm diseases found only in their countries.

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