CAN CHEMOTHERAPY BE EXTENDED TO INCLUDE THE
INTRACELLULAR DISEASE AGENTS?1

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The present limits of successful chemotherapy do not quite coincide with the
line of division between extracellular and intracellular disease agents. Thus
chemotherapeutic benefit in experimental murine typhus has recently been re-
ported by Moragues, Pinkerton, and Greiff (1944), and in human epidemic
typhus by Yeomans and others (1944). Chemotherapeutic benefit is well au-
thenticated for infections with viruses of lymphogranuloma venerum, mouse
pneumonitis, trachoma, inclusion conjunctivitis, and most recently is reported
for experimental ornithosis and psittacosis (Heilman and Herrell, 1944). Yet
the pathogenic rickettsiae and the viruses of the lymphogranuloma-psittacosis
group are obligate intracellular parasites. Nor is the limit of current chem-
otherapeutic effectiveness coincident with the limit of simple cellular organiz-
ation of the disease agent (Mudd and Anderson, 1944); vaccinia virus and even
colliphage have been shown by electron microscopy to possess cell-wall, inner pro-
toplasm, and even finer structural differentiation; yet no authenticated success in
chemotherapy with viruses other than those cited above is known. It is interest-
ning and possibly significant that those intracellular agents which now respond to
chemotherapy are the rickettsiae, which are intermediate in size between patho-
genic bacteria and the viruses, and members of the lymphogranuloma-psittacosis
group, which are the very largest of known viruses.

Several approaches to a chemotherapy of intracellular disease agents can be
formulated:

(1) Direct chemical action on the disease agent. The long list of empirical trials
has recently been reviewed by Kramer, Geer, and Szobel (1944). See also
Parker and Diefendorf (1944) and Klein, Kalter, and Mudd (1945).

(2) Interference with the key-to-lock relationship of virus to susceptible host cell.
This is precisely what specific antiphage (antiviral) antibody does when used
prophylactically, as the experiments of Kalmanson and Bronfenbrenner (1943)
on reversible serum inactivation of bacterial virus show. It is hard to imagine,
however, what more (if as much) could be expected in this connection from any
chemical agent than is now achievable by specific antiserum—namely, prophyl-
axis which is efficient, but therapeutic action which is very limited indeed.

(3) Rational investigation of cell metabolic systems and their selective inhibition.
This approach seems to the writer incomparably the most promising one. The
essential attribute of a chemotherapeutic agent is a selective action on a com-
ponent essential to the continued existence of the parasite within its host. Sir
Henry Dale in a recent review of the history of chemotherapy (1943) clearly

1 Presidential Address of the Society of American Bacteriologists given with additions
before the New York City Branch, December 28, 1944, the Maryland Branch, April 5, the
points out that in a large proportion of chemotherapeutic cures "the infection is brought to an end by stopping the further multiplication of the parasites, rather than by killing them outright. Another factor in an effective chemotherapeutic action...[is] the need for a sufficiently prolonged and continuous action. This need is almost implied in the conception of the process as essentially an arrest of the multiplication of the parasites rather than an immediately lethal action on them. What is required is not the sudden attainment of a concentration sufficient to kill most of the parasites, at the risk of a concomitant injury to the host's tissues, but the long-continued maintenance of a much lower and safer concentration, just sufficient to suppress the propagation of the parasites, without harming the cells of the host."

Evidence with respect to the mode of action of successful chemotherapeutic agents is in agreement with the original conception of Ehrlich and with Dale's conclusion that they act essentially by arrest of the multiplication of the parasites. A definition of objective then becomes possible. For successful chemotherapy of an infection due to an intracellular parasite there may be sought a metabolic inhibitor or inhibitors which selectively inhibit a reaction or reactions essential to the intracellular multiplication of the parasite, but (at least temporarily) inessential to the survival of the host cell.2

Concerning the mechanism of action of the sulfonamides two theories are current:

1. The Woods-Fildes theory that sulfonamides inhibit an enzyme or enzymes concerned with the anabolism of p-aminobenzoic acid as an essential metabolite.

2. The theory proposed chiefly by Sevag and coworkers (1942) that sulfonamides act by inhibiting in the susceptible bacteria certain respiratory enzymes which normally mediate reactions yielding the energy essential to bacterial cell division.

The fundamental proposition that substances similar to but not identical in configuration with particular components in a metabolic reaction can compete for essential reaction sites and thereby inhibit the metabolic reaction is not questioned. (See Fildes, 1940, 1941; Woods, 1940; Roblin et al., 1945, for references.) This fundamental principle is valid and applicable, however, whether the enzyme inhibitor resembles and competes with a substrate, or, in the case of the oxidative enzymes, with a coenzyme. The Woods-Fildes theory has been of the utmost importance in emphasizing configurational correspondence as a basis for enzyme inhibition in chemotherapy, and in stimulating the discovery of new instances and applications (e.g., see McIlwain, 1944; Woolley, 1944). In the writer's belief, however, the balance of evidence very definitely now indicates that in the special case of the mechanism of sulfonamide action

2 An increased resistance to experimental poliomyelitis in animals on thiamine-deficient diets seems to be well authenticated (Foster, Jones, Henle, and Dorfman, 1942, 1944a, 1944b; Rasmussen, Waisman, Elvehjem, and Clark, 1944; Editorial, J. Am. Med. Assoc., 1945). Could it be that mechanisms essential to the intracellular multiplication of this parasite are peculiarly sensitive to thiamine deficiency? Could mechanisms essential to parasite reproduction in host cells eventually prove to be peculiarly vulnerable to specific deficiencies, whether produced by dietary restrictions or by administration of drugs (Woolley, 1944)?
the critical competition is with respiratory coenzymes for sites on the oxidative enzyme proteins rather than for a hypothetical enzyme mediating p-aminobenzoic acid anabolism. The subject is comprehensively reviewed by Sevag and coworkers (1942, 1944, 1945) and Henry (1943, 1944).

Examination of such literature as is available on the mode of action of chemotherapeutic agents leads, at least tentatively, to the challenging conclusion that in cases in which critical enzyme inhibitions correlated with inhibition of growth have been identified experimentally, these critical sites of chemotherapeutic action have been found to be within the system of respiratory enzymes which mediate the aerobic and anaerobic oxidation of glucose and its derivatives.

The respiratory enzymes are proteins reversibly combined with coenzymes; the coenzymes contain as essential structural units certain components of the B vitamin complex, notably nicotinic acid amide, riboflavin, and thiamine; or, in the case of the cytochrome-cytochrome oxidase system, the iron porphyrin compound heme is the prosthetic group.

These relationships may be represented schematically as in figure 1. Thiamine for instance becomes by phosphorylation cocarboxylase (thiamine diphosphate) and riboflavin becomes riboflavin phosphoric acid. The enzyme carrier protein combines reversibly with its coenzyme and specific substrate, thus enabling the substrate to reduce the coenzyme, the substrate itself being oxidized.

We suppose that sulfanilamide has affinity for the respiratory enzyme protein.

In this connection the similarity of structure of sulfanilamide to other pharmacologically active substances is of interest. Compare for instance the structure of p-aminobenzenesulfonamide with that of the p-aminophenol derivatives (antipyrines and analgesics), procaine, ethyl and butyl p-aminobenzoates (local anesthetics), carbarsone (amebacide), and tryparsamide (trypanocide). It is suggested that widely distributed reaction sites exist with which compounds possessing the general configuration of p-aminobenzenesulfonamides, p-aminobenzoates, and p-aminophenylarsonates and their appropriate derivatives can combine reversibly with a resulting depressant effect. It is suggested that these reaction sites on oxidative enzyme proteins are of critical significance in chemotherapy.

However, in calling attention in this address to interrelationships based upon configurational correspondence, no implication is intended that all cases of inhibition and antagonism involve such configurational correspondence, which is obviously not true. In enzyme chemistry as in immunochemistry, however, the interrelationships depending upon specific configuration (Sevag, 1945) are perhaps more interesting and more stimulating to further discovery than those involving only nonspecific relationships.
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<th>CHEMOTHERAPEUTIC</th>
<th>COENZYME</th>
<th>GROWTH ACCESSORY SUBSTANCE</th>
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<tr>
<td>Sulfanilamide</td>
<td>Cocarboxylase</td>
<td>p-Aminobenzoic acid (?)*</td>
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<tr>
<td>Sulfathiazole</td>
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<tr>
<td>Sulfadiazine</td>
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<td>Thiamine (B₁)</td>
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*The image contains chemical structures and formulas for each category.
<table>
<thead>
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<th>Sulfapyridine</th>
<th>Cozymase</th>
<th>Nicotinic acid amide</th>
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*Fig. 2. Chemical structure of growth-accessory substances known to be precursors of respiratory coenzymes and sulfonamide drugs having configurations in common with the coenzymes.*

*The writer does not believe para-aminobenzoic acid to be a growth accessory for pathogenic bacteria.*
and is supplemented in the newer sulfonamides by the N₁ substituted group (pyridine, thiazole, pyrimidine) which structurally resembles the coenzyme or part of it, and competes with the coenzyme for its reaction site on the enzyme protein. (See figures 1, 2, and 3.) The proposal herewith suggested is that the sulfanilamide and the N₁ substituted group are capable of combining, respectively, at two reaction sites, in appropriate steric relationship, on the enzyme protein. Such a proposal is not without analogy since dipeptidase action is believed to depend on three reaction sites on the enzyme in very definite steric relationship (Schmidt, 1938). The drug-protein or drug-protein-coenzyme complex we suppose to be inactive enzymatically. Quantitative antagonism between drug and coenzyme thus becomes understandable, and similarly antagonism between vitamin and drug, since the vitamin is a precursor of the corresponding coenzyme. The superiority of sulfapyridine, sulfathiazole, and sulfadiazone over sulfanilamide is intelligible in these terms but difficult to account for on the p-aminobenzoic acid theory.

The following critical sites of chemotherapeutic action may be cited as indicated by experiment.

Dorfman and Koser (1942) showed that the respiration of dysentery bacilli was increased by nicotinamide and inhibited by sulfapyridine (cf. figure 2). MacLeod (1939) found that the dehydrogenase activity of pneumococci for glycerol, lactate, and pyruvate was inhibited by sulfapyridine. Acquisition of sulfapyridine fastness was associated with marked loss of dehydrogenase activity for these compounds. Fox (1942) working with isolated enzyme systems of Escherichia coli demonstrated inhibition of lactate, aerobically, and dismutation of pyruvate, anaerobically.

**Flavoprotein dehydrogenases** (riboflavin phosphoric acid proteins). Sevag and Green (1944) found that growth of certain strains of *Staphylococcus aureus* in the absence of added glucose is inhibited by sulfathiazole, and that this inhibition is antagonized by riboflavin. Atabrine has been shown by Haas (1944), working with isolated enzyme systems of yeast, to inhibit oxidation mediated by flavoprotein, and this inhibition is antagonized by the coenzyme (cf. figure 3). Selective inhibition of flavoprotein enzyme systems by atabrine has been indicated by tissue respiration experiments (Wright and Sabine, 1944).

**Carboxylase** (thiamine pyrophosphate protein). Sevag, Shelburne, and Mudd (1942, 1945) found that sulfathiazole selectively inhibits the action of carboxylase from both yeast and staphylococcus; this inhibition is antagonized by cocarboxylase (cf. figure 2). Penicillin has been shown by Welshimer, Krampitz, and Werkman (1944) to inhibit selectively the dismutation of pyruvic acid.

**Cytochrome-cytochrome oxidase** system (heme-containing enzymes). Cytochrome oxidase has been shown by Haas (1944) to be inhibited by atabrine. Zephiran has been shown by Sevag and Ross (1944) to inhibit the cytochrome-cytochrome oxidase system of yeast cells, and this action is correlated with inhibition of growth. These are probably instances of nonspecific inhibition.

The Pasteur effect has been shown to be strongly inhibited by certain substituted guanidines and amidines which are powerful trypanocides (Dickens, 1939).
Fig. 3. Riboflavin and Its Corresponding Coenzyme

Riboflavin, atabrine, the acridine ring, adenine, and adenosine are suggested as substituents on the N1 nitrogen of sulfanilamide for new sulfonamides of theoretical interest and possible practical value.
Steps in the enzymatic oxidation of glucose thus represent critical sources of the energy required for cell division, which may be vulnerable to the action of chemotherapeutic agents. In view of the great clinical success of the sulfonamides in which pyridine, thiazole, and pyrimidine are substituents on the N^1 nitrogen of sulfanilamide, and of the relationships schematized, it would be extremely interesting to have series of sulfonamides in which alloxazine, atabrine, or acridine are substituents on the N^1 nitrogen of sulfanilamide (figure 3). Because of the critical role in glucose oxidation of phosphorylation, mediated by the adenosine phosphoric acid enzymes, it would be exceedingly interesting also to have series of sulfonamides in which adenine and adenosine are similarly coupled with sulfanilamide.

The oxidations of glucose into its intermediate products, however, may proceed by several different pathways (for details see Potter, 1944), and for the further transformation of pyruvic acid seventeen different reaction paths are already known. *Multiple simultaneous inhibitions*, rather than single inhibitions, may then be necessary for the suppression of the intracellular multiplication of a disease agent and therefore may afford the key to successful chemotherapy. These considerations afford a rationale for systematic study of the *synergistic action of various chemotherapeutic agents*: sulfonamides, penicillin, atabrine, quinine, arsenicals, diamidines, and heavy metal compounds. Instances are already on record of two chemotherapeutic agents together accomplishing what neither one can do alone (Ungar, 1943; Bigger, 1944; Soo-Hoo and Schnitzer, 1944; Harned, Miller, Wiener, and Watts, 1944; T'ung, 1944; Kirby, 1944; Hobby and Dawson, 1945; Roblin et al., 1945). However, since synergistic action producing multiple inhibitions on host cell enzymes essential to intracellular multiplication of the parasite might be also injurious to the host, any application of this type of study to man, pending thorough investigation *in vitro* and in animals, would seem premature and dangerous.

The investigation of bacterial respiratory and other metabolic systems as a basis for a rational chemotherapy will bring our discipline into close association with studies of nutrition, normal and abnormal growth, and the physiology of muscular, nervous, and glandular function. The mature synthesis which will eventually result will surely make manifold return in understanding of fundamental life processes and in applications to human well-being.

*4* In the acridine derivatives listed by Northey (1940) substitution was on the N^4 nitrogen.

Pyrazine, which forms the central ring of riboflavin, has been used in the pyrazine analog of sulfapyridine (Ellingson, 1941). Sulfapyrazine is reported to be of the same order of efficacy as sulfadiazine (Robinson, Siegel, and Graessle, 1943).

After the above was written, Dr. E. H. Northey kindly furnished the writer references recording the synthesis of acridine derivatives of sulfanilamide (Ganapathi and Nandi, 1940; Das-Gupta, 1941; Coggeshall and Maier, 1942). Ganapathi (1940) writes: "The compound, 2-N'-sulphanilamidothiazol, which has since been reported by Fosbinder and Walter [sulfathiazole] and 2:8-sulphanilamidoacridine [italics the author's] possess striking protective action in experimental streptococcal and pneumococcal infections in mice."

Ganapathi (1941) has also recorded the synthesis of 7-N'-sulphanilamidoalloxazine which he reported to be therapeutically inactive in mice. It does not follow of course that among related derivatives an active one might not be found.
REFERENCES

Coggleshall, L. T., and MAIER, J. 1942 Effect of various sulphonamides, sulfones, and other compounds against experimental influenza and poliomyelitis infections in white mice. J. Pharmacol., 76, 161-166.


