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COMMENTARY

ALDEHYDE REDUCTASES: MONOMERIC NADPH-DEPENDENT OXIDOREDUCTASES WITH MULTIFUNCTIONAL POTENTIAL

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The aldehyde and the ketone reductases collectively comprise the aldo-keto reductases [1], a group of monomeric NADPH-dependent oxidoreductases with similar chemical and physical properties, which catalyze the reduction of aldehydes and ketones to the corresponding alcohol. The catalyzed reaction is overwhelmingly in favour of alcohol formation and the reverse reaction occurs only to a very limited extent, hence the name reductase as opposed to dehydrogenase. Equilibrium constants of the order of 10^{-6} to 10^{-11} M typically are found [2, 3]. The enzymes are designated as either aldehyde or ketone reductases on the basis of a preferential, but not mutually exclusive, substrate specificity, and this overlapping specificity coupled with indeterminate physiological roles has made it difficult to arrive at a more specific nomenclature for these enzymes.

Within each separate category of aldehyde or ketone reductases are several enzymes which early workers in the field considered to be sufficiently different in substrate specificity and in postulated physiological roles to be given specific names. Examples of such enzymes are aldose reductase, an aldehyde reductase involved in the polyol pathway, an accessory pathway of glucose metabolism [4], and L-hexonate dehydrogenase, an aldehyde reductase responsible for the reduction of D-glucuronate in the glucuronate-xylulose pathway [5]. At the present time, however, it is the author's view that, until more specific physiological roles can be determined, it is inappropriate to name any of these enzymes by anything more specific than aldehyde or ketone reductases. This commentary will be concerned with those enzymes that fall into the category of aldehyde reductases.

The preference of all aldehyde reductases for aromatic aldehydes, the broad overlapping substrate specificity for other substrates including naturally occurring ones, and the fact that many of the substrate specificity studies have been carried out with crude enzyme preparations have resulted in a great deal of confusion concerning the actual number of different aldehyde reductases occurring in a given tissue. Uncertainty as to the number of distinct enzymes in a tissue and whether or not they are the same as similar enzymes in other tissues has also made it difficult to establish specific physiological roles. Nevertheless, aldehyde reductases have been implicated in the metabolism of biogenic aldehydes [6–8], succinic semialdehyde [9, 10], long chain aliphatic aldehydes [11], and aldosugars [3, 12, 13]. Whether there is a specific metabolic role for these enzymes or whether they simply play a general role in the detoxication of naturally occurring and xenobiotic aldehydes will be discussed in this paper.

Comprehensive accounts of both aldehyde and ketone reductases have been published recently [14, 15]; therefore, an extensive review of the literature is not necessary at this time. This commentary will be in the nature of a personal perspective of the most significant and seminal work carried out so far in the field, together with observations on the possible physiological significance of this group of enzymes. Since the aldehyde reductases are also sensitive to inhibition by anti-convulsants, this commentary will also present a biochemist's viewpoint of the potential pharmacological importance of aldehyde reductase inhibition.

Aldehyde reductases have been investigated in greatest detail from three tissue sources: brain, kidney and liver.

Brain aldehyde reductases. Interest in brain aldehyde reductases has centered around the metabolism and possible importance of aldehydes derived from the biogenic amines. In particular, the sensitivity of brain aldehyde reductase to anti-convulsant drugs [7, 16–19] has suggested that brain aldehyde reductase activity is closely associated with the pharmacological actions of drugs affecting the central nervous system. Such studies, however, are complicated by the presence of several aldehyde reductases in brain and by the different inhibitory effects that anti-convulsant drugs have on these enzymes. There are two major forms of NADPH-dependent aldehyde reductase in mammalian brain [7, 9, 20–22] the predominant form of which has been called the high-K_m form [7]. This form is almost certainly identical with pig kidney [23], pig liver [24] and human liver [25] aldehyde reductase—an enzyme which was also known previously as L-hexonate dehydrogenase [26]. The other major form in brain is the low-K_m form which is less sensitive to drugs and can use NADH as well as NADPH as coenzyme. It is becoming increasingly evident, however, that the low-K_m form may be identifiable with aldose reductase [22, 27, 28].

The subcellular localization of the different enzyme forms of aldehyde reductase has also posed something of a problem, mainly because until recently a systematic study had not been carried out. In rat brain, the low-K_m form has been reported to be located in mitochondria [29, 30] but a recent study by Ryle and Tipton [31] clearly shows that both the high-K_m and the low-K_m forms are cytosolic. The

reason that the low-K_m form was assumed to be in mitochondria has been shown to be simply due to the choice of substrate used. A number of earlier studies used *p*-nitrobenzaldehyde in the presence of NADH to identify the low-K_m form and to distinguish it from the high-K_m form [29, 30]. These results, however, became questionable following the report of Köchli *et al.* [32] of an NADH-dependent nitro reductase activity in rat brain with activity towards *p*-nitrobenzaldehyde. Using a modification of the classical five fraction procedure of de Duve *et al.* [33] and with pyridine-3-aldehyde and *p*-carboxybenzaldehyde as substrates, Ryle and Tipton [31] clearly show that both the high-K_m and the low-K_m forms are cytoplasmic in location.

It is becoming increasingly evident, also, that there are more than two forms of aldo-keto reductase in mammalian brain, together with an enzyme responsible for the reduction of succinic semialdehyde (SSA) to γ-hydroxybutyrate (GHB) [9, 34]. The high-K_m form also exhibits activity towards SSA [9, 10]. In human brain [9] and in pig brain (J. Cromlish and T. G. Flynn, unpublished results), four aldehyde reductases may be identified. Hoffman *et al.* [9] have designated these as AR1, AR2, AR3 and SSA reductase respectively. AR1 is a ketone or carbonyl reductase [35] and has been characterized quite extensively by Wermuth [36]. AR3 is identifiable with the high-K_m form of aldehyde reductase and it is now likely that AR2 is identical with aldose reductase [9, 37]. Recent work in our laboratory has demonstrated that in the pig, for example, AR3 and antiserum to pig kidney aldehyde reductase (PKAR) cross-react with complete identity whereas no reaction at all is evident between pig brain AR2 and antiserum to PKAR. On the other hand, pig brain AR2 cross-reacts with complete identity to antiserum to a recently identified pig muscle aldehyde reductase [38]. It appears, therefore, that not only are AR3 and AR2 not the same enzyme but they are not related from the point of view of antigenic structure. Since antigenic determinants are both sequentially and conformationally determined [39], it is possible that the rapid evolution of the aldehyde reductases in general [40] is responsible for the seemingly large differences in primary and tertiary structure that are apparent between these obviously related enzymes. The active sites of all the aldehyde reductases, including the carbonyl reductases since they also reduce aldehydes to some extent, must be similar in that their substrate specificities are very much the same. It is likely, therefore, that the multiple forms of aldehyde reductase represent the products of different genes—genes that have arisen by duplication from a single primordial form and have been subsequently modified by deletion and insertion. The predominance of the high-K_m form in all species so far examined [40] makes this an attractive candidate for the original gene product.

The high-K_m form (AR3) and the low-K_m form (AR2) can be distinguished on the basis of their substrate and coenzyme specificities and by their sensitivities to various inhibitors. For example, the low-K_m form is relatively insensitive to sodium valproate, which may provide an effective means of discriminating between the two forms [28]. Both forms of aldehyde reductase have a broad substrate specificity which includes aromatic and aliphatic aldehydes, aldosugars, ketones and steroids with 17-aldol side chains [3, 7, 9]. Many of the best substrates for the high-K_m form contain a free carboxyl group [24], and it has been suggested that this enzyme is involved in the reduction of SSA to GHB [24, 41]. However, the recent work of Tipton and his colleagues makes this unlikely to be the case [10, 42]. The K_m value of the high-K_m form for succinic semialdehyde is considerably greater than that determined for succinic semialdehyde dehydrogenase [10]. The specific activity of SSA dehydrogenase in rat brain homogenates is also much greater than that of the high-K_m form of aldehyde reductase [10, 43]. These results make it unlikely that the high-K_m form of aldehyde reductase plays a significant role in the reduction of SSA in the cell. In terms of substrate specificity, the physiological role of the high-K_m form is not at all clear even though it constitutes by far the major reductase activity in brain. Most of the best substrates for this enzyme are synthetic, i.e. they do not occur physiologically, and the work of Whittle and Turner [28] and of Anderson *et al.* [29] suggests that this enzyme under normal physiological circumstances is also not involved in the reduction of aldoses or of catecholamine-derived aldehydes. The role of this major form of aldehyde reductase in brain remains most perplexing. It may simply play a scavenging role in the detoxication of general aldehydes or in drug metabolism [24, 44, 45]. However, to many in the field such a finding would be a disappointment. A more attractive and optimistic view is that of Whittle and Turner [28] who suggest the possibility that the high-K_m form may be involved in some as yet unidentified metabolic sequence.

The high-K_m form is the most sensitive of the brain aldehyde reductases towards anti-convulsant drugs [46, 47]. This aldehyde reductase is sensitive to a wide variety of anti-convulsants including barbiturates [7, 16], hydantoins, succinimides and oxazolidinediones [17]. In addition, benzodiazepines and sodium valproate inhibit the enzyme [18, 19] and, more recently, Whittle and Turner [37] have shown that carbamazepine and phenacamide are also potent inhibitors. This study also demonstrated the considerable inhibitory effects of analogues of sodium valproate and of several flavonoids such as quercetin and morin, hitherto better known as aldose reductase inhibitors [48]. It is interesting that of the latter group the commercially prepared aldose reductase inhibitor Alrestatin (Ayerst, AY22, 284) was quite effective in inhibiting the high-K_m form of aldehyde reductase. The ineffectiveness of this compound in clinical trials with human diabetes [49] may perhaps be explained by its lack of specificity towards aldose reductase alone. The fact that aldehyde reductase activity is sensitive to a wide variety of anti-convulsant and other inhibitors led to the suggestion that the physiological effect of these drugs is an alteration in brain aldehyde metabolism [18, 19]. However, as has been discussed, the major effect of anti-convulsants is on the high-K_m form and, until a physiological role is assigned to this enzyme, correlations between the action of anti-convulsants and inhibition of the high-K_m form cannot be made.

Kidney aldehyde reductase. The high-K_m form of kidney aldehyde reductase was the first of the aldehyde reductases to be purified and characterized to any extent [50], but recognition of a distinct aldo-keto reductase [51] in kidney had been made previously. Together with the liver enzyme [24, 25] it has become the aldehyde reductase of choice for physico-chemical studies, mainly because it is easier to extract and purify in large amounts from easily available tissue. The first extensive examination of aldehyde reductase was made by Flynn *et al.* [23] using pig kidney enzyme (PKAR). These workers purified the enzyme by a method similar to, but significantly different from, that of Bosron and Prairie [50]. However, it is interesting now in retrospect that neither of these preparations was homogeneous by the criterion of polyacrylamide disc gel electrophoresis (PAGE) and that both contained at least one additional enzyme exhibiting aldehyde reductase activity. At the time, such additional bands were looked on as contaminants but it is highly likely that these were the additional forms of aldehyde reductase that are observed in other tissues like brain. Indeed, in a reexamination of the number of aldehyde reductases in pig kidney, we have found recently (J. Cromlish and T. G. Flynn, unpublished work) that as many as four aldo-keto reductases may be present in this tissue. One of these forms has a molecular weight of 35,000 in comparison to a molecular weight of 40,200 obtained for PKAR, and this enzyme cross-reacts with complete identity with antiserum to PKAR. However, in the presence of phenylmethylsulfonylfluoride and EDTA, this lower molecular weight form is not detected. Clearly, when a tissue contains multiple forms of enzymes, care has to be taken to ensure that such forms are the expressed gene products and not the result of proteolytic processing. Turner and his group [52] and Tipton and his colleagues (K. Tipton, personal communication) seem to have been aware of this possibility but it is not apparent from the literature that other workers have. Improvements on the purification procedure of Flynn *et al.* [23] have been published recently [53, 54] which effectively remove all other aldehyde reductase forms. The purification procedure of Morpeth and Dickinson [54], while an improvement over the earlier procedure of Flynn *et al.* [23] does not seem to offer significant advantages over that of a more recent protocol of Flynn *et al.* [53]. The latter authors used a blue dextran-Sepharose 4B step and Turner and Hryszko [52] have also used a procedure involving the use of a nucleotide affinity matrix, in this case the dye Procion Red HE3B, to great effect in the purification of rat liver aldehyde reductase. There are several advantages in using a purification procedure involving chlorotriazinyl dyes, especially those bound to a support like dextran. They are easy to prepare and much cheaper to use than affinity resins in which the ligand is $NADP^+$, and they have greater capacities for enzyme adsorption [47, 55]. They have been used with repeatedly consistent results in the preparation of PKAR in our laboratory and in the preparation of several other aldehyde reductases from other species and tissues [24, 27, 56, 57].

Notwithstanding the method of preparation, PKAR has been shown, by the criteria of gel filtration and sodium dodecylsulfate (SDS) PAGE, to be a monomer with a molecular weight in the region of 40,000 [23, 50, 54]. Thus, aldehyde reductase is one of the few known monomeric oxidoreductases. Despite several efforts, the precise molecular weight of PKAR remains somewhat uncertain. By the criterion of SDS PAGE, the molecular weight has been reported to be 38,000 [50], 36,900 [23], 36,700 [53] and 41,700 [54]. Analytical ultracentrifugation has yielded values of 30,200 [50] and 43,700 [54]. The most widely disparate results have been found with gel filtration methods where values of 25,000 [50], 28,500, 35,000, 36,000 [54] and 33,000 [23] have been found. In recent work in our own laboratory, we have obtained a value of 40,200 by SDS PAGE. Clearly, the results of Morpeth and Dickinson [54] are the most consistent and, as these authors point out, the reason for the much lower and less consistent values obtained with gel filtration is probably an interaction between the enzyme and the gel-filtration matrix. In this connection, it is of interest that after many attempts we can detect no carbohydrate associated with the enzyme. It seems that an accurate molecular weight will not be produced until the amino acid sequence has been determined.

The amino acid composition of PKAR [23] shows no unusual features except that there is a relatively large number of proline residues for a protein of this size. A low degree of α-helicity is therefore indicated, which is confirmed by circular dichroism [23]. The amino acid compositions of the high-K_m aldehyde reductases from the kidneys and livers of several mammalian species, together with the compositions of the aldehyde reductases from fruit fly and yeast, have been analyzed for evolutionary relatedness [58]. Among mammals it was not surprising to find that considerable homology existed among the different aldehyde reductases. However, what was surprising was the finding that by comparison with the rates of evolution of oligomeric dehydrogenases these enzymes comprise the most rapidly evolving family of oxidoreductases. This is probably not related to the dispensability of these enzymes but rather to the monomeric nature of the aldehyde reductases, since Davidson and Flynn [58] showed that there is a direct correlation between the number of subunits and the rate of evolution. Thus, for the oxidoreductases at least, subunit interactions, and not functional dispensability, determine the rate of evolution. Therefore, once again, we have to conclude that the aldehyde reductases do have an indispensable metabolic role. In terms of relationships among the aldehyde reductases, it is now perhaps more important to examine relationships among the different kinds of aldehyde reductase in a given tissue, for example, between the high-K_m and low-K_m aldehyde reductases. Relationships among enzymes are best determined by amino acid sequence comparisons. The sequence of PKAR is being determined in our laboratory and is partially complete. The amino acid sequence of the human liver high-K_m form is also being undertaken [59]. By far the most interesting sequence comparison will be that between the high-K_m form and the aldose reductase from the same tissue and species. Here are two very similar

enzymes in many respects and yet they do not cross-react immunologically [4]. Their amino acid compositions, therefore, will not be similar. It is likely that the cofactor binding sites, the catalytic centres and, to a lesser extent, the substrate binding sites will exhibit some sequence and structural homology. The accuracy of these predictions awaits the outcome of the sequence studies.

PKAR, the pig liver enzyme [24], and human liver aldehyde reductase [25] have been used for studies on the active site. It has been known for some time that sulfhydryl reagents are inhibitors of PKAR [50, 51] but the first systematic investigation of the involvement of SH groups in the activity of the enzyme was that of Flynn *et al.* [23] who came to the conclusion that aldehyde reductase resembled several oligomeric dehydrogenases in that combination of the most reactive thiol groups with the sulfhydryl reagent *p*-chloromercuribenzoate (PCMB) was not accompanied by loss of catalytic activity. The addition of high concentrations of thiol reagent, however, caused complete inhibition. It is possible that high concentrations of PCMB cause denaturation of the enzyme. Similar conclusions may be arrived at with regard to human liver aldehyde reductase [25]. In more recent work, Morpeth and Dickinson [54] also failed to demonstrate an essential thiol group in PKAR.

Other potentially essential residues at the active site of PKAR have also been investigated recently by a chemical modification approach [54, 60–62]. Using the arginine specific reagent, butane-2,3-dione, Davidson and Flynn [60] showed the presence of a single essential arginine at the active site which does not serve in a binding capacity for NADPH. This residue probably serves to orient the coenzyme into position for binding and subsequent ternary complex formation. Chemical modification with pyridoxal 5′-phosphate has also demonstrated the presence of an essential lysine at the active site of PKAR [61, 62]. This lysine is involved in the binding of NADPH, and, moreover, from protection studies with NADP analogues and from the sequence of a small peptide containing the essential lysine [61], it is likely that the lysine interacts with the pyrophosphate bridge portion of the coenzyme.

In contrast to the results of Morpeth and Dickinson [54], we have obtained evidence for the involvement of a single essential histidine residue at the active site of PKAR [62]. Chemical modification with diethylpyrocarbonate resulted in inactivation of the enzyme, with the pattern of protection afforded by NADP and various analogues of the coenzyme suggesting that the modified histidine was at the active site. These results also suggested that the essential histidine was in the region of the binding site for adenosine ribose and distal to the site of hydride transfer.

Thus, results to date indicate the presence of an essential histidine, lysine and arginine at the coenzyme binding site of PKAR. No information is yet available on the nature of the substrate binding site which is, of course, the primary area of concern, not only for active site-directed researchers but also for all of us interested in the substrate specificity of the aldehyde reductases. Elucidation of the substrate binding site will be difficult because of the inability of substrate to bind in the absence of NADPH [63]. A substrate analogue capable of forming non-productive binary and ternary complexes would be enormously helpful for such a study.

Kinetic analysis of PKAR has shown that the enzyme obeys compulsory order kinetics for the reduction of D-glyceraldehyde [63] and pyridine-3-aldehyde [2] with NADPH binding before aldehyde. Morpeth and Dickinson [2] suggested on the basis of hyperbolic Dixon plots for inhibition by the alcohol product that a random or partly random order of product release occurred. We have found no evidence for this (T. G. Flynn, unpublished results) nor did Rivett and Tipton [10] in kinetic studies on rat brain aldehyde reductase (high-K_m form). Interesting differences have also been observed by different workers with respect to barbiturate inhibition of PKAR and other high-K_m form aldehyde reductases. Davidson and Flynn [63] and Rivett and Tipton [10] found linear non-competitive kinetics with phenobarbital and sodium barbitone, respectively, whereas Morpeth and Dickinson [2] observed hyperbolic inhibition effects of sodium barbitone on PKAR. In view of a possible relationship between the metabolism of biogenic aldehydes by aldehyde reductases and the mechanism of action of psychoactive drugs [20, 64], more work is needed to clarify the interactions of barbiturates and aldehyde reductases.

In discussing the classification of the various NADP-linked mammalian aldo-keto reductases, Walton [65] had suggested that classification of these reductases might be aided by determining the stereospecificity of reduction of aldehyde substrate by NADPH. Using D-glyceraldehyde as substrate, Walton [65] showed that glycerol dehydrogenase, a monomeric aldehyde reductase from rabbit muscle [3], transferred the *pro*-4R hydrogen of NADPH to D-glyceraldehyde. It has since been demonstrated that PKAR [23] and the high-K_m form from the kidneys of several other species [40], together with the enzyme from pig [24], human [25] and rat and rabbit liver [15], are also *pro*-4R or "A" specific. These results are interesting by comparison with the results of Felsted *et al.* [66] who found that the majority of rabbit liver keto or carbonyl reductases were B-hydrogen specific. As pointed out by Felsted and Bachur [15], such criteria may be an important way of distinguishing between the aldehyde and ketone reductases. It is interesting in this context that a correlation need not necessarily exist between the stereospecificity of an oxidoreductase for its substrate and a shift in the absorption maximum of NADPH consequent upon binding of the coenzyme to the enzyme. Fisher *et al.* [67], for example, have observed that for dehydrogenases with B-hydrogen specificity a red shift of the absorption maximum occurs upon binding of coenzyme. A red shift apparently occurs with PKAR [54] and with the aldehyde reductase from pig [24] and human liver [25] even though these enzymes have been shown unequivocally to be A-hydrogen specific.

PKAR or the high-K_m form of aldehyde reductase has been the only form isolated and purified from kidney. However, it is apparent from the work of

Flynn *et al.* [23], of Bosron and Prairie [50] and of Culp and McMahon [51] that other forms are present. In recent work in our laboratory (J. Cromlish, unpublished work) we have demonstrated that the low-K_m form is also present in pig kidney together with a lower molecular weight form which may have arisen by proteolysis of PKAR. This work is continuing as it is important to establish how many aldehyde reductases are present in a given tissue.

Liver aldehyde reductase. Two major forms of aldehyde reductase have been found in the livers of mouse [68] and rat [43] and in human liver [25] and pig liver [24]. Four aldehyde reductases have been isolated from rabbit liver cytosol, two of which may be identical with 3(17)β-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase respectively [69]. Guinea pig liver contains three aldo-keto reductases, two of which also may be steroid dehydrogenases [56, 57]. The major form in both rabbit liver and guinea pig liver appears to be identifiable with the high-K_m form of aldehyde reductase, but the substrate specificity of the other enzymes in these tissues suggests that they are not identifiable with any of the well characterized mammalian aldehyde reductases and may be more typical of the keto-reductases or even true aldo-keto reductases.

The best characterized of the mammalian liver aldehyde reductases from a physico-chemical point of view are the enzymes from human liver [25] and pig liver [24]. It is apparent that these enzymes are identical with the high-K_m form from rat liver [42, 52] and with PKAR [23]. They are also probably identical with daunorubicin reductase, a barbiturate-sensitive aldehyde reductase from rat liver [44, 45]. Both the pig and human liver enzymes are very similar to PKAR in many respects. They are both monomeric oxidoreductases with a molecular weight of the order of 35,000; a coenzyme preference for NADPH; pH optima and isoelectric points that are similar; and the same stereochemistry of reactions catalyzed. Some interesting results were obtained by Branlant and Biellman [24] with respect to the substrate specificity of liver aldehyde reductase. These workers showed that, in general, substituted benzaldehydes with an electron withdrawing group are the best nonphysiological substrates, but in the case of the high-K_m form (reductase I of Branlant and Biellman [24]) *p*-carboxybenzaldehyde proved to be a better substrate even though the carboxyl group is less electron withdrawing than the nitro group. On the basis that the carboxyl group may play a role in the binding of substrate, Biellman and his co-workers [70] postulated that a positively charged group, such as an arginine, might be present at the substrate binding site. Chemical modification of pig liver aldehyde reductase with phenylglyoxal resulted in complete inactivation of the enzyme but, in complete contrast to the work of Davidson and Flynn [60], a single arginine residue, not at the coenzyme binding site, was modified. Moreover, this residue was implicated in the binding of uncompetitive inhibitors like (±)2,3-dimethylsuccinic acid rather than in the binding of a carboxyl group bearing substrate. In fact, a second anion-binding site was postulated as being responsible for the binding of such substrates. Since it is extremely probable that PKAR and pig liver aldehyde reductase 1 are the same enzyme, some additional investigations are required to clarify these conflicting results.

It has been stated that the enzyme aldose reductase is not present in mammalian liver [68, 71, 72] but it has been suggested [24] that the second major form in liver (reductase II of Branlant and Biellman [24]; AR2 or low-K_m form of Rivett *et al.* [43]) may well be aldose reductase. To establish once and for all whether liver does contain aldose reductase, it will be necessary to purify the putative enzyme from liver and cross-react it with authentic aldose reductase from the same species. Identity with the low-K_m form in brain could also be made in this way.

Studies on liver aldehyde reductases first led to the suggestion that the high-K_m form was identical with L-hexonate dehydrogenase, an enzyme involved in the biosynthetic pathway leading to ascorbic acid and L-xylulose [5, 13]. Thus, it was postulated that the physiological role of the major form of aldehyde reductase was the reduction of D-glucuronate to L-gulonate in the glucuronatexylulose pathway. This role is questionable, however, because the enzyme is present in both man [25] and guinea pig [56], two mammals known to be unable to synthesize L-ascorbic acid [73]. It is possible, of course, that aldehyde reductase in man and guinea pig is a vestigial remnant of a once active ascorbic acid biosynthetic pathway but the ubiquitous presence of this enzyme in tissues and species [40] makes it difficult to accept a vestigial role for the enzyme. In the absence of a specific role, however, it has to be reiterated that the physiological function of the high-K_m form may well be that of general aldehyde detoxication, particularly for the enzyme in liver, an organ which is the seat of detoxication mechanisms in the body.

Aldose reductase and the complications of diabetes. This commentary would not be complete without a consideration of the role of aldose reductase (probably identifiable with, or closely related to, the low-K_m aldehyde reductase of brain) in the polyol pathway [71, 72] and in the etiology of some of the complications of diabetes. Studies in experimental animals, and in man, strongly suggest that in hyperglycemic conditions there is an excessive conversion of glucose to sorbitol catalyzed by aldose reductase, and that the accumulation of sorbitol is of etiological significance in the pathogenesis of several diabetic complications [74]. The tissues which suffer most of the damage in diabetes are also those in which the polyol pathway is active, for example, lens [75], retina [76], nerve [77] and kidney [78]. These are tissues which are freely permeable to glucose. In contrast, sorbitol, which accumulates in these tissues, penetrates cell membranes very poorly [79] and, once formed, is trapped intracellularly together with any fructose into which it is converted. Since fructose is poorly metabolized in these tissues and also penetrates membranes very poorly, the net effect is an accumulation of both sorbitol and fructose in the cell. This leads to hypertonicity and the resulting osmotic changes which occur produce the complications of diabetes, e.g. diabetic cataract and peripheral neuropathy. Aldose reductase appears to be highly localized within certain cell types—in lens epithelium [80], in the Schwann cell in peripheral

nerve [81], and in the papilla of kidney [82]. These localizations enable a correlation to be made between the site of sorbitol formation and the site of the observed abnormality.

The use of inhibitors of aldose reductase has played a crucial role in developing the concept that the enzyme is involved in the complications of diabetes, particularly in sugar cataractogenesis. Known inhibitors of aldose reductase include flavonoids and complex heterocyclics, e.g. Alrestatin or 1,3-dioxo-1H-benz-(de)-isoquinoline(3H)-acetic acid [48] and Sorbinil or d-6-fluoro-spiro[chroman-4,4'-imidazolidine]-2',5'-dione [83]. Alrestatin, for example, can inhibit aldose reductase *in vitro* at very low concentrations (10^{-5} M [84]). These inhibitors are not related either structurally or chemically to the substrates or products of aldose reductase and are found to be non-competitive inhibitors [85]. While aldose reductase inhibitors are effective in relieving the complications of diabetes in experimental animals (see review by Varma [48]), they have not yet been shown to be very effective in humans [49, 86, 87].

The difficulties that are encountered in inhibiting aldose reductase *in vivo* in humans may be directly related to the different susceptibilities that the aldose reductases from different species, and even from different organs within the same species, show towards various inhibitors [88]. These differing susceptibilities may be related to small differences in the primary structures of the enzymes from various species. The inhibitors used to date are all of the non-competitive type and they probably bind non-specifically to parts of the enzyme remote from the active site. We have shown that the aldehyde reductases, in general, are rapidly evolving [58], i.e. their primary structures show little relationship to each other even though they are all monomeric NADPH-dependent oxidoreductases of broad substrate specificity. Thus, while the bulk of the protein chain of these enzymes has suffered change over evolutionary time, their active sites have remained relatively constant. Non-competitive inhibitors will of necessity have different effects because the different primary structures of these enzymes will have different binding properties. It seems clear that attention has to be paid to the physical and chemical properties of the active site of aldose reductases so that a more rational approach, i.e. one based more on the chemical and physical properties of the enzyme, may be taken towards developing a therapeutically effective inhibitor.

Physical and chemical studies of aldose reductase have not been extensive. The enzyme has been partially purified from calf lens [89], brain [90] and liver [91], bovine [92] and human brain [22, 35], pig brain [27], seminal vesicle and placenta of sheep [93] and from *Rhodotorula*, a species of yeast [94]. Characterization of aldose reductase has been done largely with these partially purified preparations and with homogeneous preparations from bovine lens [95] and pig brain [27]. The various studies to date, however, have revealed several features of aldose reductase which are common to the enzyme from all mammalian sources. Aldose reductase is a monomeric NADP-specific oxidoreductase having a broad substrate specificity and a molecular weight of the order of 37,000. In these respects, the enzyme is clearly a member of the aldehyde reductase family. However, it is also clear that this enzyme is not identical with the high-K_m form aldehyde reductase [22, 27, 35, 95]. It is becoming increasingly evident from the work of Hoffman *et al.* [35], Branlant and Biellman [24] and Whittle and Turner [28] that aldose reductase is identical with, or closely similar to, the low-K_m form of aldehyde reductase.

Concluding remarks. Apart from the essential journeywork of fully characterizing the major forms of aldehyde reductase, the outstanding problems that face workers in this field are those of nomenclature and physiological role. Mammalian tissues contain at least two major forms of aldehyde reductase. The predominant form has been variously designated as the high-K_m form, AR1 and AR3. However, this enzyme is also identical with L-hexonate dehydrogenase (EC 1.1.1.19). The other major form has been designated as the low-K_m form or AR2. It is becoming increasingly evident that this form may be identical with aldose reductase (EC 1.1.1.21), an aldehyde reductase implicated in the complications of diabetes, but this is not yet proved. Brain and other tissues also contain an SSA reductase—that is an enzyme distinct from the high-K_m form which is also capable of metabolizing SSA. A carbonyl reductase with some ability to reduce aldehydes is also present in brain. Also, there appear to be additional forms in certain tissues. The question of identity and nomenclature of these different aldehyde reductase forms is extremely confusing. However, at the present stage of research in this field, the confusion may be unavoidable and it may be that application of a coherent nomenclature should not be attempted until more stringent diagnostic criteria are applied to the enzymes, e.g. immunological cross-reactivity. At present a numbering system is not appropriate because, for example, the designation AR1 from one laboratory does not coincide with AR1 from another. The high-K_m and low-K_m designations are attractive, but these were arrived at from values of K_m obtained with certain substrates. Other substrates would not necessarily yield the same designation and what, for example, does one name an enzyme with intermediate-K_m values? Specific names like aldose reductase have their uses but, if this enzyme is clearly shown to be identifiable with the low-K_m form, then which name should be retained? Ideally, a meaningful nomenclature has to be arrived at from a consideration of the metabolic roles of these enzyme forms and, as has been discussed, a definitive physiological function has yet to be assigned to any of these aldehyde reductase forms.

The multiplicity of aldehyde reductases in brain and in other tissues and their widespread distribution throughout animal tissues make it unlikely that these are vestigial enzymes, i.e. enzymes with a once critical function which have gradually lost their essentiality and metabolic usefulness over evolutionary time. It is possible, however, that, even though once critical to an organism's metabolic function, where their functions were, say, the elimination of toxic aldehydes and ketones, they have been superseded by more efficient mechanisms and that the aldehyde

reductases have been retained as a result of neutral selection. That is, they have sustained a large number of amino acid substitutions but because of their partial usefulness to an organism have not been subject to selective elimination. Whatever the case may be, the aldehyde reductases are present in extant organisms where their physiological role remains the predominant feature for future investigation.

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