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A Review of Recent Advances in Chemotherapy*

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TWO types of interaction are integrated in a biological system between the cells of the microbic invaders, on the one hand, and the drug that is introduced into the system to combat them, on the other. The first type is basically physical and the second chemical. Illustrative of the former are such phenomena as osmosis, diffusion, hydration, dehydration, solution, adsorption, corrosion and altered surface tension; while illustrative of the latter are such phenomena as precipitation, absorption, oxidation, reduction, fusion and disintegration. In a general sense the action of alkaloids simulates the former sort of interaction in which the drug bombards and after performing its action, is then released and eliminated unchanged; whereas the action of glycosides simulates the latter. In this instance chemical interaction between the constituent molecules occurs and as a consequence one or more of the above-named chemical changes takes place. Disintegration of the drug molecule and arrest or death of the invading cells with a sparing of the body cells result if the remedial drug is efficacious as it would be at therapeutic levels; while destruction of the cells of the host may ensue at toxic levels.

Until the time of Schmiedeberg, confusion reigned with regard to an appreciation of these fundamental emphases; and, as a result the fortunes of pharmacology rested insecurely in the empirical hands of the clinician. As Henri Bergson, in his "Creative Evolution" said of the philosopher that he can focus upon and clarify but one of the manifold facets of the cosmic jewel in his lifetime so it seems in science that only in exceptional cases are more vistas than one, of the unknown at the periphery of nature's known, explored by any single investigator. With nothing more than dim adumbrations of the chemical approach men like Schmiedeberg, Bernard and Cushny have traversed the first pathway and exploited its rich resources until Pasteur, Koch and Lister came upon the scene bringing with them instruments that were right then being hammered out on the anvil of a dawning creative chemistry. The chemical approach to pharmaco-dynamic enterprises came to full-bloom in the brilliant researches of Ehrlich; and chemotherapy has since become an intriguing adventure in the study of the drug-host-invader complex. Ehrlich directed his attack on trypanosomiasis with the arsenicals. Then came Sazerac and Levaditi in their experiments with bismuth on the trepanema pallidium. The theoretical basis of Ehrlich's historic achievements is quite well known to students of immunology. Equipped with certain structural configurations complimentary to those of the invading micro-organism the drug in question is supposed to attach itself to the invader acting thereby as substitute for the cells of the host; and by means of this selective chemical affinity the host is spared. The therapeutic-toxic ratio of a chemotherapeutic agent is enhanced if and when, in addition to engaging the attachment mechanism of the invader, the drug is capable also of stimulating the body cells to the production of "receptors," or antibodies which may be thrown into the circulation; the proliferation of the reticuloendothelial cells; and the mobilization of the other phagocytic systems of the body to offer added protection to the host. Ehrlich's hypothesis postulated complete sterilization with but a few doses. But while interesting, this was an eventuality which, unfortunately, actual experience failed to validate.

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The most illuminating modification of Ehrlich's view is that propounded by Voegtlin and Smith[1] and Voegtlin, Dyer and Leonard.[2] According to this group of investigators the inactive arsenical represented by the formula R—As=As—R is partially oxidized to form arsenoxide (R—As=O) in the system and this substance as such exercises lethal effects on the parasites. Subsequently, these and numerous other investigators have found that in the presence of the new chemical the defense mechanisms of the body are apt to be markedly increased, c.f., Cooper and Tatum.[3] With variations in specific emphases these modifications lie back of all modern approaches to problems in chemotherapy. The added features seem to serve as the determinative factors differentiating a chemotherapeutic from an antiseptic agent.

The history of the preparation of the sulfonamides dates as far back as thirty-five years in spite of the fact that their definitive utilization as chemotherapeutic agents is but a matter of a few years. Regarding their preparation Goodman and Gilman[4] state: "In 1909, Hoerlein and coworkers, of the I.G. Farbenindustrie, synthesized the first azo dyestuffs containing sulfonamide and substituted sulfonamide groups and noted them to be superior in color-fastness to similar dyes without the sulfonamide group. The firm combination which the complex azo dyes formed with the proteins of wool and silk suggested the possibility that these agents might react with bacterial protoplasm, and in 1913 Eisenberg discovered the bactericidal action in vitro of chrysoidine and suggested its use in chemotherapy. In the following year, Tchichibabin and Zeide (1914) synthesized a red dye, pyridium, from chrysoidin, which subsequently was to be introduced as a urinary antiseptic (Ostromyslensky, 1926). In the years immediately following, scarlet red, another azo dye, came into prominence and chemical advances in the synthesis of azo dye derivatives of cupreines were made by Jacobs and Heidelberger (1917) and Heidelberger and Jacobs (1919). The same investigators also prepared para amino benzene sulfonamide according to the method of Gelmo and postulated that this substance would be liberated by the breakdown in the tissues of sulfonamido chrysoidin. Although they commented on the high bactericidal potency of their compounds, unfortunately the work was not continued. In 1930, another azo compound known as serenium (2—4 diamino 4—ethoxy azo benzene) was introduced by Ostromyslensky as a urinary antiseptic."

In his attempt at clarifying the conceptual difference between antisepsis and chemotherapy, René Dubos[5] states: "It appears that the typical antiseptic behaves as a gross protoplasmic poison destroying the general . . . cellular mechanisms. On the contrary, most chemotherapeutic agents have a very selective effect on some specific metabolic steps."

In this report we shall consider two of the more recent groups of chemotherapeutic agents. Illustrative of drugs belonging in the first category are the sulfonamides of which prontosil, neo-prontosil, sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, sulfaguanadine, succynil sulfathiazole (sulfasuccidine) and sulfamerizine are types; while illustrative of drugs belonging in the second category are penicillin and various other metabolites or so-called antibiotics extracted from bacteria, fungi and other biological material. The chief representatives of the group are penicillin, gramicidin, tyrocidin, tyrothricin, actinomycin, proactinomycin, citrinin, streptothricin, gliotoxin, fumigacin, clavicin, lactonin, and lysozyme.

In 1933 Foerster[6] administered prontosil to an infant suffering with streptococcic septicemia and experienced a dramatic cure. However, it was not until 1935 that Domagk[7] proposed the introduction of prontosil and prontosil soluble into clinical medicine. In the body these dyes break down to para amino benzene sulfonamide which latter substance was found by the Tréfouëls, Nitti and Bovet[8] to constitute their active principle. Then it was that Fourneau[9] and his associates at the Pasteur Institute[8] began preparing sulfanilamide which with the other derivatives subsequently produced is widely employed in clinical medicine.

Early investigtaions by Zahl, Hunter and Cooper[10] have firmly established the fact that in varying degrees the sulfonamides confer limited protection against the lethal action of endotoxins of numerous gram-negative organisms in general without interfering with their immunizing processes. Specifically the sulfonamides have been employed to clinical advantage in the treatment of infections from beta-hemolytic streptococcus especially in puerperal fever, erysipelas, septicemia, meningococcic sore throat, surgical infections with

gram-negative organisms, and also in gonococcic, typhoid and paratyphoid and all types of Brucella infections. When employed with the specific anti-serum the results in pneumococcic, meningococcic and streptococcic infections are better than the results with either form of therapy alone. In an investigation of this adjuvant action Long and Bliss[11] state that while the sulfonamide drugs prevent the growth and spread of the invading microorganism, the serum neutralizes the toxic products of their growth. As such they may be said to be complimentary; in that, by restricting growth the sulfonamides limit the quantity of toxic substances produced and thereby help to mitigate, if not to abort, the toxemia, fever and rash which those toxic substances are calculated to produce.

In a report on the mechanism of action of the sulfonamides, Schmelkes and his associates[12] indicate that the efficiency of this class of compounds increases with their increasing alkalinity from pH values above 7 in the direction of 9. On this point, however, there is as yet a lack of general agreement. Cases of clinical acidosis resulting from their employment have been reported by numerous investigators, Long and Bliss,[13] Strauss and Southworth,[14] Towsley and Engelfried,[15] and Beckman.[16] This acidosis appears to be the result of an increase in the renal excretion of sodium and potassium due, apparently, to a loss of efficiency in the reabsorption capacity of the tubular epithelium with respect to fixed bases; and associated with a decrease in the carbon-dioxide combining power of the plasma.

Occasionally, when this acidosis remains uncompensated, there develops a definite hyperpnea. As a consequence the proponents of this school of thought advocate the concurrent administration of sodium bicarbonate.

Over against this approach to the problem there is the position taken by such investigators as Hartmann, Perley and Barnett.[17] These men hold that in sulfonamide medication there occurs a loss of serum carbon dioxide which results in an alkaline shift of the serum; and the kidney seeks to correct this imbalance by excreting an alkaline urine. With this explanation hyperpnea falls in line with the loss of serum carbon dioxide. As such, the exhibition of a hyperventilation alkalosis should therefore serve as a contraindication to the routine use of sodium bicarbonate. They insist that under appropriate conditions the administration of sodium bicarbonate might cause an uncompensated alkalosis. In support of this contention Hartmann[18] induced an acid excess type of acidosis by administering ammonium chloride to a normal subject. But this acidosis was equilibrated by tubular reabsorption of the urinary sodium bicarbonate. When this was followed by the administration of sulfanilamide the urinary excretion was not alkaline. To Hartmann[18] this was convincing evidence that tubular reabsorption of sodium bicarbonate is elicitable when changes in the acid-base balance demand such reabsorption. Beckman and his associates[19] have since then reinvestigated the problem. Their findings are that sulfanilamide causes a loss of sodium in the urine and a smaller loss of potassium but with no loss of chloride. They conclude that sulfanilamide hyperpnea is an event secondary and complimentary to depletion of plasma bicarbonate resulting from the loss of fixed bases in the urine and that the associated acidosis is an expression of the body's alkali deficit. The disturbance, they say, is of only minor clinical significance and is correctible by an adequate intake of sodium chloride. The correct electrolytic pattern is thereby maintained and there obtains a functional adjustment of the renal activity.

The mechanism of action of the sulfonamides in combination with urea is also intriguing. Tannenbert and his associates[20] have made the observation that organisms rendered resistant to the sulfonamides by para-amino benzoic acid were again rendered susceptible when urea was given with the sulfonamide in question; and this, even in the presence of the chemical inhibitor. They have found, also, that staphylococci which are resistant to sulfathiazole have showed susceptibility to a combination of sulfathiazole and urea. The action of urea in this connection is not dissimilar to that of azochloramid and such purine bodies as hypo-xanthine and adenine, which, when used in combination with the sulfonamides even in the presence of the inhibtor render the sulfonamide bacteriostatic. The final explanation as to whether or not this similarity of action is based on the same mechanism, and what the mechanism is, is still being sought.

At the borderline between bacterium and virus there hovers an aura of haziness regarding the functional efficiency of the sulfonamides. The

problems that arise and might arise in this area of indefiniteness seem destined by their clarification to make for a concurrent re-classification of the virus diseases. It has been demonstrated by Findley[21] and Jones and his coworkers[22] that sulfathiazole and sulfadiazine are both effective against the virus of lymphogranuloma venereum. Since it is true that the sulfonamides are effective against but a few of the virus or virus-like diseases it has been felt that insight gained into this differential effectiveness might indirectly offer some clue to the mechanism of action of these compounds. Accordingly Rake, Jones and Nigg[23] have carried out a series of experiments using these two sulfonamide compounds in mouse pneumonitis. These investigators found that sulfathiazole and sulfadiazine are even more effective in this disease than they are in lymphogranuloma venereum. On the other hand neither compound has showed significant effectiveness against meningo-pneumonitis in mice. The etiologic agents of lymphogranuloma venereum and mouse pneumonitis are strikingly susceptible to the sulfonamides; as also are the agents that produce trachoma, inclusion blenorrhea and "heart-water" fever. These are classed with the rickettsiae. But most of the other virus agents are not so classified today. Meanwhile, evidence is accumulating which tends to separate the lymphogranuloma venereum-psitticosis group from the true virus diseases. All investigations thus far have failed to establish any effectiveness of the sulfonamides against the true viruses.

THE PENICILLIN GROUP

The mould penicillium notatum produces a substance which exerts a powerful inhibitive action against gram-positive bacteria, both rods and cocci. In vitro experiments have occasionally demonstrated such action in dilutions as weak as one in one billion. Streptococci are inhibited in dilutions of 1:25,000,000 and some preparations of pencillin have exhibited activity, according to Bloomfield, Rantz and Kirby[24] in dilutions of 1:100,000,000. Against gram-negative cocci inhibitive action is also demonstrable but only in considerably higher strengths, 1:1,000 for instance. Anaerobic organisms, also, and even the treponema pallidum show varying degrees of susceptibility to penicillin. Powell and Jamieson[25] found this antibiotic an effective agent against sulfonamide-fast pneumococci in mice, and Dawson and Hobby[26] speak for its efficacy, clinically, in patients who show a prohibiting sensitivity to sulfonamide therapy, and also in patients with marked renal insufficiency and secondary anemia, in both of which conditions the sulfonamides are usually contraindicated. For staphylococcic infections penicillin is a happy discovery in that while being remarkably refractory to the sulfonamides the organism is highly sensitive to penicillin. A therapeutic agent of such high and wide effectiveness must, of necessity, arouse intense toxicological interest since, as is not infrequently the case with very active agents, the therapeutic-toxic coefficient is sometimes prohibitively narrow. In this particular respect penicillin represents a striking exception. Penicillin is remarkably non-toxic to cells of animal tissues. Summarizing many "isolated reports" Dubos[5] indicates that it affects neither their growth nor metabolism; and Dawson and Hobby[26] supporting the thought state that: "a variety of experimental observations have indicated that penicillin is completely devoid of toxic effects in concentrations far beyond those necessary for therapeutic purposes." In addition, these investigators point out that: "prolonged administration has not led to the development of any intolerance or sensitivity . . . or cumulative effect . . . (nor has) the infecting strain showed . . . evidence of becoming resistant to the action of penicillin."

On the other hand, tyrothricin, the original crude gramicidin obtained from bacillus brevis which yields two other active substances, gramicidin and tyrocidin when purified, while exhibiting marked action against both gram-positive and gram-negative bacteria completely inhibits the oxygen consumption of bovine spermatozoa and also their motility in acid Ringer's phosphate and Ringer's bicarbonate solutions. Tolerance to this agent is readily acquired by staphylococci; and, with tolerance, a correlevant reduction of its therapeutic efficiency. Although tyrothricin has not been found thus far to be toxic towards other animal cells which have been tested yet the presence of pus, serum, gram-negative bacteria in abundance and also its own hemolytic properties and high toxicity act as limitations to its therapeutic applicability. On account of these peculiarites tyrothricin (and also gramicidin and tyrocidin) will probably be restricted in their employment

to local administration. Little[27] has reported on the successful use of gramicidin, locally, in bovine mastitis and infected ulcers.

Actinomycin A and B are two substances isolated by Waksman and Woodruff[28] from A. antibioticus. Actinomycin-A is highly selective as a bacteriostatic agent while actinomycin-B is reputed to be largely bactericidal. Actinomycin is antagonistic to many bacteria and fungi. However, because of its rapid disappearance from the blood stream and also its toxic action against all animal species its therapeutic employment will no doubt be limited. Its in vivo effects against streptococcus hemolyticus, type-1 pneumococcus and brucella abortus in guinea pigs are not pronounced and late death through respiratory failure is not infrequently elicited.

Tyrothricin and actinomycin-A inhibit fibrolysis and plasma coagulation by beta-hemolytic streptococcus cultures or their supernatant fluids, Neter.[29] Another antibiotic, streptothricin derived from a soil actinomyces has been found to be active both in vivo and in vitro in tests with brucella abortus. Because of its low-grade toxicity for animal tissues this antibiotic gives promise as an effective agent for treating brucelliasis in animals.

Another interesting antimicrobiotic is streptomyces closely related to actinomyces griseus, which is presently being investigated. Streptomycin resembles streptothricin as to solubility in water, the manner of its isolation, its reproduction in laboratory culture; its high selectivity against gram-negative organisms, and its limited toxicity to the animal organism. The most notable differences are in their bacteriostatic spectra and their quantitative action.

Lactenin and lysozyme are good samples of antimicrobic substances derived from sources somewhat different from the agents mentioned above. The former is obtained from the albumin fraction of milk; the latter is an enzyme present in a large variety of animal tissues, and possibly plants also. Jones and Simms[30] have attributed to lactenin great activity against streptococci; and lysozyme, while especially active against non-pathogens causes lysis of many species of bacteria, according to the report of Meyer and his co-workers.[31] These workers indicate that its action is due to the hydrolysis of some mucopolysaccharide which constitutes an integral part of the structure of the susceptible cell. A successful attack, not necessarily upon the cell proper but upon those agencies which as products or components of the cell condition its pathogenicity might turn out to be of an order of clinical importance equal to a direct attack. Thus Dubos[5] observes: "there have been obtained from saprophytic micro-organisms, also from leech extract, enzymes capable of hydrolyzing the capsular polysaccharides of pneumococci and streptococci." And he notes that whereas the viability, metabolism or growth in vivo of these organisms is not affected by the destruction of their capsules yet their removal deprives the bacteria of their surface protection as well as their essential pathogenicity and renders them thereby more vulnerable to the phagocytes of the body. Of lysozyme, Dubos[5] has this to say specifically: "Lysozyme attacks a chemical component so essential to the integrity of cellular structure that it causes lysis and death of the susceptible cells. To affect the pathogenic career of an infectious micro-organism, however, it may be sufficient to attack a product of the micro-organism not essential to its vital activities but of critical importance to its pathogenicity." With such a thought in mind the conceptual difference between an agent that is bacteriostatic and one that is bactericidal appears to be merely academic.

MECHANISMS OF ACTION

Our knowledge of the mode of action of the sulfonamide group of drugs, although limited, is yet considerably in advance of our information on that of the penicillin group. We shall, therefore, begin with a consideration of the former.

The close similarity between the structural configurations of para aminobenzoic acid and the sulfonamides put over against their opposing dynamic action has served to open up the investigation into the mechanism of sulfonamide action.

In 1942 Thomas and Dingle[32] confirming the findings of Wood[33] published a report on the "Protection of Mice against Meningococcal Infection by Sulfadiazine and Inhibition of Protection by Para aminobenzoic Acid." These investigators found that in appropriate doses sulfadiazine furnishes adequate protection against a virulent strain of Group-1 meningococcus but that this protective action could be inhibited by repeated doses of para aminobenzoic acid. Similarly Maier and

Riley[34] reported that para aminobenzoic acid is capable of completely inhibiting the antiplasmodial effect of sulfanilamide in rhesus monkeys. They were also emphatic in stating that para aminobenzoic acid exerts no inhibiting action whatever against the antimalarial effects of quinine or atebrine. A definite antagonism was thus demonstrated between these two chemical substances.

The accepted explanation is that bacteriostasis is brought about in sulfonamide medication through an interference which the drug imposes in the course of the normal metabolic processes of the invading cells. Cells of the living bacteria are equipped with a complex enzyme system by means of which they undergo a series of chemical reactions on or near their surfaces in meeting their nutritional needs. Para aminobenzoic acid, a basic component of the synthetic local anesthetics of the cocaine series, appears to be necessary for the growth of the hemolytic streptococcus. This statement is also true, probably, for all organisms that show susceptibility to the action of the sulfonamide group of compounds. When introduced into the para aminobenzoic acid, compete with the latter and largely replace it in the enzymatic process. By so doing they probably prevent the completion of the reaction and thereby arrest the organisms in their growth.

Whereas the sulfonamides interfere with bacterial growth, penicillin seems to interfere with cellular division at some stage in this process. However, neither agent seems capable of arresting the susceptible organisms in their other vital functions; for both of them when given in concentrations even considerably in excess of the optimal for the causation of inhibition of growth and division fail to cause rapid death. Moreover, after complete inhibition of their growth and propagation, if these susceptible organisms are transferred to an auspicious environment upon a new culture medium they promptly revive and proceed to grow and divide as readily as control cells. The sulfonamides and penicillin for this reason, then, are referred to as bacteriostatic in action. But since they tend to spare the host by their bacteriostatic action they are said by some workers to be also bactericidal, especially in large doses. In fact, in addition to this bacteriostatic effect, investigators of high repute like Levaditi and Vaisman[35] and Domagk[7] speaking for the sulfonamides have attributed to these compounds the capacity to stimulate the phagocytic powers of the body. On the other hand such observers as Long, Bliss and Feinstone[36] would be inclined to deny them any such action. Regarding this moot question the opinion expressed by Marshall[36] seems to be gaining general acceptance. He says . . . "the first effect in the cure of an infected animal or man is a direct action of the drug on the parasite rather than a stimulation of defense mechanisms of the host. Such defense mechanisms may, however, operate as the final stage in the chemotherapeutic process."

Finally, individual specificities must not be overlooked. For example, gramicidin, tyrocidin, tyrothricin and actinomycin, especially, are highly toxic to certain body cells. These antibiotics will, therefore, always be limited in their therapeutic scope. But by contrast penicillin and, to a less extent streptomycin and streptothricin will continue to be available for wide systemic employment.

No attempt has been made to make complete coverage of therapeutic possibilities for these two groups of chemical compounds. However it would be well-nigh unpardonable were we to omit the fact that although the sulfonamides have exhibited no action whatever against the treponema pallidum penicillin has. Mahoney[37] reporting to the American public Health Association states that as a result of penicillin treatment there was a rapid disappearance of the spirochete from superficial lesions and also the resolution of early lesions. This observation has been verified by Bloomfield, Rantz and Kirby[24] who add that "condylomas have become free of treponemes in approximately twelve to twenty hours" and that "immediate results comparable to those obtained with full doses of arsphenamine can be achieved." Treatment for syphilis with penicillin is now being undertaken under the supervision ot committees of the National Research Council and the Committee on Medical Research of the Office of Scientific Research and Development. The final verdict regarding the question of its permanent addition to the current antisyphilitic armamentarium lies in the lap of time.

The most interesting features which this study into the chemotherapy of the sulfonamides and the penicillin group of compounds reveals are the scope of their therapeutic applicability; their sparing action on the cells and tissues of the host; and their predilection for the organisms that cause infection. Bearing these facts in mind it seems en-

tirely within the purview of the constructive scientific imagination to envisage for the therapeutics of tomorrow a mine of chemotherapeutic agents capable of covering the entire category of pathogens that attack and afflict the human body and its protection against the many ailments which they cause.

SUMMARY

1. An attempt has been made to set forth the differences between the essentially physical and the essentially chemical mechanism of action of drugs. The first category is illustrative of the action of alkaloids, which bombard and escape unscathed and unchanged. The second is of the glycosides which effect molecular changes in all the affected components in the course of a typical reaction.

2. Since the problem of antisepsis is better known than chemotherapy this knowledge of antisepsis has been employed by way of an introduction to our approach to chemotherapy. Ehrlich's idea of chemotherapeutic agents was the effecting of speedy sterilization. Since this first proposal, however, the truly definitive action of chemotherapeutic agents is bacteriostasis, first; and sterilization, later.

3. With this in mind the actions of two of the newer types of chemotherapeutic agents, the sulfonamides and the penicillin group of compounds, has been set forth with pertinent illustrations of their scopes and specifications.

4. The mechanisms of their action are discussed and illustrated. The sulfonamides seem to retard growth; penicillin group to inhibit cell division.

5. On the basis of their therapeutic range; the ability of most of them to act on the invader while sparing the host; and their remarkable antagonism with respect to infectious micro-organisms, the warfare against infectious diseases looks bright.

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