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New Applications of Microbial Products

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Summary. Microbial secondary metabolites are now being used for applications other than as antibacterial, antifungal, and antitumor agents. These applications include use against parasites (coccidia, helminths) and insects as well as for animal and plant growth stimulation, immunosuppression, uterocontraction, and other pharmacological activities. Further applications are possible in various areas of pharmacology and agriculture, a development catalyzed by the use of simple enzyme assays for screening prior to testing in intact animals or in the field.

The expression "wonder drugs," refers to the selective action that microbial chemicals exert against pathogenic bacteria, fungi, and tumors. The discovery of this selective activity ushered in the "antibiotic era," and for more than 40 years we have been the beneficiaries of this remarkable property of antibiotics. The success rate has been so high that for years the predominant application of microbial secondary metabolites was that of antibacterial, antifungal, and antitumor chemotherapy. Unfortunately, however, such a restricted view of the potential of microbial idiolites (secondary metabolites) has retarded the further development of the fermentation industry. Many industrial microbiologists have felt that antibiotic activity is merely the tip of the iceberg; that is, with regard to the potential application of microbial secondary metabolites for the benefit of humankind, the surface has only been scratched. In this article, I point out those cases in which microbial metabolites have surprising applications and also point to some challenges for the future. In this way, I hope to engender further appreciation of Jackson Foster's astute and predictive statement: "Never underestimate the power of the microbe" (*1*).

Antiparasitic Activities

One of the major economic diseases of poultry is coccidiosis, which is caused by species of the parasitic protozoan *Eimeria*. For years, this disease was treated solely by synthetic chemicals, and indeed only synthetic compounds were screened for coccidiostat activity. Although they were generally effective, resistance developed rapidly in the coccidia, and new chemical modifications of the existing coccidiostats had to be made. Then a parenterally toxic and narrow-spectrum antibiotic, monensin, was found by the group at Eli Lilly & Company to have extreme potency against coccidia (*2*). At first there were grave doubts that the fermentation process for this polyether compound could be improved to the point where monensin would become economically feasible. However, industrial genetics and biochemical engineering techniques were applied to this improvement project, and as a result the polyethers (*2a*), especially monensin (produced by *Streptomyces cinnamonensis*) and lasalocid (produced by *Streptomyces lasaliensis*), now dominate the commercial coccidiostat market.

An interesting sidelight of the monensin story is the discovery of its further use as a growth promoter in ruminants. For years, synthetic chemicals had been screened in an effort to supplement cattle and sheep diets with an agent that would eliminate the wasteful methane production and increase volatile fatty acid formation (especially propionate) in the rumen, thus improving feed efficiency. Although the concept was sound, no useful products resulted. Experimentation with monensin showed that polyethers have this beneficial activity, and now these compounds are widely used (*3*). Polyethers also have cardiovascular effects that are being studied for possible medical application.

Another major agricultural problem has been the infection of farm animals by worms. The predominant screening effort over the years was the testing of synthetic compounds against nematodes, and commercial products did result. Certain antibiotics had also been shown to possess antihelminthic activity (for example, hygromycin, antibiotic G-418, destomycin, paromomycin, antibiotic complex S15-1, antihelvencin, aspiculamycin, anthelmycin, myxin, thaimycin, and axenomycin) against nematodes or cestodes (*4*), but these failed to compete with the synthetic compounds.

Although the Merck Sharp & Dohme Laboratories had developed a commercially useful synthetic product, thiobenzole, they had enough foresight to also examine microbial broths for antihelmin-

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thic activity. They were pleasantly surprised to find a fermentation broth that killed the intestinal nematode, *Nematosporoides dubius*, in mice and was nontoxic, and that it was without antibiotic activity against bacteria or fungi (*4*). The *Streptomyces avermitilis* broth contained a family of secondary metabolites that they named "avermectins." These are macrocyclic lactones with exceptional activity against parasites; that is, the activity was at least ten times higher than any synthetic antihelminthic agent known. Despite their macrolide structure, avermectins do not inhibit protein synthesis nor are they ionophores; they appear to interfere with neurotransmission in many invertebrates (*5*). They have activity against both nematode and arthropod parasites in sheep, cattle, dogs, horses, and swine. A semisynthetic derivative, 22,23-dihydroavermectin B_1 ("ivermectin") (*6*) is 1000 times more active than thiobenzole and is already being used in certain countries. As in the monensin story, these potent molecules have additional activity as insecticides and may be useful in protecting plants (*7*).

Insecticides and Herbicides

The selective toxicity of the crystal protein (that is, the delta endotoxin) of *Bacillus thuringiensis* against insects of the order Lepidoptera has been exploited commercially for several years (*8*). Indeed, the selectivity of its toxicity against these insects has limited its commercial success because agricultural practitioners have been spoiled by broad-spectrum chemical insecticides. However, the world is becoming wary of the ecological damage done by many synthetic chemicals and the resistance that is developing in insects (*9*). In contrast, the insecticidal toxin of *Bacillus thuringiensis* has not disturbed the environment and no resistance has developed. Other intriguing applications that have not yet reached their potential include the activity of certain strains of *B. thuringiensis* (*8*) and *B. sphaericus* against mosquitoes (*10*) and *B. popilliae* against the Japanese beetle (*11*).

With regard to low-molecular-weight microbial metabolites, there are a number with activity against insects. These include inhibitors of respiration (antimycin A, patulin, and piericidines), of protein synthesis (cycloheximide and tenuazonic acid) and membrane-active agents (destruxin, beauvaricin, and polyene antibiotics). However, their toxicities have restricted their practical application. On the other hand, there is considerable interest in the potential use of nikkomycin against agricultural pests. The nikkomycins are nucleoside analogs, structurally related to the polyoxins which are being used as agricultural antifungal agents. Since these compounds function as inhibitors of chitin synthetase and since chitin is an important structural material for insects (*12*), the nikkomycins have potent insecticidal activity (*13*). Other fermentation products with insecticidal activity include the prasinons (*14*) and the milbemycins (*15*). The macrotetralide tetranactin has been in use since 1974 as a mitocide for plants (*16*).

The current agricultural use of synthetic chemicals as herbicides has worried many environmentalists. Although microbial products have not really been taken seriously as potential herbicides, there are reports of the herbicidal activity of streptomycete secondary metabolites. These include the herbimycins [ansamycins active against mono- and dicotyledonous plants (*17*)] and the herbicidins [nucleoside analogs active against dicotyledons (*18*)]. If economic problems can be solved, this certainly will be a viable commercial area for microbial metabolites in the future.

Plant Growth Regulators

Gibberellins are a group of phytotoxic mycotoxins, produced by *Gibberella fujikuroi*, which is the cause of the "foolish seedling" disease of rice (*19*). In this disease, the infected plant grows abnormally fast and then dies. The gibberellins have been used successfully in regulating the growth of plants. They are used to reduce the time needed for malting of barley, to improve the quality of malt, and to increase the yield of vegetables as well as to allow their earlier marketing. Development of biennial plants can be made so rapid that seed crops may be obtained from lettuce and sugar beets in 1 year instead of the usual 2 years.

Pharmacological Action of Microbial Metabolites

Several investigators (*20*) have pointed out the varied pharmacological activities of microbial secondary metabolites (Table 1). Unfortunately, there has been a reluctance to screen the pharmacological activities of fermentation broths for the following reasons: (i) These activities are normally assayed in living animals, and pharmacologists are reluctant to administer crude broths to their animals. Pharmacologists prefer screening synthetic chemicals since there are fewer side effects and, if activity is observed, they immediately know the structure of the active agent. (ii) There is a bias that microbial metabolites are only useful in solving microbial problems. Although the first reason is quite justifiable, the second reason is not. However, as I describe below, certain microbial metabolites are indeed useful in medicine and it is a source of wonder how their activities were ever detected in view of the above restrictions (i) and (ii).

Some of these metabolites were detected because the products had antibacterial or antifungal activity although they were not suitable for use as antibiotics. Since the products had been purified during the attempt to develop them as antibiotics, there was no reluctance to test such purified materials for pharmacological activities in animals. As a result, cyclosporin A (an antifungal antibiotic produced by *Tolypocladium inflatum*), is used today as an immunosuppressive agent in human organ transplants (*21*). Cyclosporin A has received interest in cases of heart transplantation in that it can block production of white cells that cause rejection but not those fighting infectious microbes. It has also been associated with improvement in the effectiveness of kidney and liver transplants.

A well-known example of the nonantibiotic use of microbial secondary metabolites is that of the ergot alkaloids (*22*). It is remarkable that this group of mycotoxins, responsible for widespread and fatal poisoning of people eating bread from contaminated grain or animals feeding on contaminated grain or infected grass throughout the ages, has also been used for the benefit of humankind. Ergot alkaloids are used for uterocontractant activity in obstetrics, to treat migraine headaches, hypertension, serotonin-related disturbances, to inhibit prolactin release in treating agalactorrhoea, and to disrupt implantation in early pregnancy. They are produced by various species of *Claviceps* in large-scale industrial fermentations. New applications of this large and potent group of fungal products are still being uncovered, especially in the treatment of Parkinsonism and cancer (*23*). Alkaloids are also produced by actinomycetes (*24*) and have antihistaminic, hypotensive, and hypoglycemic activities.

Another mycotoxin whose potent ac-

tivities have been harnessed is zearelanone, produced by *Gibberella zeae* (*25*). This compound is an estrogen and is used as an anabolic agent in cattle and sheep to improve both growth and feed efficiency.

Some recently discovered pharmacological activities of other microbial metabolites follow.

Anti-inflammatory activity. A number of actinomycete and *Bacillus* products have anti-inflammatory activity as measured by inhibition of rat foot pad edema induced by carrageenin (*26–29*). One of these is amicomacin A, a *Bacillus pumilus* antibiotic that shows both anti-inflammatory and anti-ulcer activities (*28*); others are forphenicine and esterastin (*29*). Other compounds found to show anti-inflammatory activity are the pyrrothine antibiotics produced by *Streptoverticillium* sp. (*30*).

Hypocholesteremic activity. The ability to inhibit cholesterol formation in the liver of rats has been detected with citrinin, a metabolite of *Pythium ultimum* and with compactin, an antifungal agent produced by *Penicillium brevicompactum* and *Penicillium citrinum*. Compactin has low acute toxicity and shows activity also in hens and dogs (*31*). More recently discovered metabolites include monacolin K from *Monascus ruber*, a nontoxic metabolite which is structurally similar but four to five times more active than compactin (*32*). Menivolin, discovered independently as a product of *Aspergillus terreus*, is identical to monacolin K (*33*). The dihydroderivatives of mevinolin (*34*) and of compactin (*35*) have been isolated from *Aspergillus terreus* and *Penicillium citrinum*, respectively, and resemble the activities of their parent compounds.

Hyperlipidemic activity. Hyperlipidemia is one of the causes of coronary heart disease in humans. A synthetic drug, clofibrate, has been used but it has side effects and marginal activity. An antibiotic, ascofuranone, produced by *Ascochyta viciae*, is orally active in rats, reducing serum cholesterol, triglycerides, phospholipids, free fatty acids, and cardiac cholesterol content. Ascofuranone does not induce hepatomegaly, the main side effect of clofibrate, and shows only weak acute toxicity in mice and rats (*36*).

Hypotensive activity. Dopastin, produced by a *Pseudomonas* strain, shows a hypotensive effect in spontaneously hypertensive rats (*37*); it is of low toxicity. Another hypotensive agent is oudenone, a product of low toxicity produced by *Oudemansiella radicata* (*38*). Compound II of *Corynespora cassiicola* is a nontoxic, nonantibacterial agent that is hypotensive. Its structure is 2,3,5-trihydroxy-6-(3-hydroxy-*n*-butyl)-7-methoxy-1,4-naphthaquinone (*39*).

Vasodilator activity. Vasodilators WS-1228A and B have been isolated from *Streptomyces aureofaciens* (*40*). Testing of the B component revealed no antibiotic activity and low acute toxicity. Both compounds contain a *N*-hydroxytriazene moiety and are thought to be the first natural compounds containing a triazene group.

Table 1. Some pharmacological activities of microbial secondary metabolites.

ACTH-like	Diuretic	Hypolipidemic
Anabolic	Edematous	Hypotensive
Anesthetic	Emetic	Hypersensitizing
Analeptic	Erythematous	Immunomodulating
Anorectic	Estrogenic	Leukemogenic
Anticoagulant	Fertility enhancing	Parasympathomimetic
Antidepressive	Hallucinogenic	Photosensitizing
Anti-inflammatory	Hemolytic	Relaxant (smooth muscle)
Antispasmodic	Hemostatic	Sedative
Carcinogenesis inhibition	Herbicidal	Serotin antagonist
Coagulative (blood)	Hormone releasing	Spasmolytic
Complement inhibition	Hypocholesterolemic	Vasodilatory
Dermonecrotic	Hypoglycemic	

Enzyme Inhibitors

As stated above, it is extremely difficult for the microbiologist to get adequate pharmacological testing of microbial broths. Yet, it is known that such broths exhibit interesting pharmacological activities. A solution to this dilemma was proposed by Umezawa a number of years ago (*41*). He suggested that in vitro enzyme assays be used to detect inhibitory compounds in microbial broths. Since known pharmacological agents do inhibit enzymes and since some diseases are associated with excessive or unregulated enzyme activities, it was reasoned that enzyme inhibitors from microbial broths might exhibit valuable pharmacological activities; most important, this was a means of "liberating" the microbiologist from the pharmacologist with respect to the discovery of new, possible nonantibiotic, agents with potential application in medicine.

As a result of the initiative of Umezawa and subsequent extensive studies by his group (*41*) and others, a large number of extremely potent enzyme inhibitors have been isolated and identified; some 50 inhibitors were found by the Umezawa group alone (*42*). There is no longer any doubt that given a simple enzymatic assay, extremely potent inhibitors can be found in microbial broths, some of which are orders of magnitude more active than previously known inhibitors, either synthetic or derived from higher animals and plants. Some of these microbial inhibitors are described below.

Inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase. This rate-limiting enzyme of cholesterol synthesis has been successfully used as an assay to isolate hypocholesterolemic agents. Such agents, for example, monacolin K (mevinolin), which have been described above are extremely active in animals (*43*) and appear to be headed for clinical use.

Inhibitors of dopamine β-hydroxylase, tyrosine hydroxylase, and catechol-O-methyltransferase. Broths screened for activity in these assays have yielded products showing hypotensive activity in animals (*37, 39, 44*).

Inhibitors of complement. An inhibitor of the complement activation cascade is known as K-76 monocarboxylic acid, a sesquiterpene derivative that is an oxidation product of the natural compound produced by *Stachybotrys complementi* (*45*). The action of this compound is rather specific; that is, there is no inhibition of trypsin or plasmin. K-76 monocarboxylic acid acts by inhibiting formation of the chemotactic factor for polymorphonuclear leukocytes in human complement serum. It inhibits nephrotoxic nephritis in rats and may be useful in immune complex diseases, allergic diseases, and inflammation.

Inhibitors of intestinal glycosidases. Agents inhibiting amylase or invertase might be useful for persons who should only consume restricted quantities of carbohydrates to avoid hypoglycemia and increased synthesis of triglycerides in adipose tissue, liver, and the wall of the intestine, that is, patients suffering from carbohydrate-dependent diseases such as diabetes, type IV hyperlipoproteinaemia, and obesity. Such a compound is acarbose (BAYg5421), pro-

duced by *Actinoplanes* sp. (*46*), which is awaiting approval by the German government. Another is product S-AI of *Streptomyces diastaticus* subsp. *amylostaticus* which inhibits α-amylase and glucoamylase but not β-amylase or pullulanase (*47*).

A specific inhibitor of *Streptococcus mutans*, dextran-sucrase, has been isolated from a streptomycete. Since dextran-sucrase is thought to play a role in the initiation of dental caries, the fungal product (ribocitrin) may have application in preventing cariogenicity. Ribocitrin has no antibiotic activity and appears to have no acute toxicity (*48*).

Inhibitors of pancreatic esterase. Esterasin, an inhibitor of pancreatic esterase, is produced by *Streptomyces lavendulae*; it is nontoxic and possesses no antibiotic activity (*49*). It suppresses delayed-type hypersensitivity and antibody formation.

Inhibitors of cholinesterase. A compound (I-6123) inhibiting cholinesterase is produced by *Aspergillus terreus* (*50*). This is of interest since known synthetic insecticides are cholinesterase inhibitors (*51*).

Protease inhibitors. The possible role of proteases of polymorphonuclear leukocytes in inflammation and carcinogenesis has been pointed out (*52*). A large number of potent protease inhibitors have been isolated from streptomycete broths (*27*); among the best known are leupeptin (*53*), antipain, chymostatin, elastatinal, bestatin and pepstatin (Table 2). They have been important in studies directed toward assessing the role of proteases in various processes. For example, the inhibition of carcinogenesis in cell culture and animals by leupeptin, pepstatin, chymostatin, elastatinal, and antipain has indicated that protease activity is a necessary step in the tumor-inducing process (*53*). Elastatinal and antipain are active against chemical mutagenesis in bacteria, apparently inhibiting a protease involved in SOS DNA repair (*54*). Carboxyprotease inhibitors include pepstatins, pepstanones, and hydroxypepstatins. Pepstatin inhibits focus formation by murine sarcoma virus on

Table 2. Inhibitory activities of protease inhibitors of microbial origin (*27*). IC_{50} is the concentration which inhibits the enzyme by 50 percent. [Courtesy of the *Japanese Journal of Antibiotics*]

Enzyme	Substrate	IC_{50} (μg/ml)					
		Leupeptin	Antipain	Chymostatin	Elastatinal	Pepstatin	Bestatin
Trypsin	Casein	2.0	0.26	>250.0	>250.0	>250.0	>250.0
Plasmin	Fibrinogen	8.0	93.0	>250.0	>250.0	>250.0	>250.0
Papain	Casein	0.5	0.16	7.5	>250.0	>250.0	>250.0
Chymotrypsin	Casein	>500.0	>250.0	0.15	>250.0	>250.0	>250.0
Elastase	Elastin–congo red	>250.0	>250.0	>250.0	1.8	>250.0	>250.0
Pepsin	Casein	>500.0	>250.0	>250.0	>250.0	0.01	>250.0
Thermolysin	Casein	>250.0	>250.0	>250.0	>250.0	>250.0	>250.0
Cathepsin A	Z-Glu-Tyr*	>500.0	1.2	62.5	>250.0	>125.0	>250.0
Cathepsin B	BAA†	0.44	0.6	2.6	—	>125.0	>250.0
Cathepsin C	Ser-Tyr-NA‡	>250.0	>250.0	>250.0	>250.0	>250.0	>250.0
Cathepsin D	Hemoglobin	109.0	>250.0	49.0	>250.0	0.01	>250.0
Renin	Peptide§	>250.0	>250.0	>250.0	>250.0	4.5	>250.0
Aminopeptidase B	Arg-NA‖	>250.0	>250.0	>250.0	>250.0	>250.0	0.05
Leucine aminopeptidase	Leu-NA¶	>250.0	>250.0	>250.0	>250.0	>250.0	0.01

*Carbobenzoxy-L-glutamyl-L-tyrosine. †N^{α}benzoyl-L-arginine amide hydrochloride. ‡L-Seryl-L-tyrosine β-naphthylamide. §His-Pro-Phe-His-Leu-Leu-(^{3}H-Val)-Tyr-Ser. ‖L-Arginine 2-naphthylamide. ¶L-Leucine 2-naphthylamide.

Table 3. Enzymes which are potential targets for new drugs.

Enzyme	Target	Reference
Cholinesterase	Myasthenia gravis, insect diseases of plants	*50, 51, 83*
Monoamine oxidase	Depression	*84*
Serine protease	Fertility	*57*
Protease	Inflammation	*27, 57*
Elastase	Pulmonary emphysema	*56–59*
Collagenase	Glomerulonephritis	*57*
Proteinase	Demyelinating diseases	*57*
Cathepsins B and D	Muscular dystrophy	*57*
Cyclo-oxygenase of prostaglandin synthetase	Inflammation	*85*
Viral proteases	Viral disease	*86*
3-Hydroxy-3-methylglutaryl-CoA-reductase	Hypercholesteremia	*43*
Dopamine β-hydroxylase, tyrosine hydroxylase, catechol-*O*-methyltransferase	Hypertension	*37–39, 44*
Complement activation	Nephritis, immune diseases, allergic disease, inflammation	*45*
Glycosidase	Hypoglycemia, diabetes, type IV hyperlipoproteinemia, obesity	*46, 47*
Dextransucrase	Dental caries	*48*
Esterase	Hypertension	*49*
Protease	Mutagenesis, carcinogenesis	*27, 29, 42, 53–55, 64*
Pepsin	Ulcers	*62*
Cyclic AMP phosphodiesterase	Cancer, hypertension, asthma, cholera, diabetes	*68–70*
Prolyl-4-hydroxylase	Fibrotic disease	*71–73*
Ornithine decarboxylase	Psoriasis, chronic nonsuppurative prostatitis, cancer	*74, 75*
Angiotensin-converting enzyme	Hypertension	*78, 79*
S-adenosylmethionine decarboxylase	Cancer, psoriasis, chronic nonsuppurative prostatitis	*80*

YH-7 mouse cells and ascitic accumulation in cancer (*55*).

Elastase appears to be involved in chronic obstructive lung diseases such as emphysema (*56, 57*) as well as in pancreatitis, acute arthritis, and various inflammations. Elasnin, a nonantibiotic metabolite of *Streptomyces noboritoensis*, inhibits human granulocyte elastase but is relatively inactive on pancreatic elastase, trypsin, chymotrypsin, thermolysin, and papain (*58*). Another elastase inhibitor is the peptide, elastatinal, produced by an actinomycete (*59*). Serine- and thiol-protease inhibitors from microbial broths show anti-inflammatory activity (*27, 57*).

Pepstatin, a peptide product of several streptomycetes, is an inhibitor of acid protease [especially pepsin (*60*)] and has a strong diuretic effect, probably due to inhibition of renin (*55*). Other streptomycete-derived pepsin inhibitors include SP-I (*61*) and the pepsinostreptins [isobutyryl-, propionyl-, and acetyl-(valyl-valyl-4-amino-3-hydroxy-6-methylheptanoyl-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid)]. Pepsinostreptin prevents gastric ulceration in rats (*62*).

A specific inhibitor of the metallic endopeptidase, thermolysin, is phosphoramidon, a nonantibiotic, nontoxic metabolite of *Streptomyces tanashiensis*. Its structure is *N*-(α-L-rhamnopyranosyloxyhydroxyphosphinyl)-L-leucyl-L-tryptophan (*63*).

Bestatin [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-(*S*)-leucine is a specific inhibitor of aminopeptidase B and leucine aminopeptidase, and is produced by *Streptomyces olivoreticuli* (*64*). Since these enzymes are cell surface enzymes in lymphocytes, they could conceivably be involved in the immune response, and compounds binding to such enzymes might be immunomodulators. Bestatin was found to enhance delayed-type hypersensitivity in vivo, activation of peripheral blood lymphocytes by concanavalin A, and the activity of antitumor agents in animals (*27*). It increased the number of antibody-forming cells in mice and inhibited slow-growing solid tumors such as the Gardner lymphosarcoma and IMC-carcinoma. In clinical studies, bestatin enhanced immunity in cancer patients (*29*) and showed a number of other beneficial effects (*42*).

Other surface enzymes are alkaline phosphatase and esterase and microbial inhibitors of these enzymes have immuno-modulation activity (*42*). Amastatin is an inhibitor of aminopeptidase A and leucine aminopeptidase and is produced by *Streptomyces* sp. Its structure is (2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoyl-L-valyl-L-valyl-L-aspartic acid. Amastatin is nontoxic and has no antibiotic activity (*65*). Forphenicine is an inhibitor of alkaline phosphatase and is produced by *Streptomyces fulvoviridis* var. *acarbodicus* (*66*). Its structure is 4-formyl-3-hydroxy-phenylglycine (*67*). Both products increase the number of antibody-forming cells. Forphenicine enhances delayed-type hypersensitivity and shows activity against solid tumors (*42*).

Agents affecting cyclic adenosine monophosphate (AMP) levels. Since cyclic AMP concentrations are altered in cancer, hypertension, asthma, cholera, and diabetes, there has been some interest in identifying agents that bring about these alterations. Of special interest have been cyclic AMP–increasing agents that might increase fat cell lipolysis and bronchodilation. As a result, screening efforts have been directed toward the detection of rabbit brain cyclic AMP phosphodiesterase inhibitors, and several streptomycete products have been isolated. These include 3-carbamoyl-1,2-dihydro-4-hydroxy-5-methoxy-3-[*H*]-pyrrolo [3,2-*e*]indole-7-carboxylic acid and its 3-acetyl derivative from *Streptomyces* sp. (*68*) and reticulol (6,8-dihydroxy-7-methoxy-3-methylisocoumarin) from *Streptomyces mobarensis* (*69*). A useful agar plate assay in which beef brain cyclic AMP phosphodiesterase is used has been described (*70*).

Prolyl-4-hydroxylase inhibitors. Fibrotic collagen accumulation causes fibrotic disease of connective tissue. Lung and liver fibrosis in animal models is prevented by proline analogs. Prolyl-4-hydroxylase is thought to be the target enzyme since there is a hydroxyproline requirement for collagen secretion and thermal stability (*71*). Since there are no known nontoxic inhibitors of collagen synthesis, it is of interest that an inhibitor, P-1894B, has been isolated from *Streptomyces* sp. (*72*) and found to be identical to antitumor antibiotic vincomycin A_1 (*73*). P-1894B showed some acute toxicity in rats (LD_{50} = 100 to 200 milligrams per kilogram) when given intraperitoneally but was nontoxic orally.

Ornithine decarboxylase inhibitors. Polyamines such as putrescine, spermine, and spermidine play a mysterious but essential role in cell growth, differentiation, and multiplication. Interfering with their synthesis could be useful in diseases in which abnormally rapid proliferation of cells occurs (*74*). Since L-ornithine decarboxylase is the rate-controlling enzyme of polyamine synthesis in mammalian cells, it is a good target for diseases such as psoriasis, chronic nonsuppurative prostatitis, and cancer. An initial screening of microbial broths led to the isolation from *Streptomyces neyagawaensis* of two known compounds, dihydrosarkomycin and sarkomycin (*75*); the latter is an old antitumor antibiotic (*76*). This successful isolation of an antitumor agent by a simple enzymatic assay suggests further successful application of this system to the discovery of more effective agents.

Other in vitro Tests

Simple means of detecting potentially active pharmacological agents also include in vitro tests that are not based on inhibition of a known enzyme. One such technique involves platelet aggregation induced by various compounds such as adenosine diphosphate or soluble collagen. Inhibitors of aggregation such as the pyrrothine antibiotics appear to have anti-inflammatory activity (*30*). Herquline is an inhibitory alkaloid produced by *Penicillium herquei* (*77*).

Other Enzyme Assays

A number of enzymes appear to be involved in disease processes, yet they have not been seriously used for detection of pharmacological agents in microbial broths. This section highlights such enzymes (Table 3).

Angiotensin-converting enzyme. Blood pressure is normally regulated by the renin-angiotensin system. Angiotensinogen (a plasma protein) is cleaved by trypsin and renin to form an inert peptide angiotensin I. This is cleaved by the angiotensin-converting enzyme, a zinc exopeptidase, to angiotensin II, the most powerful vasoconstrictor known. Overproduction of angiotensin II appears to be a major cause of hypertension. A leading synthetic oral hypotensive drug, captopril (*78*), and newer compounds (*79*) act by inhibiting the enzyme. These are small peptide derivatives and it is very possible that improved compounds might be detected in microbial broths.

S-*Adenosylmethionine decarboxylase*. Polyamine synthesis requires the activity of *S*-adenosylmethionine decarboxylase and for the reasons listed earlier in the ornithine decarboxylase inhibitor section, this enzyme might be a good target for diseases featuring rapidly proliferative and abnormal growth (*80*). This enzyme assay has not yet been applied to microbial screening.

Final Comments

There is no doubt that microorganisms are capable of producing nonantibiotic secondary metabolites with activities against parasites, insects, weeds, and enzymes, as well as controlling plant growth and exhibiting various pharmacological effects. In addition, microbes can be exploited for their ability to produce new industrially valuable polysaccharides (*81*), enzymes, and flavor and aroma (*82*) compounds. In my mind, the only factors limiting the discovery of useful compounds of the future are our own commitment, ingenuity, and ability to devise simple in vitro screening procedures for desirable activities.

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