ANTIMETABOLITES IN MEDICINE

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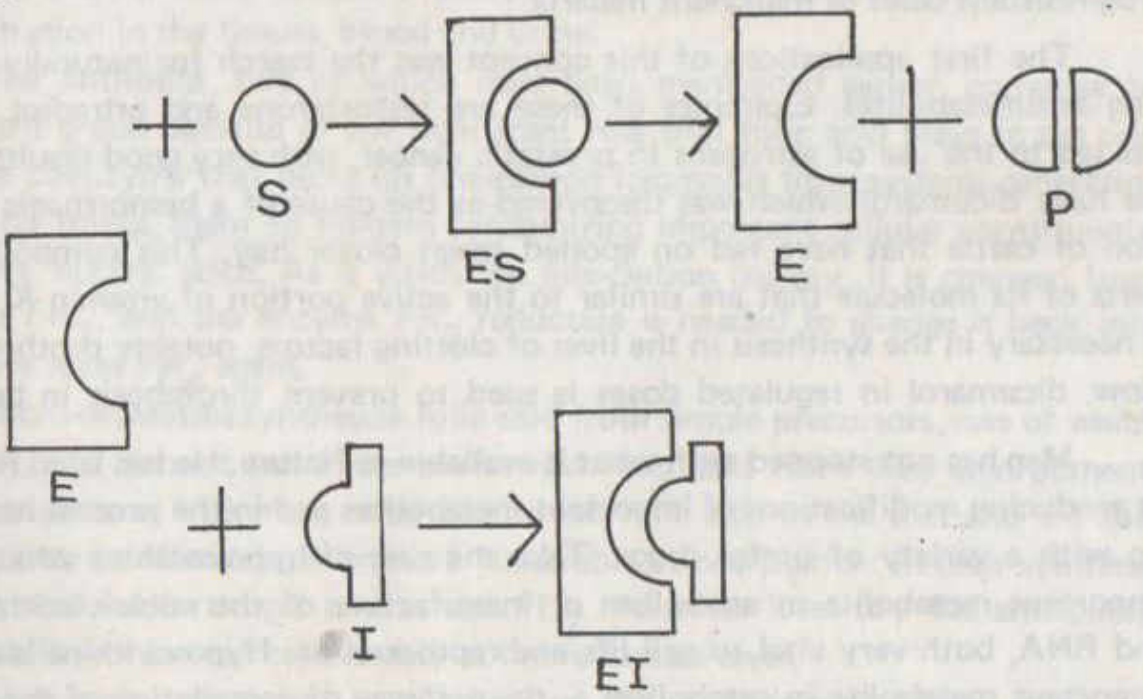
Antimetabolites as a group received a boost in medicine when sulfanilamide was discovered to be bacteriostatic, and Woods in 1940 explained its action on the basis of its structural similarity to PABA. He speculated that sulfanilamide is able to compete against PABA in an enzyme reaction that must be important for the survival of the bacteria and thereby prevents bacterial growth. This explanation based on the phenomenon of metabolic antagonism was borne out by later work. The concept is that, whereas the enzyme is specific in its choice of substrate in terms of catalysis of a given reaction, it exhibits much less specificity for simple combination with compounds, so that as long as a compound bears enough structural similarity to the substrate, such a compound can bind with the enzyme at its active site. This is diagrammatically illustrated as follows:



There is however, a difference in the affinity of the enzyme to the substrate on the one hand and to the inhibitor (or substrate analog or antimetabolite) on the other. This is expressed quantitatively by the Inhibitor Index (I.I.) which is the ratio in moles/Li : $\frac{[I]}{[S]}$ that will produce 50% inhibition of the reaction. This has a value which is usually greater than one, which means that the affinity of the enzyme is usually greater for the substrate than for the antimetabolite. This I.I. is useful in determining the therapeutic use of a drug. As examples, I can cite the diaminopyrimidines: pyrimethamine and trimethoprim. These are antimetabolites of dihydrofolic acid (FH_2) in the enzyme

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reaction catalyzed by dihydrofolic acid reductase to produce the one-carbon carrier, formyl-tetrahydrofolic acid ($f.FH_4$). The reductase is an important enzyme that is present in all living cells for on its presence depends the function of folic acid in supplying one-carbon moieties in biosynthetic reactions such as those for nucleic acid synthesis.

The I.I. of pyrimethamine (Daraprim) for mammalian and bacterial FH_2 reductase show relatively little difference, but the I.I. for human FH_2 reductase is 2000 times that for plasmodial FH_2 reductase. It has therefore become useful as a drug for the prevention of malaria with an effective dose as small as 1 mg/person/week.

For trimethoprim, its I.I. for human liver FH_2 reductase is 100,000 times that of *Proteus vulgaris* enzyme. It is therefore effective as an antibacterial agent, especially when combined with sulfonamides as in Septrin and Bactrim. Its I.I. for human reductase enzyme is also much greater than that for the malarial parasite enzyme, so it has been used, also with sulfonamide, against drug-resistant cases of malignant malaria.

The first applications of this concept was the search for naturally occurring antimetabolites. Examples of these are testosterone and estradiol, which has led to the use of estrogens in prostatic cancer, with very good results. Then we have dicumarol which was discovered as the cause of a hemorrhagic condition of cattle that have fed on spoiled sweet clover hay. This compound has parts of its molecule that are similar to the active portion of vitamin K, which is necessary in the synthesis in the liver of clotting factors, notably prothrombin. Now, dicumarol in regulated doses is used to prevent thrombosis in patients.

Man has not stopped with what is available in Nature. He has tried his hand at producing modifications of important metabolites and in the process has come up with a variety of useful drugs. Take the case of hypoxanthine which is an important metabolite in anabolism or manufacture of the nucleic acids, DNA and RNA, both very vital to cell life and reproduction. Hypoxanthine is also an important metabolite in catabolism – the pathway of degradation of the purine bases of nucleic acids towards the production of uric acid which is finally excreted in the urine.

By modifying the molecule to produce 6-mercaptopurine (6MP), we have an antimetabolite that has been found to compete with hypoxanthine in the anabolic pathway to nucleic acid synthesis. It proved to be effective in systems of rapidly multiplying cells, and in medicine, it has found a use in leukemias of children, bringing about remissions of the disease. Since most of the drug administered to patients is destroyed in the liver making it necessary to give large doses to be able to reach the leukemic cells, scientists sought to protect it from liver destruction by substituting a protective radical for the H in the SH group. Azathioprine (Imuran) was chosen for its good balance of lability and stability. When tested in leukemia, however, it was found to be no better than

6 MP. The drug got a new lease of life when it was tried and found successful in suppressing the immune reaction in organ transplants. The first human patient with kidney transplant who received Imuran was able to survive for 2 years. Since then, the main component of the immunosuppressive regimen that has been instituted for the thousands of kidney transplants and hundreds of heart transplants is Imuran.

Another molecular modification of hypoxanthine, allopurinol (Zyloprim), has proved useful in a different way. It serves as an antagonist in the catabolic pathway – the oxidation of the purine ring catalyzed by xanthine oxidase. It has therefore become a useful therapeutic agent in combatting hyper-uricemia. Increase uric acid in the body leads to gout, kidney stones and other complications of cancer chemotherapy. Various ways of ridding the body of the difficulty soluble uric acid have been tried. Interference with purine synthesis affects all body cells which is not advisable. Zyloprim interferes not with synthesis but with the process of degradation so that the end product becomes a mixture of uric acid and its more soluble precursors resulting in a reduction of uric acid concentration in the tissues, blood and urine.

The antifolics, two of which have been mentioned earlier, comprise an important group because of the significant role that folic acid plays in the cell. It is the coenzyme that picks up one-carbon fragments from systems generating these and brings them to systems synthesizing important cellular constituents, especially nucleic acids. As it yields the one-carbon moiety, it is changed from FH_4 to FH_2, and the enzyme FH_2 reductase is needed to change it back into the active form FH_4 again.

Micro-organisms synthesize folic acid from simple precursors, one of which is PABA, and cannot assimilate preformed folic acid from their environment. Higher organisms like man, need preformed folic acid in the diet and are able to assimilate it. Micro-organisms are vulnerable at two points : in their synthesis of folic acid (sulfa drugs) and at the FH_2 reductase level (by the antifolics). Vulnerability of human cells is only at the reductase level.

As I have mentioned in the case of the diaminopyrimidines, there are subtle differences in the FH_2 reductases of man, bacteria and malarial parasite which are manifested by their different affinities to an inhibitor. Such an antimetabolite that has much less affinity to the enzyme of man than to that of a parasite, whether it be a virus, bacterium, protozoan or helminth, can therefore be used as a drug to effectively suppress the parasite without affecting the host which is the patient.

Cancer cells pose the problem of a parasite derived from the patient's own cells. Here, one takes advantage of differences in quantitative requirements between the cancer cell and the normal cell. Since cancer cells multiply much more rapidly than normal cells, their rate of nucleic acid synthesis is much greater. An antifolic acid drug that has found use in cancer therapy is methotrexate which is 4-amino, 10-methyl pteroyl glutamic acid.