

CHAPTER 11

THE AZOMETHINE DOUBLE BOND

The azomethine condensation, like the free radical and the chain oxidation to which the latter is related, have not found much utility in past biochemistry except as we have employed them in our Postulate. If the criticism is offered that we are giving them too much speculation, the answer comes right back, that we are Postulating processes in matters that were not understood before, nor mastered until these Hypotheses were applied. The azomethine double bond is present in many vitally important molecules. Thiamine has it three times, adenine three times, flavine twice, pyridoxal once, cytochrome twice, porphyrin twice, cytosine twice, vitamin B₁ four times, and it is found in many others and their derivatives. In view of its wide occurrence and great versatility it warrants thorough study. We have used it as a first step in the process of energy transfer and its block in reactions that belong to the high efficiency system oxidations. Recently, however, it is being written in the preliminary steps to certain hydrolytic reactions as where the Carbonyl group of pyridoxal phosphate condenses with an amine group to co-factor certain transaminations and the decarboxylations of amino acids. (Snell and Metzler, 1952). Here the azomethine double bond is hydrolyzed to reproduce an amine group and a Carbonyl group, whereas we use it in preparation for the oxidative destruction of the amine group and the restoration of Carbonyl. We regard it as a bridge for energy transfer and as an activator of dehydrogenations that lead to peroxide free radical formation and oxidative amine destruction. We also use it in preparation for the Amadori reaction as a first step in sugar oxidation. It impressed one as significant that guanidine and methyl guanidine contained it, and so we supposed it played a part in the oxidative cleavage that yielded urea, and in the synthetic reactions as where guanidine was built into guanine. So we noted the possibility that the parathyroid glands might have to do with the formation and destruction of this bond. Whether this supposition is correct or not, further investigation will open up a big field of information.

Glucose is more difficult to oxidize than fructose, and the consensus is now beginning to shift to the idea that all glucose is converted into fructose before it is burned. Let us assume that the functional mechanism where sugar is attached to be burned, presents an activated amine group as in creatine, adenine, etc., much the same way as it has the highly active FCG. This amine would condense with the Carbonyl group of fructose to form an azomethine double bond, which would undergo an Amadori rearrangement, placing the double bond between carbon atoms C₂ and C₃. This would mobilize the hydrogen atom attached to C₄ and subject it to easy removal with production of a free radical that would add molecular oxygen to become a peroxide free radical. This would cause cleavage of the molecule into ketoglyceric acid and glyceric acid, with reversal of the Amadori reaction and regeneration of the functional mechanism's amine group. If

plenty of oxygen were at hand, there would be no interruption; simultaneous further dehydrogenations would produce more peroxide free radicals that would break the molecules down into carbon dioxide and water. If oxygen failed, the reduction chemistry in dominance would change the glyceric acids to lactic acid. These would remain until oxygen came along. This, of course, is not the fermentation process, and in the presence of oxygen no intermediaries would be trapped. Oxygen would be the electron acceptor right on the job. One finding regarding the effects of parathyroidectomy gave clues that led to a successful attack on the cancer and virus problems, with ramifications touching on the most pressing questions in the basic medicine of today. These orient us with regard to a Least Common Denominator in disease production.

The recent support to our Postulate offered by the structures and actions of antimetabolic and antibiotic agents is strengthened by the phenomena and structure of the prosthetic group of Diamine Oxidase, as revealed by Zeller in 1951 ("The Enzymes" — edited by Sumner and Myrback — Vol. II, Part I, page 554 — 1951 Edition). Here Zeller shows that Diamine Oxidase removes one amine group from the toxic diamines by an oxidation process started with the dehydrogenating action of a proven Carbonyl group. The action is thus of the order of our Postulated FCG, and its range of action is similar, since it acts on loosely combining amines, but is inactivated by the firmly combining guanidines. Thus, it detoxicates putrescine, cadaverine, agmatin, etc. . . . but is inactivated by condensing with guanidine, just as the Postulated FCG is inactivated. This condensation blocks the oxidations and causes failure of function and then death, in line with our Postulate. For this reason, Diamine Oxidase is produced in greatest quantities in the placenta, the intestinal mucosa, and the liver. It is found in the blood of pregnant women, and, when its content falls, death of the fetus threatens. Its production in the intestinal wall protects the muscle and secreting glands of the mucous membrane from block in energy production for function. This is particularly necessary because the diamines are produced within the intestine by the decarboxylation of amino acids by bacteria, especially in an acid reacting (pH 3.5 - 6) colon. It is produced especially in the liver, too, to protect the tissues in general from toxic diamines entering the portal circulation. The action of the diamines is of the same order as those of virus, carcinogenic amines, guanidine, and other toxic amines of our Postulate. Their detoxication is likewise initiated clearly by the dehydrogenating action of the Carbonyl group, — oxidation. However, Zeller does not go far enough to identify free radical and peroxide free radical action, which would be the normal chemical sequel to dehydrogenation. He rather lets the enzyme conception carry the burden of explanation.

He also assumes the enzymatic activation of the two hydrogen atoms attached to the carbon atoms alpha and beta to an amine group, and that these are the hydrogen atoms that are removed by the enzymatic Carbonyl group. He assumes that a double bond is thus produced between these carbon atoms, and that this double bond is shifted to the

amine group to form an imide group that is then hydrolyzed off as ammonia. Thus a Carbonyl group takes the place of the amine group, and the detoxication is accomplished. Prof. Zeller gives no thought to a condensation between the Carbonyl group of the enzyme and the amine group of the toxin to form an azomethine double bond. Nor does he refer to the oxidation, we have Postulated that replaces the amine group of the toxin with a Carbonyl group. However, the products of the reaction are ammonia and hydrogen peroxide, as would be expected from a hydrolysis in an enzyme system that operates in solution instead of on the grana surfaces, where oxidations would be expected, as in the case of the FCG system. This difference in position determines the reaction mechanism.

Diamine oxidase is inactivated by various guanidines and diguanidines, imidazole, such dyes as pyocyanine, methylene blue, streptomycin, pyridoxamine, by cyanide, and by the Carbonyl reagents, semicarbazide, hydroxylamine, phenylhydrazine, etc. The enzymatic Carbonyl group does form an azomethine bond with the latter, the Carbonyl Reagents, and with guanidine in agreement with our Postulate, so it can also form the same azomethine bond with the other amines except, as the direction of the reaction is determined enzymatically. Such enzymatic forces, however, are not able to prevent an inactivating condensation of the Carbonyl group with guanidine, phenylhydrazine, hydroxylamine, etc., which take place outside the physiological range of control, just as they can inactivate the FCG. Both inhibited systems, that of the grana FCG, and of the enzymatic Diamine Oxidase Carbonyl group, require the oxidative rescue via a high O/R potential Survival Reagent, which indicates that they are both inhibited in the same way by a firm azomethine condensation. This will be demonstrated in the case histories.