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BIOLOGICAL ANTAGONISMS BETWEEN METABOLICALLY IMPORTANT COMPOUNDS AND THEIR STRUCTURAL ANALOGS[1]

D. W. WOOLLEY

Associate, Rockefeller Institute for Medical Research

DURING the past few years compounds which are very similar in structure to vitamins, hormones, and other metabolically important substances, have been synthesized or found to exist in nature. These structural analogs have the property of calling forth in various living organisms some or all of the signs associated with deficiency of the metabolite to which they are related. Tonight I wish to discuss with you some of the facts in this regard which we have chanced upon, and to trace very briefly the origins of the concepts. I would also like to explore with you some of the theoretical and practical aspects in biology arising from this work.

My own interest in the antagonism between structurally related compounds goes back to the winter of 1937 and 1938. At that time, following our identification of nicotinamide as the pellagra-preventative factor (1), a number of chemicals related to nicotinic acid were assayed for vitamin-potency in order to gain some basis for an opinion of the relationship of structure to vitamin-activity (2). Surprisingly enough, it was found that 3-acetylpyridine was quite poisonous to nicotinic acid-deficient dogs, but was harmless to normal animals. At that time no explanation of these results could be made. At the present time, as we shall see in that which follows, 3-acetylpyridine is believed to cause nicotinic acid deficiency by interfering with the action of the vitamin. In looking back over the literature one can see that other investigators likewise had made a few scattered notes on the antagonism which certain structurally similar compounds exerted against

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certain biologically important substances. Some of these findings seemed to run so counter to modes of thought then in vogue that doubts were cast on the validity of the observations. Such was the case as late as 1940 with regard to Kuhn's claim that the sex-determining factor in certain algae was not a single agent but rather the ratio between cis and trans dimethyl crocetin (3). Today, the dependence of a specific biological response on the *ratio* between two closely allied compounds does not seem so heretical.

In 1937 Clark (4) very clearly set forth his idea that antagonism which could be demonstrated between certain structurally similar pharmacological agents was due to competition between them for a specific part of the cell. Clark's views did not become widely known until very recently.

Attention was centered on the competition between structural analogs by Woods' discovery that the bacteriostatic action of the sulfonamides could be negated by additions of p-aminobenzoic acid (5). This latter compound was quickly shown to be a normal constituent of cells, and an essential growth-factor for many microorganisms. It was postulated that sulfanilamide owed its bacteriostatic action to the production of a crippling deficiency of the essential metabolite, p-aminobenzoic acid. This action was possible because of the analogous chemical structures of the two substances.

It was the involvement of a dramatic new therapeutic agent namely, sulfanilamide, in the slowly forming concepts of competition between metabolites and compounds related to them structurally, that focused attention on this biological phenomenon. Without this impetus, progress would have been much slower.

Following Woods' discovery, Fildes (6) showed that alteration of the tryptophane-molecule to give indole acrylic acid would yield an antibacterial agent. McIlwain demonstrated that the same structural change involved in passing from the metabolite, p-aminobenzoic acid to sulfanilamide would, when applied to nicotinic acid, give rise to a bacteriostatic agent, pyridine-3-sul-

fonic acid, the action of which was antagonized competitively by nicotinic acid (7). Snell observed the same situation with pantothenic acid and thiopanic acid (8). The structural relationships involved in these examples may be seen in the first slide (figure 1).

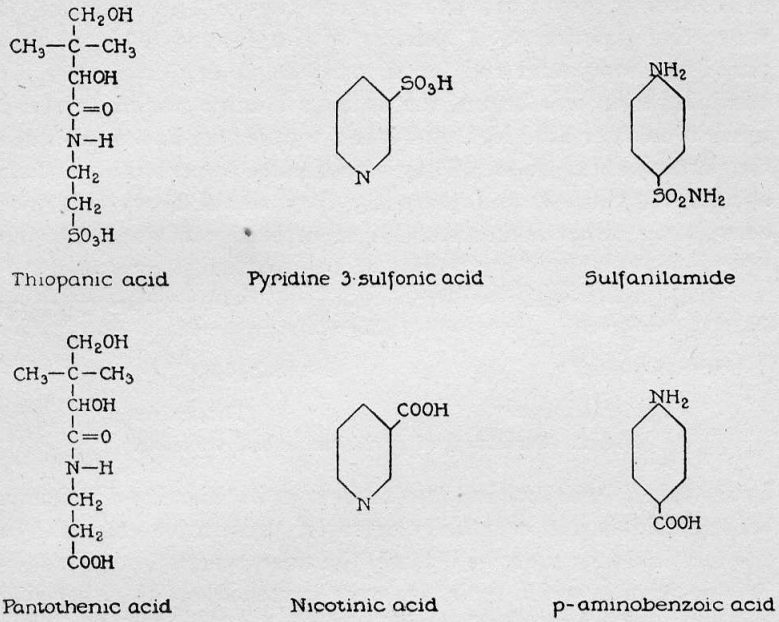


FIG. 1. Structural formulae of some metabolites and related analogs.

We also had been concerned with thiopanic acid in relation to pantothenic acid (9), and had done experiments on the possible usefulness of this agent in the treatment of virus diseases. Therefore, it was natural to look for some more compounds that would compete with vitamins or other metabolites. Let us now examine some of the things that were found.

A series of compounds was soon discovered which would call forth in animals the signs characteristically associated with specific vitamin deficiencies. Let us discuss in some detail one of

the first of these, for many of the features of antagonism between structurally related compounds will thus be illustrated.

When the sulfur atom of thiamine is replaced with a vinyl group, the pyridine analog of the vitamin, pyrithiamine (10), is obtained. The relationship in structure between these two substances is shown in the next slide (figure 2). Following a suggestive experiment of Robbins with a fungus (11), we prepared this compound and fed it to mice. A few days after the administration was begun, the animals became unable to stand upright on their hind legs, and would topple over backward when they attempted to do so. They soon became hyperirritable, their appetites dwindled, and presently they would be thrown into convulsions either spontaneously, or especially when picked up

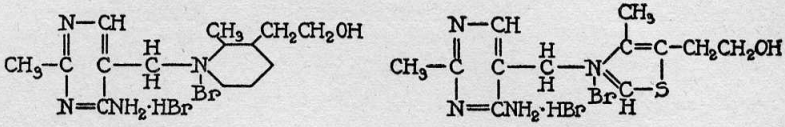


FIG. 2. Structures of pyrithiamine and thiamine.

by the tail. Opisthotonos, familiarly seen in thiamine deficiency, became evident and eventually extensive prostration ensued. In the terminal stages of the disease, the mice assumed a characteristic position in which their legs were stretched out on either side at right angles to the body, and only the head was responsive to stimuli. Death soon followed. These signs have been seen frequently in various species as the characteristic manifestations of thiamine deficiency. When increased amounts of this vitamin were added to the basal diet, the disease called forth by pyrithiamine administration was averted. Likewise, thiamine was effective in curing animals, and would even resuscitate them dramatically from the terminal stages. Data to illustrate the competition between pyrithiamine and thiamine in mice are shown in the next slide (table 1).

Pyrithiamine also proved to be a rather active agent for the suppression of growth of many microorganisms. Here too its

action was prevented by increasing the thiamine-content of the medium (12).

From a quantitative standpoint, the relationship between this pair of compounds is interesting, for it can be seen that the action of pyrithiamine did not depend on the absolute amount present, but, for any given species, it was decided by the ratio of pyrithiamine to thiamine. In other words, pyrithiamine competed with thiamine for the attention of the organism. Such a competitive

TABLE 1

Response of Mice to Various Doses of Thiamine and Pyrithiamine

Pyrithiamine	Thiamine	Animals showing deficiency signs	Average change in weight
gamma per day	*gamma per day*	*per cent*	*gm. per wk.*
0	1.6	0	+3.0
600	1.6	100	−0.2
300	1.6	100	+1.9
100	1.6	100	+2.5
100	2.0	75	+2.4
50	2.0	0	+3.5
600	61.6	0	+3.1
2000	60.0	0	+3.6

situation is almost universal between metabolites and their inhibitory structural analogs.

Although it may seem irrelevant, I cannot refrain from a few words about the importance to these studies of a knowledge of nutrition, and more especially, the development of highly purified adequate diets for animals, and chemically defined media for microorganisms. Most, if not all of the phenomena we are to discuss tonight would not have been found had it not been for the tedious and difficult work which has been done to ferret out, to isolate, and to characterize the essential nutritive requirements of animals and microorganisms. It was necessary to know and to manipulate the thiamine-content of the ration in order to carry out the experiments with pyrithiamine. In like manner, syn-

thetic diets and media have been essential to the elucidation of the other facts to be discussed tonight.

After the demonstrations with pyrithiamine, analogs of other vitamins were produced which were found to cause manifestations of disease similar to those seen in deficiency of the related vitamin. These diseases were believed actually to be deficiencies not only because of the similarities in pathology, but also because they were prevented or cured by adequate amounts of the vitamins concerned. Hand in hand with these experiments on ani-

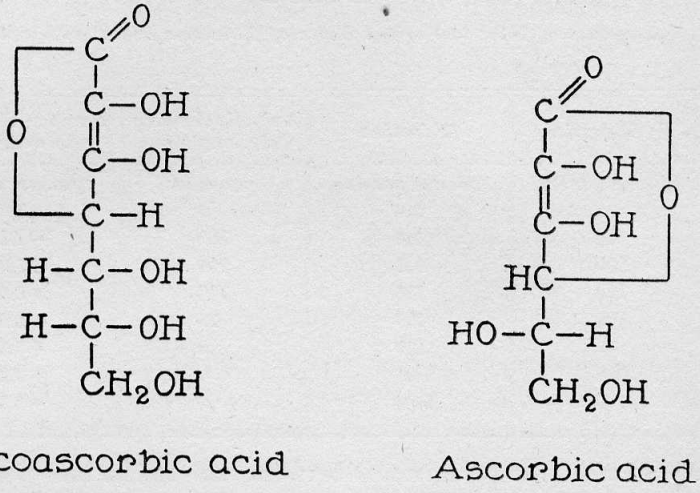


FIG. 3. Structures of glucoascorbic acid and ascorbic acid.

mals a number of studies were made with microorganisms as the biological means of detecting and exploring the phenomenon of antagonism. Let us briefly mention some of these cases.

Glucoascorbic acid, the structure and relationship of which to ascorbic acid are shown in the next slide (figure 3), was found to cause a condition in mice with many of the signs of scurvy as seen in animals susceptible to that disease (13). Now, the mouse, like most other animals, does not need ascorbic acid in the diet. Therefore, a scorbutic mouse has never been recognized, but the individual shown in the next slide (figure 4) had most of the

types of lesions seen in scorbutic guinea pigs or primates. Guinea pigs were also susceptible to the action of glucoascorbic acid, and in this species ascorbic acid counteracted its effect (14).

Shortly thereafter benzimidazole was investigated because the pharmacological action of it on animals had just been reported by Goodman and his associates (15). This drug caused extensive

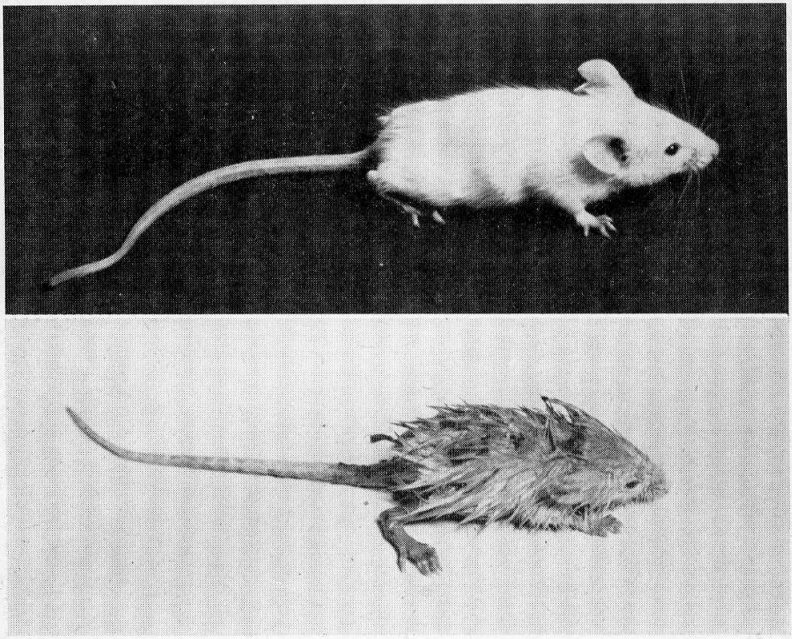


FIG. 4. Mouse treated with glucoascorbic acid compared to control.

loss of muscular tone, sufficient to immobilize the animal. It was therefore of interest to find that the benzimidazole was an inhibitor of microbial growth, and that adenine or guanine antagonized this action (16). Data to illustrate these points are shown in the next slide (table 2). The structural relationship of the drug to adenine may be seen in the next slide (figure 5). The reason I referred to this case as interesting will appear when it is recalled that adenine in the form of adenosine tri-

TABLE 2

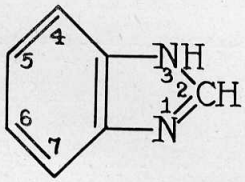
Inhibitory Effects of Graded Amounts of Benzimidazole on Growth of Saccharomyces cerevisiae and Their Reversal by Adenine

Benzimidazole	Adenine sulfate	Turbidimeter reading*
γ *per cc.*	γ *per cc.*	
0	0	69
100	0	69
200	0	66
300	0	84
500	0	96
600	0	99
1000	0	100
600	1000	69
600	600	83
600	300	93
1000	2000	80

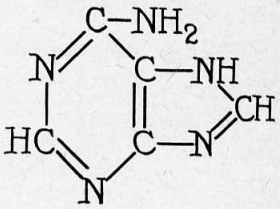
* Turbidimeter readings were expressed as per cent of the incident light transmitted by the cultures, and hence were inversely proportional to the amount of growth.

phosphate and adenylic acid plays a prominent role in muscular contraction, and muscular contraction is inhibited by benzimidazole. Nevertheless, adenine will not overcome the action of benzimidazole in animals, but will do so only in microorganisms.

To continue with examples of antagonism, signs of riboflavin-deficiency were produced in mice as well as in various bacteria



Benzimidazole



Adenine

FIG. 5. Structures of benzimidazole and adenine.

by the administration of the phenazine analog of the vitamin shown in the next slide (figure 6) (17). This compound was prepared and tested because of the previous results with benzimidazole. Since the exchange of a pyrimidine ring in adenine for a benzene ring to form benzimidazole had produced a successful antagonist, the same type of structural alteration was applied to riboflavin, and the result was the same. It was the pyrimidine ring contained in the right hand side of the riboflavin structure

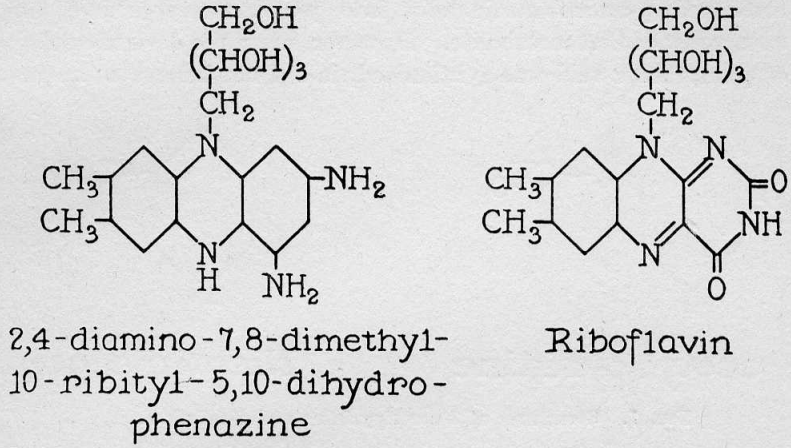


FIG. 6. Structures of riboflavin and its phenazine analog.

that was replaced by the benzene ring in order to form the phenazine.

During all this time the experiments with acetylpyridine in nicotinic acid-deficient dogs had not been forgotten. These were the ones outlined at the beginning of this lecture. The matter was taken up again, and it was soon demonstrated that this compound did act in competition with the vitamin (18). Since mice do not require dietary nicotinic acid, no one knows how the deficiency should manifest itself in this species. Nevertheless, the feeding of 3-acetylpyridine called forth such signs in mice as fiery red tongues, diarrhea, dermatitis, loss of weight and death. Such findings are characteristic of pellagra and black tongue. These

manifestations were prevented by the inclusion of more nicotinic acid in the ration. The next slide (figure 7) will show the structural similarity of the two substances.

Now, if one applied to pantothenic acid the same type of structural change as was involved in passing from nicotinic acid to 3-acetylpyridine, the formation of an inhibitory structural analog of pantothenic acid might be expected. This is particularly so when it is known that Auhagen (19) observed that this alteration converted p-aminobenzoic acid into p-aminoacetophenone, an antagonist of that metabolite. However, when the desired analog of pantothenic acid was synthesized, it did not behave as an in-

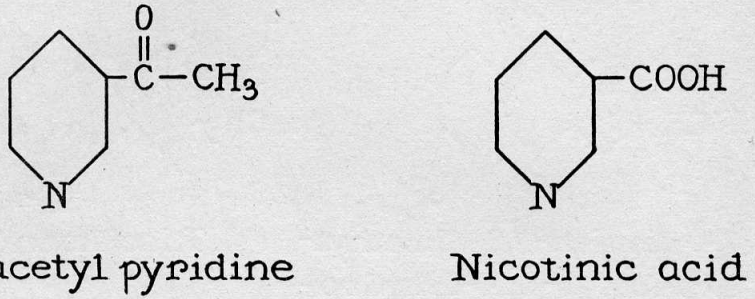


FIG. 7. Structures of 3-acetylpyridine and nicotinic acid.

hibitor. More careful examination of the cases of 3-acetylpyridine and of p-aminoacetophenone indicated that while these were aromatic type ketones and hence somewhat more acidic than aliphatic ketones, the pantothenic acid analog was a purely aliphatic substance. Therefore, an aromatic type of analog was formed by synthesizing phenyl pantothenone, and this proved to be a valuable competitor to pantothenic acid (20). Its value will appear later in the discussion. The structure of phenyl pantothenone and its relationship to that of pantothenic acid are shown in the next slide (figure 8).

I have bedecked this slide purposefully with many formulae, not only to show you the three separate ways in which the analog was prepared, but also to call attention to a phase of the work which is not being discussed tonight. A few of the compounds

which one would wish to test as metabolite antagonists can be purchased from supply houses. A few more can be synthesized according to directions in the chemical literature. By far the majority, however, have never been described, and it is there-

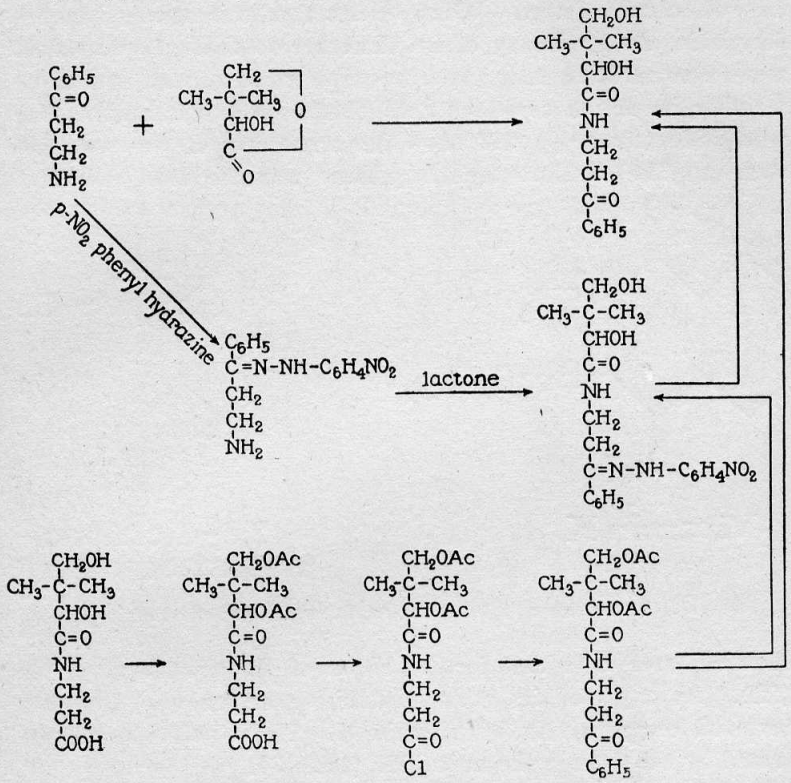
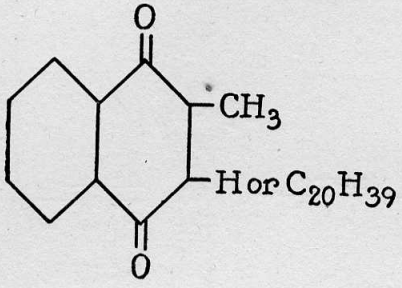


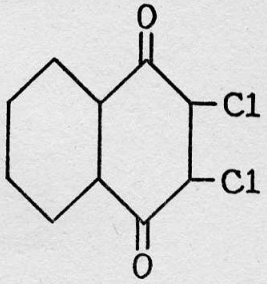
FIG. 8. Structure and modes of synthesis of phenyl pantothenone.

fore necessary to conduct investigations in pure organic chemistry before the biological work is even attempted. For this reason, a major part of the effort involved has been in synthetic organic chemistry. Since the title of my paper tonight begins with "biological," little can be said of the purely chemical aspects.

As a final example of an antagonist to a vitamin let us consider the case of 2,3-dichloro-naphthoquinone. During the war, the U. S. Rubber Company introduced this compound as an antifungal agent (21). It was developed empirically just as were the sulfonamide drugs. When we heard of this new and useful fungicide, the similarity of its structure to that of vitamin K seemed so striking that experiments were begun to determine whether its action could be antagonized by the vitamin. The next slide (figure 9) will show the structures of the two substances. The dichloro-naphthoquinone was the most powerful



Vitamin K



2,3-dichloro-naphthoquinone

FIG. 9. Structures of 2,3-dichloro-naphthoquinone and vitamin K.

chemical ever examined for inhibition of the growth of yeasts (22). As expected its growth-inhibiting action on yeast was reversed competitively by vitamin K. These points are illustrated by the data in the next slide (table 3).

Let us turn now from a brief scanning of examples of antagonism between metabolites and their analogs to a consideration of some general features of this biological phenomenon. I shall attempt to state briefly some of the general principles that have appeared from a mass of data which has been collected by various workers over the past few years. Few of these generalizations will be found free of exceptions. Indeed, our knowledge of this field is so fragmentary that in a few years the picture may look

quite different from the one before us tonight. Therefore, I shall endeavor to reduce hypotheses to a minimum. The task of outlining the general principles of antagonism between structurally related compounds is similar to that of the biochemist who attempts to summarize the salient points concerning enzyme-action. In both instances there is no single criterion which can be set up

TABLE 3

Effect of 2,3-dichloro-naphthoquinone and of 2-methyl-naphthoquinone singly and together on the growth of Saccharomyces cerevisiae

2,3-dichloro-naphtho-quinone	2-methyl-naphtho-quinone	Turbidity* of culture
μg. per cc.	*μg. per cc.*	
0.0	0.0	39
0.002	0.0	78
0.005	0.0	93
0.01	0.0	99
0.005	0.04	60
0.005	0.02	68
0.005	0.01	77
0.005	0.005	85
0.002	0.02	48
0.002	0.01	66
0.0	0.05	46
0.0	0.20	65

* Turbidity is expressed as per cent of the incident light transmitted by the culture when the uninoculated basal medium is considered to have 100 per cent transmission.

to define the phenomena, because exceptions to almost every rule can be found. About all which can be said is that there are a number of features usually associated with biological antagonism between structural analogs, just as there are with enzyme action; and that a majority of these characteristics apply to any given specific case.

In the first place, antagonism between a metabolite (such as a vitamin or hormone) and its structural analog is usually com-

petitive. This is best stated by saying that the biological action of the agent is dependent, not on the absolute amount present, but rather on the ratio of the quantity of the analog to that of the metabolite. This ratio is constant, at least over a limited range of concentration, and when this is so the antagonism is said to be competitive. The ratio between the concentration of analog necessary to cause a biological response and that of the metabolite needed to reverse or negate it exactly is called the inhibition index. It follows that for a series of inhibitory analogs of a given metabolite, the smaller the inhibition index, the more active is the inhibitor. The index is a function of the particular biological system, and may vary widely from species to species for a given inhibitor-metabolite pair. With but few exceptions the index is greater than 1. In other words, much more of an inhibitory analog must be added to an organism than there is of metabolite present. Failure to appreciate this point has led to several faulty conclusions. For example, it would be hazardous to state that an analog of, let us say, vitamin K was not an inhibitory agent, if only 10 or 100 moles of the analog had been supplied for every mole of vitamin in the organism since inhibition indices greater than 10,000 are not uncommon.

From a consideration of these facts one can see that the potency of any given inhibitor depends on two things: first, the inhibition index, and second, the potency of the metabolite concerned. If the metabolite is very active, such as is biotin, and thus only a minute amount suffices to the organism, the chances of producing a very active inhibitory structural analog are greater than if the metabolite is a relatively inactive one such as ascorbic acid. One reason why the sulfonamides are so effective is because of the high potency and consequent low cellular concentration of p-aminobenzoic acid.

Competition between structural analogs cannot be taken as the sole criterion of the phenomenon we are discussing (i.e., of antagonisms). If the action of an agent is reversed by its structurally related metabolite, there is no question; but if reversal is not possible one cannot conclude that the effect of the analog

is divorced from that of the metabolite. For example, analogs such as phenyl pentothenone and glucoascorbic acid behave competitively with the related vitamins in some species but not in others. Even in the classical case of sulfonamides and p-aminobenzoic acid there are bacteria known for which reversal of the action of the drugs cannot be effected (23). What shall we conclude when we have tested an analog of some metabolite and have found that it is indeed inhibitory to bacterial growth or otherwise pharmacologically active, but is not counteracted by the related metabolite? If all that we can observe is an inhibition of microbial growth, it does not seem to me that we can conclude with certainty anything about the mode of action. However, if the analog calls forth in animals more or less specific manifestations which have been associated with a deficiency of the related vitamin or hormone, there is some reason to view the agent as interfering with the metabolite even though no reversal can be demonstrated.

A second general feature of antagonism between metabolites and their analogs is the fact that in many instances the only organisms which are affected by the analog are those for which the metabolite is a nutritive essential. If the animal or bacterium can synthesize the metabolite, the structurally similar agent is ineffectual. This dependence of action on nutritive requirement may be seen in the case of pyrithiamine acting on a variety of microorganisms shown in the next slide (table 4) (12). Here it can be seen that the forms which require the vitamin are very susceptible to the growth-inhibition by the analog. Those which are a little less exacting nutritionally, and can get along nicely on the pyrimidine portion of the vitamin-molecule are about ten times more resistant to the action of pyrithiamine. Those which need only the thiazole moiety of thiamine, or the pyrimidine and thiazole parts are even more resistant to the agent. Finally the species which have no requirement nutritionally for the vitamin are unaffected by the analog, and many of these can grow in concentrations of the drug several million times the inhibitory dose for the exacting organisms.

There are several exceptions to this generalization. Inhibitory analogs such as the sulfonamides, benzimidazole, and 2,3-dichloronaphthoquinone do not depend for their action on the nutritional requirements of the organisms. A middle course between these

TABLE 4

Inhibitory Power of Pyrithiamine for Various Microbial Species

Organism	Inhibition index $\frac{\text{Pyrithiamine}}{\text{Thiamine}}$	Thiamine requirement
Ceratostomella fimbriata	7	Intact thiamine
Ceratostomella from London plane tree	19	" "
Ceratostomella pennicillata	10	" "
Phytophthora cinnamomi	12	" "
Chaloropsis thielavoides	11	" "
Endomyces vernalis	130	Pyrimidine
Mucor ramannianus	800	Thiazole
Saccharomyces cerevisiae	800	Pyrimidine and thiazole
Staphylococcus aureus	2000	" "
Salmonella gallinarum	1000	" "
Neurospora crassa	Greater than 400,000	None
Escherichia coli	" " 2,000,000	"
Clostridium butylicum	" " 2,000,000	"
Lactobacillus arabinosus	" " 40,000	"
Lactobacillus casei	" " 5,000,000	"
Lactobacillus delbruckii	" " 5,000,000	"
Lactobacillus mesenteroides	" " 5,000,000	"
Lactobacillus pentoaceticus	" " 5,000,000	"
Streptococcus lactis R	" " 5,000,000	"
Propionibacterium pentosaceum	" " 5,000,000	"
Hemolytic streptococcus H69D	" " 4,000,000	"

two extremes seems to be followed by analogs such as phenyl pantothenone and glucoascorbic acid, for these act upon all species tested regardless of nutritional needs, but are reversed or competed with by the related vitamin only in the case of those species which require the growth factor. An elucidation of the

underlying mechanisms of these phenomena would be valuable to biochemical understanding.

In order to avoid having this lecture drift off into generalities, I shall discuss only one more common feature of biological antagonism between analogous compounds. Now, although much has been said about the competition between metabolites and compounds similar to them in structure, many of these same analogs are antagonized by naturally occurring compounds totally unrelated to them. The finding of Harris and Kohn (24) that methionine would reverse the microbiostasis caused by the sul-

TABLE 5

Responses of Mice to Added Tryptophane and 3-Acetylpyridine

dl-Tryptophane added	3-Acetylpyridine	Survivals	Average change in weight
per cent of ration	*mg. per day*	*per cent*	*gm. per wk.*
0.0	0	100	+4.0
0.0	4	8	.....
2.0	4	100	+5.2
0.3	4	91	+6.1
0.1	4	83	+4.6

fonamides was the first example of this. We have observed that the pharmacological effects of glucoascorbic acid can be counteracted by a nitrogenous substance found in certain fresh plant tissues (13) and that the pellagragenic action of 3-acetylpyridine is as readily negated by tryptophane as it is by nicotinic acid (25). Data to illustrate this are shown in the next slide (table 5). Furthermore, the antimicrobial action of phenyl pantothenone may be reversed by the amino acids histidine, glutamic acid, or proline as well as by pantothenic acid. There may be some significance in the fact that these structurally unrelated antagonists are so frequently specific amino acids. Furthermore, it is noteworthy that these dissimilar antagonists are effective in the cases where the related metabolite fails to counteract the agent. For example, histidine, or glutamic acid, or proline will relieve

the inhibition of growth caused by phenyl pantothenone in either *Saccharomyces cerevisiae* or *Lactobacillus casei,* although, as was discussed above, pantothenic acid is only effective for the latter (or pantothenic acid–requiring) species (26). In like manner, methionine will reverse those bacteriostases by sulfonamides which are not antagonized by p-aminobenzoic acid.

We all wonder about the mechanism of antagonism between structurally related compounds. How does it work? Probably all of you know of the hypothesis advanced by Quastel and Wooldridge (27) and by Clark (4), and made popular by Woods (5) and Fildes (28), which states that the analog competes with the metabolite for a specific site in an enzyme. Either compound can occupy this site at the expense of the other since both, because of structural features in common, can react with the groups involved. When the metabolite combines, it passes normally through the metabolic reactions for which the system is adapted, but when the antagonist unites, it cannot do this. Like the fabled dog in the manger the analog denies the biological system the use of the metabolite. Whether the metabolite or the analog combines with the enzyme depends on relative concentrations in accordance with the law of mass action.

It is not my intention to defend this hypothesis. A few experimental observations have been made which are very difficult to explain in terms of it. Furthermore, in nearly every instance, the postulated enzymes are not known, and so the hypothesis is difficult to examine experimentally. A few models have been set up to demonstrate that the structural analog can actually displace the metabolite from combination with specific proteins. The competition between oxygen and carbon monoxide for hemoglobin, and the recent observation of Dittmer and du Vigneaud (29) that the antagonist biotin sulfone displaces biotin from its combination with antibiotin, may be cited. If these findings can be extended they will add greatly to the prestige of the hypothesis. Since the postulate has been most stimulating to research, I feel that we should adopt it as a working hypothesis until a better one is forthcoming.

Let us turn now to a consideration of the uses to which the knowledge of antagonism between metabolites and their analogs may be put in the understanding of natural processes and in the treatment of disease. Here, although hope exceeds realization, some typical examples of what has already been done will serve to indicate current trends and point the way ahead.

Because the phenomenon of antagonism was popularized by the discovery that the sulfonamides acted in competition with p-aminobenzoic acid, the belief has arisen (28) that other chemotherapeutic agents may be developed against infectious diseases by producing analogs of metabolites. We have already noted that another useful antimicrobial agent, namely 2,3-dichloronaphthoquinone, was found to act in competition with vitamin K, but this discovery came only after the drug had been developed quite empirically. McIlwain and Hawking (30) showed that very large doses of thiopanic acid, an analog of pantothenic acid, were effective in protecting rats against induced streptococcal infection. While this demonstration indicated that there was a possibility of finding a therapeutic agent among metabolite-analogs, thiopanic acid was much too weakly active to be of practical importance.

Now, we had been conducting experiments along this line for several years, and in particular were attempting to develop an inhibitory analog which would be useful in the prevention of infection by obligate, intracellular parasites. For such parasites, in contrast to the situation with pathogenic bacteria, there are, in the main, no therapeutic means of control. For streptococcal or pneumococcal infections the sulfonamides or penicillin seem to be doing a good job; but for poliomyelitis or influenza there are no effective chemotherapeutic agents. I cannot tell you tonight of a drug which is efficacious in these infectious diseases, but there is one which works against an obligate intracellular parasite.

In collaboration with the Survey of Antimalarial Drugs it has been found that phenyl pantothenone, which you will recall is an inhibitory structural analog of pantothenic acid, is about as

active as quinine in the treatment of malaria either avian or human. Some derivatives of phenyl pantothenone have been produced which are more active than quinine. Time and opportunity will not permit the telling of this story, except to say that the trial of phenyl pantothenone was prompted by observations on the functioning of pantothenic acid in the metabolism of the parasite. It is only with the conclusion of the war that these matters may be mentioned.

Chemotherapeutic agents are not limited to drugs which are effective against infectious diseases. Indeed, these anti-infection agents form only a small part of the chemotherapeutic arsenal. The application of our knowledge about deficiency diseases, and about their production by inhibitory structural analogs of metabolites, can lead to the formation of new series of pharmacological agents. For example, the specific manifestations which result from a given deficiency of a hormone or vitamin are becoming well known. Some of these signs may have therapeutic desirability. If this is so, it might be possible to produce an antagonistic analog which, as we have seen would bring about the desired sign of deficiency.

But you may ask why the production of signs of deficiency can ever be desirable. Isn't everyone, with vitamin pills and hormone injections, trying to prevent signs of deficiency? To answer this, let us look backward at the case of 3,3′-methylenebis-(4-hydroxycoumarin), the so-called dicoumarol. This agent was observed to produce the specific signs seen in vitamin K deficiency (31). Only after this was established, and it was shown that vitamin K would antagonize the hemorrhagic action of the drug, was it realized that dicoumarol was an analog of vitamin K. The clinical application of dicoumarol is based on the fact that it produces in animals the signs of vitamin K deficiency. With this backward glance to fortify us, let us look ahead to discover whether new series of pharmacological agents may be found among inhibitory structural analogs of metabolites. The history of pharmacology shows that the finding of new types of drugs depends primarily on chance, and that once the first member of the series

is thus uncovered, other and possibly more useful derivatives may be synthesized. Perhaps we have a means of coming to the first member of a series without waiting for chance.

As you will recognize, this is nothing but a bright hope. Nevertheless, we do have some experimental models to indicate that it may not be too far-fetched. In setting up these models we attempted to produce a selective pharmacological agent which would elicit a type of response that could be predicted before the compound was synthesized and which had never before been called forth by a drug.

Tocopherol recommended itself as a metabolite for such a

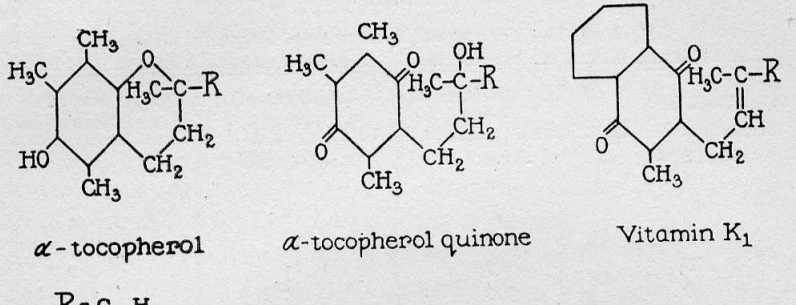


FIG. 10. Structures of α-tocopherol quinone and vitamins E and K.

model, because in female mice a deficiency of this vitamin is apparent only in the pregnant individual, and is characterized by the resorptive interruption of pregnancy during the latter part of gestation. The deficiency is not fatal to the mother, and furthermore, does not produce readily demonstrable signs of disease in non-pregnant mice. A successful antagonistic structural analog of tocopherol should therefore produce resorptive interruption of pregnancy in mice, and should be without effect on non-pregnant individuals, α-tocopherol quinone proved to be just such an agent (32). Its structural resemblance to tocopherol and also to vitamin K is shown in the next slide (figure 10). It is at the same time an analog of both vitamins. The data in the next slide (table 6) will show that daily oral ad-

ministration, or a single parenteral dose caused the desired pharmacological effect. Curiously enough, its action was not reversed by tocopherol, but was by vitamin K. The size of the dose required of α-tocopherol quinone was so large that there is little possibility of it being of practical importance. Nevertheless, as an experimental model it may point the way to new and useful avenues of attack.

Now let us examine a piece of work which is just in progress at the present time, and which tends to show that inhibitory structural analogs of metabolites occur naturally in foods, and

TABLE 6

Effect of dl-α-Tocopherol Quinone on Pregnant Mice

Oral dose	Intraperitoneal dose	Incidence of litters	Ave. size of litters
mg. per day	*mg.**	*per cent*	
0	0	100	6
10	0	100	4
50	0	50	6
100	0	14	3
0	200	67	5
0	400	0	.....

* This was the total amount of material used per gestation.

contribute to the production of disease. In order to do this I would like to talk very briefly about the etiology of pellagra. Our discovery in 1937 (1) that nicotinamide was the pellagra-preventative vitamin seemed to clear up the mystery of the cause of this disease, for the syndrome could be viewed as resulting from a lack of nicotinic acid in the diet. Indeed, it was shown that pellagragenic diets were low in this vitamin. Now, the eating of corn has been recognized for a long time as being intimately associated with the occurrence of pellagra. This has recently been forcefully pointed out by Aykroyd and Swaminathan (33), who showed that human diets containing corn and supplying 15 mg. of nicotinic acid per day were pellagragenic, while

corn-free rations yielding only 5 mg. of the vitamin never produced pellagra. Krehl, Tepley and Elvehjem (34) then clearly demonstrated that corn would reduce the rate of growth of rats, and that this action was overcome by nicotinic acid. They explained these observations largely on the basis of interference with the intestinal flora. However, some of the experiments which we had done with 3-acetylpyridine as a pellagragenic agent in mice led us to postulate that corn contains a structural analog of nicotinic acid which acts as a positive etiological factor in pellagra (25, 18). A substance has now been demonstrated in corn which causes pellagra-like manifestations in mice, and which is counteracted by nicotinic acid. This pellagragenic agent has been concentrated about 100,000 times, and appears to be a pyridine compound. The inference from this work therefore is that pellagra is a deficiency disease which results partly from a lack of sufficient nicotinic acid, but more especially from the action of an antagonistic agent in corn which competes with nicotinic acid, and thereby intensifies the deficiency.

Now that we have examined briefly some of the things which may be done with antagonistic analogs, we may wish to know what types of structural change applied to a metabolite will convert it into such an agent. I believe that certain generalizations can be made about the kinds of alteration which will do this (35). Most of the compounds described at the outset of this lecture as well as many others were predicted from these generalizations, and when they were finally synthesized, were found to have the forecasted biological action. Therefore, it may be justifiable to consider these generalizations briefly.

The first general type of structural change which will convert metabolites into inhibitors is the replacement of a carboxyl by some other more or less acidic grouping. This latter may be sulfonamide or sulfonic acid as in the case of the sulfonamide drugs and of thiopanic acid. On the other hand, it may be an aromatic ketone group as in the case of 3-acetylpyridine or phenyl pantothenone. These relationships are summarized in the next slide (figure 11).

The second general method of converting metabolites into inhibitors involves the exchange of one or more atoms in a ring system for some other atom. Examples of this type are shown in the next slide (figure 12). Here one can see that a sulfur atom may be replaced by two carbons as in the case of pyrithiamine; or carbons may be traded for nitrogens as in benzimidazole; or oxygens for carbons as in dicoumarol. A particularly effective method is to replace an atom in a ring with nothing at all, and

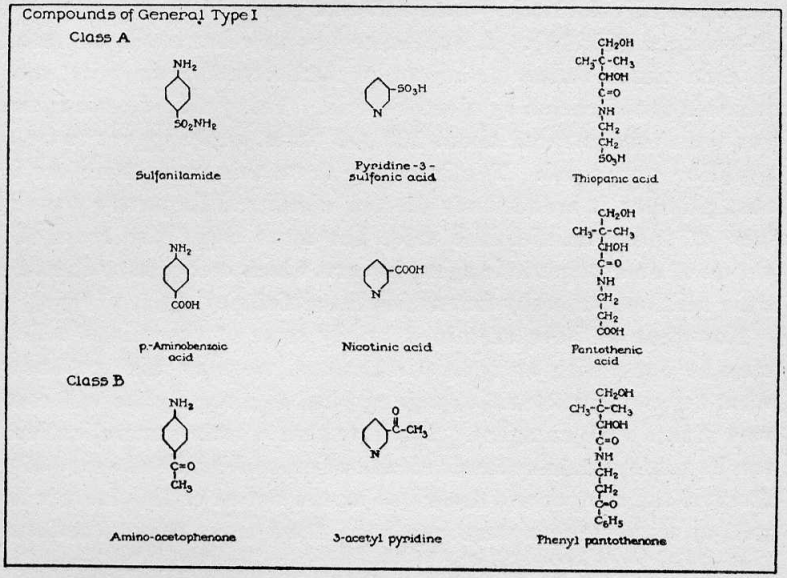


FIG. 11. Classification of analogs. Compounds of General Type I.

thus arrive at an open chain-compound. Good examples of this may be seen in the desthiobiotin-derivatives with which du Vigneaud and his collaborators (36) have worked, or in α-tocopherol quinone viewed as an analog of vitamin K as we have discussed earlier. Numerous other examples of this class are known from the works of many investigators but time has permitted the mention of only a few.

A third general method of realizing inhibitory structural analogs from metabolites is to replace alkyl side chains attached to

aromatic nuclei with halogen-atoms. This is the type of alteration involved in the formation of 2,3-dichloro-naphthoquinone, an antagonist of vitamin K. Kuhn, Weygand and Möller (37) have produced competitors to riboflavin in this fashion, and there are other examples as well.

Finally, there are a number of miscellaneous types of structural alteration which have been found to result in the formation

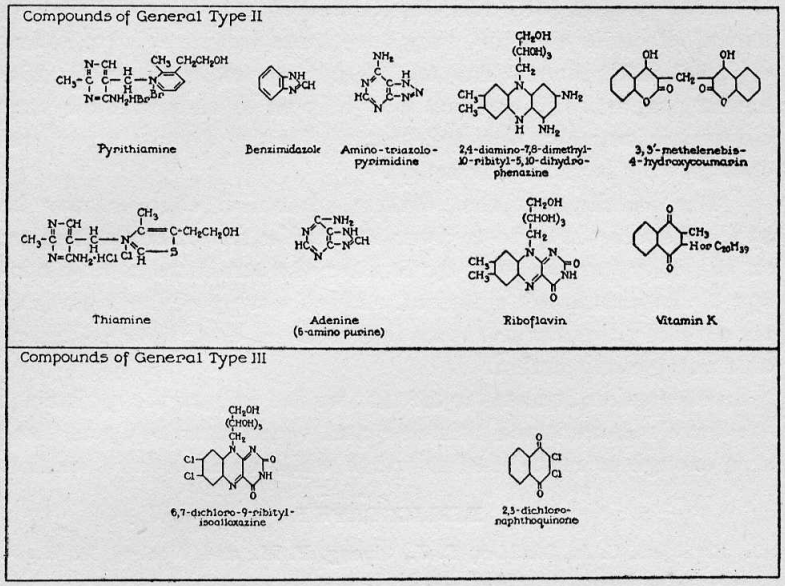


FIG. 12. Classification of analogs. Compounds of General Types II and III.

of antagonistic compounds when applied to metabolites. As work progresses, some of these may be found to be generally applicable. An example of these miscellaneous cases is the addition of an extra carbon to the chain of ascorbic acid with the resulting formation of glucoascorbic acid.

In viewing these relationships of chemical constitution to biological activity, I want to emphasize that there is apparently no unique manner in which the structure of a metabolite must be altered in order to achieve an antagonist (35). Several inhibi-

tory analogs of a single vitamin have been made by altering the metabolite in different ways. Interestingly enough, these varying antagonists of the same vitamin have certain qualitative differences in biological action.

For example, dicoumarol, 2,3-dichloro-naphthoquinone and α-tocopherol quinone are all analogs of vitamin K produced by divergent types of structural change. While all three can be shown to compete with the vitamin and thus to have certain biological effects in common, they have some very decided pharmacological differences. The fact that they do have certain biological properties in common may help us to understand a very old puzzle, namely, that substances of quite diverse structural changes may have similar action.

Despite the fact that diverse structural changes may be applied successfully to a given metabolite in order to produce an inhibitory analog, merely to alter the structure in any nonspecific fashion is not sufficient. Much testing of derivatives of the several vitamins has shown that many of these have no detectable antagonistic action.

In conclusion, it seems to me that we are only at the beginning. We have reconnoitered this new and virgin territory and have seen enough to make it seem worth while to explore it further.

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