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SOME NEW ASPECTS OF THE RELATIONSHIP OF CHEMICAL STRUCTURE TO BIOLOGICAL ACTIVITY*

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ALTHOUGH for many years it has been recognized that relationships exist between the chemical structures of certain compounds and their pharmacological properties, those interdependencies[1] which have been observed have served more to correlate existing data in many isolated sectors of the field of drug action than to serve as guideposts on the road of medicine and research ahead. Therefore it has seemed desirable to examine some of the facts recently uncovered in nutritional research in an effort to gain new vantage points from which to survey the field of the relationship of chemical structure to biological activity. From these vantage points it may be possible to see some of the roads along which future progress may be made.

Much of the material from which conclusions will be drawn in this paper had its origin in the observation of Woods,[2] who in 1940 reported that the bacteriostatic action of sulfanilamide was reversed competitively by *p*-aminobenzoic acid. These two antagonistic substances are very closely related structurally, since they differ only in the fact that the sulfonamide group of the former is replaced by a carboxyl group in the latter. The hypothesis was

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[1] Thus while many studies have been made of the relationship of such factors as the length of side chains to a given biological activity in a series of compounds, these studies have followed the original empirical discovery of an active member of the series.

[2] D. D. Woods, *Brit. Jour. Exp. Path.*, 21: 74, 1940.

advanced that the bacteriostatic action of the sulfonamides was to be explained by the competition of the drugs with *p*-aminobenzoic acid at some vital stage of metabolism in which *p*-aminobenzoic acid was concerned. This hypothesis grew in stature when it was shown that *p*-aminobenzoic acid was a cell constituent and that it was actually an indispensable nutrient or vitamin for several species.[3,4,5] Pursuing the hypothesis of Woods, other investigators, in efforts to produce bacteriostatic compounds, applied to other vitamins structural changes similar to those involved in passing from *p*-aminobenzoic acid to sulfanilamide. In this way McIlwain[6] found that pyridine-3-sulfonic acid inhibited bacterial growth in competition with nicotinic acid and that α-aminosulfonic acids acted similarly in competition with α-amino acids.[7] Likewise, Snell[8] and subsequently Kuhn *et al.*[9] and McIlwain[10] showed that thiopanic acid (pantoyltaurine, N-[α,γ-dihydroxy-β,β,-dimethylbutyryl]-taurine) inhibited bacterial growth in competition with pantothenic acid. In all of these instances it could be said that the sulfonic acids prevented growth by the production of specific deficiencies, since the action of the drugs was nullified by sufficient amounts of the vitamin or metabolite concerned.

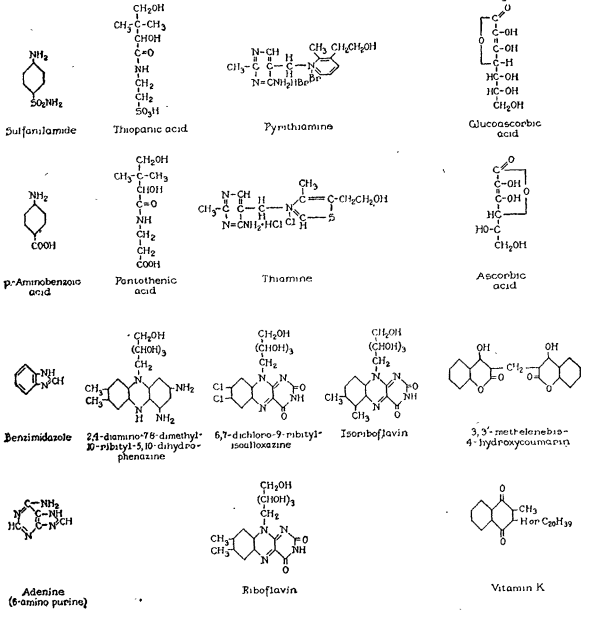

FIG. 1

Thus far, the work involved one type of structural change, and was limited by the desire to produce only inhibition of microbial growth. Although the techniques of bacteriology have been and will continue to be of first rate importance in this field, it must be remembered that bacteriostatic drugs form only a small part of the list of useful chemotherapeutic agents, and that the same principles used to produce bacteriostatic substances may serve to guide the search for useful drugs of other categories.

The next advance was made when it was shown that certain compounds related structurally to the vitamins or other metabolites produced typical signs of deficiency diseases in animals. The first clear recognition of this fact appeared when Woolley and White[11] showed that pyrithiamine caused the production of typical signs of thiamine deficiency in mice, and that the disease so produced was preventable or curable by sufficient amounts of thiamine. At about the same time Woolley and Krampitz[12] reported that glucoascorbic acid caused the production of a disease with most of the characteristic signs of scurvy.[13] In guinea pigs, this disease was preventable by ascorbic acid.[14] Some time before this Overman *et al.*[15] had shown that the signs of vitamin K deficiency which are produced by 3,3′-methylenebis(4-hydroxycoumarin) were prevented by vitamin K to which the coumarin has some structural similarity. Furthermore, Emerson and Tischler[16] have shown that isoriboflavin caused signs of riboflavin deficiency in rats which were reversed by adequate amounts of the vitamin. Woolley[17] reported that the structurally-related phenazine analog of riboflavin caused typical riboflavin deficiency in mice and that the disease was preventable by excess of the vitamin. Finally, the production of nicotinic acid deficiency in mice with β-acetylpyridine and its prevention with nicotinic acid has been observed recently.[18]

Interspersed with these observations on animals there has been a long series of illuminating results on the inhibition of microbial growth with analogs of various metabolites, and the reversal of this effect with increased amounts of the metabolite concerned.

[3] S. D. Rubbo and J. M. Gillespie, *Nature*, 146: 838, 1940.
[4] K. C. Blanchard, *Jour. Biol. Chem.*, 140: 919, 1941.
[5] L. A. Underkofler, A. C. Bantz and W. H. Peterson, *Jour. Bact.*, 45: 183, 1943.
[6] H. McIlwain, *Brit. Jour. Exp. Path.*, 21: 136, 1940.
[7] H. McIlwain, *Jour. Chem. Soc.*, page 75, 1941.
[8] E. E. Snell, *Jour. Biol. Chem.*, 139: 975, 1941.
[9] R. Kuhn, T. Wieland and E. T. Möller, *Ber. deutsch. chem. Ges.*, 74: 1605, 1941.
[10] H. McIlwain, *Biochem. Jour.*, 36: 417, 1942.
[11] D. W. Woolley and A. G. C. White, *Jour. Biol. Chem.*, 149: 285, 1943.
[12] D. W. Woolley and L. O. Krampitz, *Jour. Exper. Med.*, 78: 333, 1943.
[13] This and subsequent studies have provided valuable clues to the mode of action of some of the vitamins or metabolites. The use of inhibitory analogs for the investigation of the mode of action of the vitamins, on the one hand, and of certain drugs on the other, is an inviting approach, but a discussion of this branch of the subject can not be included here.
[14] D. W. Woolley, *Fed. Proc.*, 3: 97, 1944.
[15] R. S. Overman, J. B. Field, C. A. Baumann and K. P. Link, *Jour. Nutrition*, 23: 589, 1942.
[16] G. A. Emerson and M. Tishler, *Proc. Soc. Exper. Biol. and Med.*, 55: 184, 1944.
[17] D. W. Woolley, *Jour. Biol. Chem.*, 154: 31, 1944.
[18] D. W. Woolley, *J. Biol. Chem.*, in press.

An exhaustive review would not be in order here, but the action of pyrithiamine reversed by thiamine,[19] of benzimidazole reversed by adenine or guanine,[20] of 6,7-dichloro-9-ribitylisoalloxazine reversed by riboflavin,[21] of iodinin reversed by vitamin K,[22] and of desthiobiotin reversed by biotin[23,24] may be noted.

With this cursory scanning of the field, let us look about for vantage points from which the roads ahead may be discerned. In particular, let us examine what new aspects of the relationship of chemical structure to biological activity may have become apparent.

The first aspect is the formation of new types of drugs the action of which may, in part, be predicted. The knowledge of the specific histological and biochemical signs produced by deficiencies of the various vitamins and hormones is being steadily increased. It has already been seen that it is possible to synthesize structural analogs of several vitamins which produce in animals the characteristic signs of deficiency of the vitamins to which the analogs are related. Thus it has been possible to produce pharmacological manifestations similar to the signs of specific vitamin deficiencies by administering suitable analogs of the vitamins. Some of the changes which these analogs or drugs elicit could be predicted at least in part, since the signs of the deficiencies have been established. Such drugs carry with them the rather unique advantage that their antidotes, that is, their related vitamins, usually are known prior to the trial of the compound.

The agents thus far produced as vitamin analogs have caused various specific pharmacological signs, but the latter have not indicated a clinical use of the substances. It is not inconceivable, however, that certain specific changes which are brought about by a given vitamin deficiency may be effective and desirable therapeutically. The study of an inhibitory analog of the vitamin in question would then be of interest. In retrospect it can be seen that the drug, 3,3′-methylenebis(4-hydroxycoumarin), which causes signs similar to those seen in vitamin K deficiency, is related structurally to this vitamin. Furthermore, the action of the drug can be reversed by the vitamin. This drug was not conceived on the basis of its relationship to vitamin K—but with the backward glance at this case to fortify us, it may be possible in the future to proceed in a less circuitous manner than was necessary for the development of this coumarin derivative. At the present time, that which has been done is the prediction and subsequent synthesis of compounds which bring about at least some anticipated pharmacological signs; that which remains to be done is the production of types of compounds having useful therapeutic action.

There are diseases known which are due to the overproduction, or to the diminished rate of destruction, of some of the hormones. It would be of interest to determine whether the administration of an inhibitory analog of the hormone concerned would effectively antagonize the extra amount of hormone, and thus combat the disease. It may be questioned whether it is possible to produce antagonistic analogs of the hormones. The results with glucoascorbic acid[12] suggest that it is possible to do this. Ascorbic acid may be regarded as a hormone in all species except guinea pigs and the primates, and it has been seen that glucoascorbic acid antagonizes the action of ascorbic acid in species for which ascorbic acid is not a dietary essential.

Until the present, attention in connection with inhibitory analogs has been focused largely on the production of chemotherapeutic agents useful in combating infections. The possible value of the vitamin analog approach to this problem has been indicated by the encouraging results of McIlwain and Hawking[25] with thiopanic acid (pantoyltaurine). It may be that not only new magic bullets against infection can be cast in this mold, but that, in addition, agents of use in other situations may be formed.

The second new aspect of the relation of chemical structure to biological activity is an outgrowth of the first. If it is possible to form specific drugs which bring about the same effects as are produced by deficiencies of vitamins or other metabolites, then it is of interest and importance to see what structural changes in the metabolite molecule will result in the production of such drugs. Thus far, two general methods of forming such compounds may be discerned. In addition, there is a third group of structural changes about which it is not as yet possible to make generalizations.

The first general method of modifying the structure of metabolites in order to produce inhibitory compounds involves the exchange of a carboxyl group of the metabolite for some other group. The most widely-studied exchange is that of a sulfonic acid or sulfonamide group for the carboxyl. Sulfanilamide and the related sulfonamides are familiar examples of this type of change in which the metabolite is *p*-aminobenzoic acid. Pyridine-3-sulfonic acid, the α-amino sulfonic acids, and thiopanic acid (pantoyltaurine) are examples of the application of this

[19] D. W. Woolley and A. G. C. White, *Jour. Exper. Med.*, 78: 489, 1943.
[20] D. W. Woolley, *Jour. Biol. Chem.*, 152: 225, 1944.
[21] R. Kuhn, F. Weygand and E. F. Möller, *Ber. deutsch. chem. Ges.*, 76: 1044, 1943.
[22] H. McIlwain, *Biochem. Jour.*, 37: 265, 1943.
[23] K. Dittmer, D. B. Melville and V. du Vigneaud, SCIENCE, 99: 203, 1944.
[24] V. G. Lilly and L. H. Leonian, SCIENCE, 99: 205, 1944.
[25] H. McIlwain and F. Hawking, *Lancet*, I: 449, 1943.

change to other metabolites. It is a matter of some mystery why analogs produced by use of this type of alteration are not able to bring about signs of deficiency diseases in animals,[26] while they are quite active in this respect in microbial species. The fact that several drugs which are active as therapeutic agents against infectious diseases come from this group (sulfonamides and pantoyltaurine) may be related to the relative immunity of animal organisms to such compounds. It is possible to achieve bacteriostatic concentrations of the drugs in the host without doing violence to the animal.

Inhibitory compounds may also be formed by replacing the carboxyl group with –CO–R. Auhagen[27] showed that such a change in *p*-aminobenzoic acid to yield *p*-amino-aceto-phenone resulted in a drug which would inhibit the growth of several bacteria competitively with *p*-aminobenzoic acid. A related ketone was chemotherapeutically active against certain bacterial infections of mice. Woolley[18] showed that 3-acetylpyridine produced nicotinic acid deficiency in animals and that phenyl pantothenone (N-[α, γ-dihydroxy-β,-β,-dimethylbutyryl]-1-amino-ethyl-phenyl ketone acted competitively with pantothenic acid. In contrast to the findings with the sulfonic acid derivatives mentioned above, acetylpyridine was not an effective inhibitor of the growth of bacteria.

The second general method of deriving inhibitory compounds involves the replacement of one or more atoms in the ring systems of cyclic metabolites. Since so many compounds of biological importance are cyclic in structure, this method of approach is particularly inviting, and has proved rather fruitful. The type of change which has been applied to the widest variety of metabolites is the substitution of C for N in six-membered rings. In this category are benzimidazole[20] related to the purines; 2,4-diamino-7,8-dimethyl-10-ribityl-5,10-dihydrophenazine[17] related to riboflavin; *o*-aminobenzylmethylthiazolium chloride[28] related to thiamine; and iodinin[22] related to certain naturally-occurring anthroquinones, and more remotely to vitamin K. Here, as in the case of the sulfonic acid or amide compounds, the method of structural change has apparently worked in every instance in which it has been tried. It will really be remarkable if this record continues to stand in the face of more varied application of the principle. Pyrithiamine,[11] the first inhibitory compound discovered to belong to this second general class of structural change, involves a slightly different type of alteration of a metabolite. In this substance –CH : CH– replaces the S atom of the thiazole ring of thiamine. Similarly, 3,3′-methylenebis(4-hydroxycoumarin) may be viewed as a structural analog of vitamin K in which one C atom in the ring system of the vitamin has been replaced by an O atom and changes have been made in the nuclear substituents. However, the relationship here is not as clear, both from a structural and a biological viewpoint, as in the case of several of the other examples cited.

As with the inhibitory compounds derived by the first general method of structural change, so with those arising from the second general method there are very many questions to which adequate answers can not yet be given. A few should be mentioned here. In the case of benzimidazole which is related structurally to, and whose antimicrobial action is reversed competitively by, adenine, the inhibitory compound differs from the metabolite not only in the ring system but also in the fact that benzimidazole lacks the $-NH_2$ group which is present as a substituent of the ring in adenine. It was thought that if benzimidazole were made more analogous to adenine by substituting an amino group in the proper position, a more active compound would result. Actually, however, this substitution did not increase the potency of the benzimidazole.[20] With the exception of pyrithiamine a similar problem concerning the nature of the proper nuclear substituents exists for all representatives of this second general class of compounds. From this it must be concluded that there are many things yet to learn about the relation of the side chains to biological activity in such substances. Another question is whether the production of inhibitory analogs by, let us say, the exchange of C atoms for N atoms is a unilaterally effective process, or whether it is equally effective to exchange N for C. Thus it appears that trading benzene rings for pyrimidine rings results in inhibitory compounds in all the cases tried. It would be well to know if the replacement of benzene rings in metabolites with pyridine or pyrimidine rings likewise would be effective. From the competitive action of aminonicotinic acid with *p*-aminobenzoic acid[29] it would seem that the process is not unilateral, but more data are required before a conclusion can be reached on this point. With a subject in its infancy as this entire one is, there are sure to be many more questions than answers, and the few answers that are available are more in the nature of hypotheses than of facts.

The third group of structural changes, *i.e.*, the miscellaneous types of alteration of metabolites which yield inhibitory compounds, need not be catalogued here since no guideposts to future research can yet be seen from it. In this group belong such substances as glucoascorbic acid,[12] α-methyl-pantothenic acid,[30] and desthiobiotin.[23] Some of these derivatives have

[26] D. W. Woolley and A. G. C. White, *Proc. Soc. Exper. Biol. and Med.*, 52: 106, 1943.
[27] E. Auhagen, *Zeit. f. physiol. Chem.*, 274: 48, 1942.
[28] R. R. Sealock and R. L. Goodland, *Jour. Am. Chem. Soc.*, 66: 507, 1944.
[29] O. H. Johnson, D. E. Green and Ruth Pauli, *Jour. Biol. Chem.*, 153: 37, 1944.
[30] M. A. Pollack, *Jour. Am. Chem. Soc.*, 65: 1335, 1943.

been of considerable value in the investigation of vitamin function, but this branch of the subject of inhibitory compounds and their uses does not concern us here.

It must not be inferred that it is only necessary to alter the structure of a metabolite in any way in order to produce an inhibitory derivative. Much testing of compounds related structurally to each of the several vitamins has shown that most of these derivatives are biologically inert and definitely not inhibitory in the systems in which they were tested.

On the other hand, there appears to be no unique manner in which the structure of a given metabolite must be altered in order to produce an inhibitory drug. For example, isoriboflavin,[16] 6,7-dichloro-9-ribityl isoalloxazine,[21] and 2,4-diamino-7,8-dimethyl-10-ribityl-5,10-dihydrophenazine,[17] three analogs of riboflavin in which the type of structural change is fundamentally different, all have been shown to produce riboflavin deficiency in various species. Also, Johnson *et al.*[29] have called attention to several ways in which the structure of *p*-aminobenzoic acid may be altered in order to produce bacteriostatic compounds.

Again it must be pointed out that the present work has been erected alongside of a new and dim trail to act as a guiding sign until the road has become a well-traveled highway, when a more substantial and pretentious marker may be raised. Furthermore, it must be stated that no attempt has been made to constrain the view with some of the hypotheses now prevalent which would picture the inhibitory metabolite analogs as competing at enzyme surfaces for the corresponding metabolites. Such hypotheses have served well as guides for further investigation, but at present seem to lack sufficient experimental support to make them more than speculations. The facts, however, must be kept clearly in view. These facts are that certain compounds related structurally to various biologically important substances cause specific signs of deficiency diseases, and that these signs are reversed by the metabolites in question when given in adequate amounts.

OBITUARY

WILLIAM WILLIAMS HENDERSON
1879–1944

FOLLOWING a confining illness of approximately two months resulting from heart trouble, Dr. W. W. Henderson, prominent and beloved teacher at the Utah State Agricultural College for twenty-one years, died on October 31, 1944.

He was born at Clarkston, Cache County, Utah, on May 23, 1879. When he was nine years of age, his parents moved the family to Robin, Idaho, where he was raised on a farm. His natural thirst for learning brought him to the Brigham Young College at Logan, and from this institution he obtained a bachelor of arts degree in June, 1903. Immediately following this, he entered the University of Chicago, later transferring to Cornell University, where he obtained a master of science degree in 1905.

Returning from Cornell University, W. W. Henderson became instructor in biology at the Brigham Young College, serving in this position from 1905 to 1910. From 1910 to 1914, he was principal of the Weber Academy in Ogden.

In 1917, Professor Henderson came to the Utah State Agricultural College as head of the department of zoology and entomology, professor of zoology and entomology and entomologist of the Agricultural Experiment Station. He continued in these positions until 1920, when he became president of the Brigham Young College, serving there until that institution was discontinued in 1926.

Obtaining his doctor of philosophy degree in 1924 from the University of California, Dr. Henderson returned to the Utah State Agricultural College in 1926, resuming his former position. With the exception of one year, 1928–29, when he was on leave of absence, he served the college continuously from 1926 until the time of his death.

Dr. Henderson took a prominent part in all campus activities of a scholastic or professional nature. For years he served as chairman of the attendance and scholarship committee. He aided in organizing and sponsoring such campus organizations as the Genetics Seminar, Zoology Seminar and the Utazoa Society. He rendered valuable service in establishing the Utah State Chapter of the Society of the Sigma Xi on the campus in 1942 and served as its president during 1943–44. He was a member of the Faculty Advisory Council and other faculty organizations and committees.

Dr. Henderson possessed a natural penchant for discovering truth and a zeal for teaching it. He searched for it in its original sources of the great out-of-doors, in the laboratory and in the works of other researchers. He was an earnest courageous champion of all truth and of the scientific method of obtaining it. However, because of the large amount of executive work required of him in most of his positions, he was prevented from doing as much original research as he desired to do. Nevertheless, he made important contributions to science, particularly with reference to the taxonomy of western Orthoptera.