medicine, but I am convinced that this difficulty can be largely overcome. The practical courses should not, in my opinion, be arranged to introduce the pupils to the intricacies of physiological experimentation, but, beyond illustrating some basic principles, they should familiarize the students with methods actually useful in medicine. The growth of the science should be emphasized, and it should be taught how to find and use original literature.

I feel very definitely that the practice adopted by a few schools of giving those students who desire it an opportunity to reestablish a close connection between physiology and medicine is very beneficial. It can be done, as I have seen, by establishing courses for older students given jointly by clinical and physiological teachers, but other methods are no doubt available.

I have been much in favor of special chairs for the study and teaching of pathological physiology, and I still think that it may be, in certain circumstances, a useful development, but it would serve as an excuse to make the preclinical teaching too academic, and when a close cooperation can be established between those who teach physiology (normal *and* pathological) and those who teach clinical medicine and surgery, I think the best results can be achieved.

It may be said that the system which I advocate would discourage students from going into physiological research. My reply would be: So much the better. Students should be discouraged from choosing a career involving research, and we should welcome only those whose urge is strong enough to overcome discouragement and difficulties.

THE MODE OF ACTION OF SULPHANILAMID[1]

By Professor PHILIP A. SHAFFER

DISCOVERY of the dramatic therapeutic effect of sulphanilamid in various bacterial infections has stimulated wide-spread interest and renewed activity in the field of chemotherapy for infectious disease. From this renewed activity there should come the discovery of still better drugs for the control of infections. Progress in this new era of chemotherapy is likely to depend a good deal on an understanding of the ways in which the drugs exert their action; without that understanding the search for more useful therapeutic substances is apt to be largely a haphazard venture. It is therefore disappointing to find that in spite of the numerous investigations with sulphanilamid and related compounds there is yet no accepted explanation of their action. In Marshall's recent review of the pharmacology of sulphanilamid,[2] he states: " . . . no satisfactory explanation of the mechanism of action has been found." Yet certain facts have been known for some time which appear to point plainly enough in the direction of a logical explanation. The purpose of this communication is to draw attention to these facts and to cite briefly some new evidence, all of which when combined seem to provide an explanation of the mode of action of sulphanilamid and of related substances.

In June, 1937, R. L. Mayer pointed out[3] that the frequent appearance of methemoglobin in the blood of animals and patients treated with sulphanilamid suggests the formation in the body of an oxidation product of the drug which is responsible for the formation of methemoglobin. He further advanced the hypothesis that the bactericidal effect is due not to sulphanilamid but to the same oxidation product which oxidizes hemoglobin to methemoglobin. In support of this idea Mayer showed that p-hydroxyl amin benzene sulphonamid is highly bactericidal, as the corresponding amine is not. It is the opinion of the present writer that this hypothesis contains the important germ of truth, and if substantiated and developed may supply a rational chemical basis for this important branch of chemotherapy. Should this expectation prove to be correct it will represent the extension of a point of view that prompted Ehrlich in his early experiments with oxidation-reduction dyes and will incorporate also some of Pasteur's ideas on the relationship of fermentation to respiration.

The following facts would seem to be conclusive evidence that sulphanilamid and sulphapyridin are not themselves bactericidal and that their therapeutic (and toxic) effects are due to oxidation products of these substances formed by atmospheric oxygen under the catalytic influence of respiring tissues or organisms.

(1) Both drugs are wholly without effect on the growth of bacteria in the absence of oxygen. Both become more or less bacteriostatic or bactericidal under certain aerobic conditions, but promptly lose this property when the culture media become anaerobic in consequence of bacterial metabolism and the resulting consumption of dissolved oxygen.

The oxidation products of a number of more or less analogous substances, for example, benzoquinone and quinhydrone, are known to be highly bactericidal under anaerobic as well as aerobic conditions, whereas the reduced forms are bactericidal only under aerobic conditions, *i.e.*, when their oxidation is possible.

[1] From the Laboratory of Biological Chemistry, Washington University School of Medicine, Saint Louis.

[2] *Physiological Reviews*, 19: 254, April, 1939.

[3] *Bull. L'Academie de Med.*, 1937, 117: 727, 1937.

(2) When sulphanilamid is given to animals infected by streptococci there is a "lag" of some hours during which little or no inhibition of bacterial growth occurs, after which period the number of bacteria in blood and tissues may rapidly decrease or disappear. This again suggests the inactivity of sulphanilamid as such, but does not prove the active substance to be an oxidation product.

(3) The presence of methemoglobin in the blood of virtually all animals or patients receiving therapeutic doses of sulphanilamid is, as Mayer pointed out, significant. Methemoglobin is the *oxidized* form of hemoglobin, and the appearance of this pigment is probably always indicative of the presence in the blood of an active oxidizing agent not normally there. It is reasonable to suppose that the agent present after giving sulphanilamid is an oxidation product of sulphanilamid.

(4) Active *oxidizing* agents, of sufficient oxidizing intensity, are known to be highly bactericidal for many organisms, provided their oxidizing action is not diverted from the bacteria by being consumed by other reducing substances in the culture fluid. A demonstration by W. M. Clark illustrates this qualification. Many times the lethal concentration of iodine may be added to bacterial cultures without bactericidal effect, if added so slowly as to be at once reduced to iodide ion, thereby avoiding an excess of I_2 and the consequent rise of the oxidation potential of the medium. Similarly, Dubos and others have shown that streptococci and various other organisms do not grow when the oxidation potential of the media is kept above certain levels. The reducing intensities of living cells, produced by the "activation" of metabolites by "dehydrogenase" enzymes, provide one environmental factor essential for growth and in the case of many bacteria essential for life. That essential environment is destroyed and the organisms are killed by such excess of active oxidant as will maintain the oxidation potential above a critical level.

(5) It has recently been reported[4] that sulphanilamid added to broth raises the electrode potential and somewhat delays its fall as growth proceeds. This effect is due to the oxygen (and possibly H_2O_2) contained at the start in the broth since it was not observed under anaerobic conditions or when cystein was added. Sulphanilamid is not an oxidant and is itself inactive toward electrodes. When, however, even a very small fraction is oxidized (as occurs slowly in aqueous solutions exposed to light and air), the product (until reduced or decomposed) imposes a high potential, as will be explained below.

(6) It has been found by Shinn, Main and Mellon[5] that the oxidation product of sulphanilamid produced by radiation (Ottenberg and Fox) destroys catalase and thus allows H_2O_2 to accumulate in respiring bacterial cultures. From this observation they advance the hypothesis that the bactericidal action is due to H_2O_2.

To the above significant observations the writer is able to add the following facts.

Although virtually unaffected by atmospheric oxygen in the absence of catalysts, sulphanilamid and sulphapyridin are readily oxidized to characteristic cherry-reddish products by a number of chemical oxidants as well as by electrolytic oxidation. The identity of the products has not been established, but circumstantial evidence suggests what they are. The products are the same regardless of the oxidizing agent used, and are therefore derivatives of sulphanilamid (or sulphapyridin) and not of the reagent used to oxidize it. In the oxidation four equivalents are consumed per mole of sulphanilamid oxidized, which points to the conclusion that the primary end-product of oxidation is nitroso-benzene sulphonamid. Among the agents used are ceric sulphate, chlorine water, sodium bismuthate, MnO_2, $KMnO_4$, ferric-ortho phenanthroline, PbO_2, and H_2O_2 (with Fe^{++} as catalyst). The last two are especially effective. Less intense oxidants, such as ferricyanide, I_2, $K_2Cr_2O_7$, vanadic acid, thallic sulphate, do not attack sulphanilamid or sulphapyridin rapidly at room temperature. Ferric ion is reduced to a slight extent. Bromine is instantly consumed by bromination of the ring.

It proves to be possible to measure the oxidation intensity of the products by platinum or gold electrodes. The products are rather unstable and do not give steady potentials. The potential falls rapidly and conventional curves characteristic of stable reversible systems are not obtained by titration. Nevertheless, there is fair reproducibility of potentials and rather good agreement among several different electrodes in the same solution. Approximate agreement of potentials is obtained with different oxidizing agents at the same pH levels. These features are regarded as justifying the belief that the observed potentials arise from and represent finite ratios of electromotively active substances, and so constitute significant and characteristic intensity levels of the substances from which the products are formed. If this belief proves to be correct, the measurement of potentials should be an important criterion in the search for new therapeutic agents of this type.

The relation of the observed potentials to "normal" potentials of the systems has not yet been established. Sulphanilamid concentration has no effect upon the potential, and the parent substance is therefore not a member of the electromotive pair. It is probably a fairly safe guess that the reversible electrode couple

[4] Fox, German and Janeway, *Proc. Soc. Exp. Biol. Med.*, 40: 184, February, 1939; and Warren, Street and Stockingen, *ibid.*, 208.

[5] *Proc. Soc. Exp. Biol. Med.*, 40: 640.

is composed of the hydroxyl amin and nitroso derivatives or the corresponding semi-quinone free radicals. The following evidence may be cited in support of this conclusion. Neither aniline nor nitrobenzene are active toward electrodes, while the corresponding intermediates, phenyl hydroxylamin and nitroso benzene, are active. Conant and Lutz[6] measured the potential of an equimolar mixture of the two last-named substances in 0.1 N HCl, (+0.605 v.). On oxidizing a solution of aniline in the same solvent by PbO_2 we find an initial potential about 0.1 v. higher which rapidly falls to and below this "normal" value. This behavior would be expected if the oxidation products were a mixture of the hydroxyl amin and nitroso forms, with the nitroso derivative decomposing at a faster rate.

The measured potentials of oxidized sulphanilamid solutions, as might perhaps be expected from the intense oxidants needed to form them (though there is no necessary connection), indicate an astonishingly high oxidizing intensity for these products. At pH 4.6 the plateau potential (E_h) of sulphanilamid oxidant is +0.59 v. At pH 1.8 (0.1 N H_2SO_4) the potential of sulphanilamid oxidation by ceric ion is +1.07 v. Measurement of potentials at pH 7 has not been successful for technical reasons. It is of interest, and is perhaps significant, that the potential of sulphapyridin at pH 4.6 is 30 or 40 mv. higher than that of sulphanilamid. For the potential measurements I am indebted to Dr. E. S. Hill.

The oxidation products are highly reactive, and fortunately the intensity levels indicated by the electrode potentials can be confirmed in a rough but convincing way by chemical reactions. The oxidized products are quickly reduced by hydroquinone, diphenylamine, p-phenylene diamine, p-amino phenol and in part by ortho-tolidine but not by benzidine, in part by iodide ion (with liberation of I_2) and by ferrous ion. They are also promptly reduced by oxyhemoglobin, which is thereby oxidized to methemoglobin, and also to other unknown products which are suggestive of the brown pigments observed in the blood of cyanotic patients treated with sulphanilamid. The intensity levels and potentials of the oxidation products of sulphanilamid therefore lie above those of the reductants named. It follows also that if these or other more reducing substances be present with sulphanilamid when it undergoes oxidation, only these and not sulphanilamid will appear to be oxidized. Metabolites activated by cells, many cell constituents and substances present in peptone broth culture media are such reducing substances. In their presence bacteria might be more or less protected from the effect of slowly oxidized sulphanilamid. But if not at once reduced, the activity and oxidation intensity of the products of sulphanilamid are such that they would certainly exert on bacteria much the same lethal effects as would like concentrations of I_2 or Br_2. (The oxidation products are, of course, highly bactericidal *in vitro*). Because of the presence in tissues and tissue fluids of many reducing substances it would appear to be hopeless to expect to find the oxidation products of sulphanilamid in living cells; perhaps it may be possible to find them in dead tissues.

The question naturally arises: Is it possible that a substance so difficult of oxidation as sulphanilamid could be attacked by oxygen in living cells? The same question might be asked about acetic acid or sugar or fat. To be sure, these are made reactive by enzymes, while there is no evidence that enzymes activate sulphanilamid. But activation of the drug is not necessary. It may be recalled that oxygen has an oxidizing intensity represented by +1.23 v. (at one atmosphere pressure and at 0 pH). For comparison with our measurements of sulphanilamid at pH 4.6, the corresponding value for O_2 at one atmosphere is +0.95 v. and at 50 mm Hg pressure +0.78 v. The energy factor raises no difficulty. Confirmation of this conclusion is found in the fact that epinephrine is rapidly oxidized in the body. Its potential (Ball, *Jour. of Biol. Chem.*, 102: 691, 1933) is in the same region as that observed in sulphanilamid oxidation.

But the question remains: How could so intense an oxidant as necessary to oxidize sulphanilamid (or adrenalin) be formed in living cells? A tentative answer may be formulated somewhat as follows. The lethal oxidizing intensity of O_2 would presumably exert itself on all respiring cells if the oxygen molecule were to react by the formation of two molecules of water; it is this reaction for which O_2 has a potential of 1.23 v. This possibility is avoided by the formation of H_2O_2 instead of water. For the formation of H_2O_2 the potential of O_2 is only +0.26 v. at pH 7. Without catalysts H_2O_2 is strangely inert at low concentration. But with metal catalysts present in cells it is an even more intense oxidant than O_2. The enzyme catalase, however, rather promptly and completely destroys H_2O_2, thereby protecting the cell from higher than about 0.26 v. (the level of cytochrome). If, however, substances are introduced which are freely permeable and are capable of oxidation even to a slight extent to reactive products the potentials of which are high, then such active oxidants may attack and oxidize functional components of the cell which are otherwise protected from O_2 and H_2O_2. The potential of the oxidation products of sulphanilamid at pH 7 (calculated from values observed at pH 4 to 5) is +0.45 v., which is considerably higher than the normal potential of any other organic substance yet measured (except sulphapyridin and adrenalone). Every reactive reducing system in cells would be attacked by so energetic an oxidant. Among the cell components which would

[6] *Jour. Am. Chem. Soc.*, 45: 1059, 1923.

be oxidized are catalase (a ferrous hemin complex), hemoglobin, glutathione, sulphhydryl groups, ascorbic acid and so forth. With catalase more or less inactivated, H_2O_2 would accumulate locally. And H_2O_2 with ferric or ferrous ion (present in serum and tissues) is a rapid oxidant of sulphanilamid and of sulphapyridin. Once slowly started, more and more of the toxic oxidation products would thus be formed, in proportion as O_2 is available and H_2O_2 is produced. Bacteria in the blood stream and in regions of rich blood supply should be most exposed to the bactericidal effects. The appearance of methemoglobin is evidence of activity in the blood. Conversely, bacteria sequestered in tissues or locations (abscesses) with poor blood supply may be expected to be less accessible to the bactericidal effects. The relative immunity of host tissues to toxic effects is perhaps due to their lower oxygen tension, their higher metabolism and to higher catalase content. A dominant reducing environment should protect tissues from the sulphanilamid products in the same way as bacteria are protected from I_2 in Clark's demonstration above mentioned. In general, these hypothetical expectations appear to be in accord with clinical and experimental experience.

As the writer sees the evidence now available, the probable mode of action of sulphanilamid is that outlined in the above statement. The drug provides a mechanism by which the sterilizing oxidation intensity of molecular oxygen is applied nearly at its maximum to bacteria and unavoidably also to some extent to host cells. It is not surprising that toxic as well as therapeutic effects are observed from the use of such substances.

If the ideas here advanced are even in part correct, the new era of bacterial chemotherapy will be directly concerned with that fund of systematic information dealing with oxidation-reduction potentials and with the relation of molecular structure of organic substances to potential levels, as well as with the fascinating complexities of reactions that occur particularly among the intermediate oxidation stages of nitrogenous compounds.

From this store of information a number of predictions can at once be made. For example, if high potentials are desired, ortho rather than para substitution of the oxidizable group may be preferable. It is unlikely that the sulphonic acid group is essential to activity, though it does raise potentials and increase solubility. Hydroxyl rather than amino compounds may be useful. The applicability of these and other similar ideas will be explored and tested in future work in this laboratory.

SCIENTIFIC EVENTS

FELLOWSHIPS IN THE NATURAL SCIENCES OF THE NATIONAL RESEARCH COUNCIL

THE National Research Fellowships Board in the Natural Sciences of the National Research Council has made the following appointments for the academic year, 1939–1940:

John Nathaniel Adkins (Ph.D., seismology, University of California, 1939). The Massachusetts Institute of Technology. "The Electromagnetic Response of an Ellipsoid Imbedded in a Conducting Material."

Daniel I. Axelrod (Ph.D., tertiary paleobotany, University of California, 1939). The United States National Museum, Washington, D. C. "The Late Tertiary Floras of the Great Basin Province."

Richard Henry Bolt (Ph.D., physics, University of California at Los Angeles, 1939). The Massachusetts Institute of Technology. "The Wave Theory Approach to Room Acoustics."

Herbert Leonard Eastlick (Ph.D., chemistry, Washington University, 1936). The University of Chicago. "A Study of Pigmentation, Muscle Development, etc., by Means of Reciprocal Heteroplastic Transplants between Different Species of Avian Embryos."

Eugene Henderson Eyster (Ph.D., physical chemistry, the California Institute of Technology, 1938). The University of Michigan. "The Application of Infra-red Spectroscopy to Problems of Molecular Structure."

Frank Junior Fornoff (Ph.D., chemistry, the Ohio State University, 1939). University of California. "The Establishment of Subgroups within the Rare Earth Group of Elements by Means of Heat Capacity Studies of Rare Earth Salts."

Jackson Walter Foster (Ph.D., soil microbiology, Rutgers University, 1939). The University of Cambridge, England. "Respiration Studies on Filamentous Fungi."

Orville Goodwin Harrold, Jr. (Ph.D., mathematics, Stanford University, 1936). The University of Virginia. "The Structure of Semi-schlicht Images of a Compact Metric Space with Especial Reference to (k, 1) Transformations. The Topology of Rectifiable Curves."

Norman Harold Horowitz (Ph.D., embryology, the California Institute of Technology, 1939). Stanford University and Hopkins Marine Laboratory. "An Investigation of the Respiratory Enzymes of Developing Marine Eggs."

John Oliver Hutchens (Ph.D., zoology, the Johns Hopkins University, 1939). The Carlsberg Laboratorium, Copenhagen, Denmark. "The Carbon and Nitrogen Metabolism of Chilomonas Paramecium."

Francis Philip Jahn (Ph.D., physical chemistry, New York University, 1938). Princeton University. "Azoethane: Preparation, Pyrolysis and Photolysis, Molecular Spectra and Thermodynamic Properties of Azoethane."

Ralph Ernest Lincoln (Ph.D., genetics, the Iowa State College, 1939). Cornell University. "Mutation in *Bacterium stewartii* Including Its Pathogenicity on Maize."

John Lafayette Magee (Ph.D., chemistry, University of