Biochemical Pharmacology, Vol. 33, No. 7, pp. 1137–1142, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

OXIDATION OF FORMALDEHYDE AND ACETALDEHYDE BY NAD$^+$-DEPENDENT DEHYDROGENASES IN RAT NASAL MUCOSAL HOMOGENATES

MERCEDES CASANOVA-SCHMITZ, RAYMOND M. DAVID* and HENRY D'A. HECK†
Departments of Pathology, and General and Biochemical Toxicology, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709, U.S.A.

(*Received* 19 *June* 1983; *accepted* 16 *September* 1983)

Abstract—Homogenates of respiratory and olfactory tissue from the rat nasal cavity were examined for their capacity to catalyze the NAD$^+$-dependent oxidation of formaldehyde (in the presence and absence of glutathione) and of acetaldehyde. Both aldehydes were oxidized efficiently by nasal mucosal homogenates, and formaldehyde dehydrogenase (FDH) and aldehyde dehydrogenase (AldDH) were tentatively identified in both tissue samples. At least two isozymes of AldDH, differing with respect to their apparent K_m and V_{max} values with acetaldehyde as substrate, were found in the nasal mucosa, one of which may catalyze the oxidation of both formaldehyde and acetaldehyde. The specific activity of FDH in the olfactory mucosa was twice that in the respiratory mucosa, whereas the specific activity of the higher K_m isozyme of AldDH was five to eight times greater in respiratory than in olfactory tissue. The specific activity of the lower K_m isozyme of AldDH was similar in respiratory and olfactory homogenates. Repeated exposures of rats to formaldehyde (15 ppm, 6 hr/day, 10 days) or to acetaldehyde (1500 ppm, 6 hr/day, 5 days) did not substantially affect the specific activities of FDH and AldDH in nasal mucosal homogenates. Glutathione is a cofactor for FDH; the concentration of nonprotein sulfhydryls in respiratory mucosal homogenates was approximately 2.8 μmoles/g and was not changed significantly by repeated exposures to formaldehyde (15 ppm, 6 hr/day, 9 days). These data indicate that the rat nasal mucosa, which is the major target site for both aldehydes in inhalation toxicity studies, can metabolize both formaldehyde and acetaldehyde, and that the specific activities of formaldehyde and aldehyde dehydrogenase in homogenates of the nasal mucosa are essentially unchanged following repeated exposures to toxic concentrations of either compound.

Formaldehyde dehydrogenase (FDH) (EC 1.2.1.1) and aldehyde dehydrogenase (AldDH) (EC 1.2.1.3) are present in most or all mammalian tissues [1, 2]. Owing to their important roles in the overall metabolism of methanol and ethanol, the investigations of these enzymes have focused mainly on the liver, and highly purified preparations of both enzymes have been obtained from this source [3, 4]. However, studies of the toxicities of inhaled formaldehyde (FA) and acetaldehyde (AA) have shown that the major target site following inhalation exposure of rats, mice, or hamsters is the upper respiratory tract, especially the nasal mucosa [5, 6,‡]. This result is not surprising, inasmuch as both aldehydes are highly water-soluble, very reactive, and are absorbed by and attain their highest tissue concentrations in the nasal passages [7, 8].

The toxic effects of FA and AA that occur in the upper respiratory tract are dependent on concentration and time. In acute or subacute studies in rats, the primary effects included degenerative changes, hyperplasia, and metaplasia of the respiratory epithelium (formaldehyde) [9] or of the olfactory epithelium, larynx, and trachea (acetaldehyde) [10]. Under chronic exposure conditions, both aldehydes have led to the development of nasal cancer [5, 6, 11,‡].

Several other compounds that are hydrolyzed or oxidatively *N*-demethylated to FA have also been shown to be carcinogenic [12]. For example, the nasal carcinogen hexamethylphosphoramide is rapidly oxidized to FA by rat nasal cytochrome P-450-dependent monooxygenases [13]. The rate of generation of FA, the accessibility of the FA to target sites, and the rate of removal of FA by metabolism or other processes may play important roles in the toxicity of such FA precursors [12, 13].

Inasmuch as the oxidation of FA and AA catalyzed by nasal mucosal NAD$^+$-dependent dehydrogenases may be a major mechanism of defense against toxic injury, it was of interest to investigate whether these enzymes are present in respiratory and olfactory tissues of the rat and to determine their activities. We report in this article that both enzymes are present in nasal mucosal homogenates, and we tabulate their specific activities and apparent K_m values, using FA and AA as substrates. Since reduced glutathione (GSH) is a required cofactor for FDH, the concentration of nonprotein sulfhydryls (NPSH) in respiratory mucosal homogenates is also presented.

* CIIT postdoctoral fellow. Present address: Microbiological Associates, River Road, Bethesda, MD.

† Address all correspondence to: Dr. Henry Heck, CIIT, P.O. Box 12137, Research Triangle Park, NC 27709, U.S.A.

‡ V. J. Feron, R. A. Woutersen and I. M. Appelman, Abstract, *First Annual Sympos. Eur. Org. Cooperation Cancer Prevention Studies*, Brussels, Mar. 25–26, 1983.

MATERIALS AND METHODS

Chemicals. Glutathione, dithiothreitol, NAD^+, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from the Sigma Chemical Co. (St. Louis, MO). The glutathione was dissolved at a concentration of 0.5 M in 0.1 M sodium pyrophosphate buffer (pH 8.0) and was stored frozen until use. DTNB (99 mg) was dissolved in 25 ml of methanol and was stored at a temperature of 4°. No change was detected in the absorbance of the DTNB solution during storage. Pyrazole was purchased from Eastman Organic Chemical (Rochester, NY). Other chemicals, including buffers and EDTA, were reagent grade.

Paraformaldehyde was obtained from the Aldrich Chemical Co. (Milwaukee, WI). Stock solutions of FA were prepared in dilute NaOH (pH 11) and were standardized by the chromotropic acid method.* Acetaldehyde was purchased from Eastman and was redistilled before use.

Animals. Male F-344 rats [CDF(F-344)/CrlBR] (Charles River, Portage, MI), weighing between 200 and 250 g, were used for the measurement of *in vitro* enzyme activities. Rats received NIH-07 laboratory diet (Zeigler Bros., Gardners, PA) and tap water *ad lib.* and were maintained on a 12 hr light : dark cycle. To determine the effect of repeated exposures to either FA or AA on the nasal mucosal enzyme activities, rats were exposed to FA (15 ppm, 6 hr/day, 10 days) or to AA (1500 ppm, 6 hr/day, 5 days). Exposures to FA were carried out in an 8 m^3 chamber operated at 1.7 m^3/min air flow. Paraformaldehyde was heated in a stainless steel cylinder enclosed within a thermally controlled oven that was maintained at approximately 90°. Formaldehyde vapor was carried by air through a heated stainless steel pipe and was introduced at a right angle into the chamber air supply. Chamber temperatures were 74 ± 1° F and the relative humidity was 45%. Exposures to AA were carried out in a 27-l glass aquarium operated at 20 l/min air flow [14]. Liquid AA was cooled in an impinger to the temperature of solid carbon dioxide. Nitrogen was passed through the impinger at a rate of 1.5 l/min, and the resulting vapor was mixed with the incoming air supply of the chamber. In both cases, chamber air concentrations were monitored by infrared spectrophotometry (Foxboro MIRAN 1-A, South Norwalk, CT).

Preparation of nasal mucosal homogenates. Rats were decapitated, and the heads were cooled in ice. Tissue dissections were performed at 4°. Respiratory mucosal samples were obtained from the naso- and maxilloturbinates, lateral walls, and septum anterior to the olfactory epithelium. Olfactory mucosa was dissected from the dorsal lining of the cavity and from the ethmoid turbinates. For the assay of enzyme activities, tissue samples from eight control or from eight treated rats were combined. Approximately 30 mg of respiratory mucosa and 40 mg of olfactory mucosa were obtained from the nose of each rat. Protein represented about 9% of the total tissue weight [15].

The combined tissue was suspended in 0.6 ml of 0.05 M sodium phosphate buffer (pH 7.4), 0.1 mM dithiothreitol, and 1mM EDTA. The sample was weighed, homogenized in a small glass grinder, and centrifuged at approximately 9000 *g* for 5 min. The supernatant fraction was removed and saved, and the pellet was resuspended and homogenized in the same buffer. After centrifugation, the two supernatant fractions were combined. Buffer was added to achieve a final ratio of 6 ml/g wet wt of tissue. The final suspension was kept in ice for the duration of the enzyme assays, which were performed immediately.

Preparation of liver homogenates. Freshly-collected whole livers of rats were minced with scissors and homogenized in the same buffer as that used for the nasal mucosa. The homogenates from three livers were combined, and the resulting suspension was centrifuged at 13,000 *g* for 30 min at 4°. To compare the results of the FDH assay in liver with those reported previously by Goodman and Tephly [16], the liver supernatant fraction was recentrifuged at 78,000 *g* for 2 hr at 4°, and the soluble fraction was saved in ice for the analysis of the enzyme activities. The assay of FDH in liver was not feasible in the absence of the latter centrifugation due to the opacity of the liver suspension. In contrast, the assay of FDH in the nasal mucosa did not require high-speed centrifugation of the homogenate.

Assay of FA and AA oxidizing capacity. The kinetics of the NAD^+-dependent oxidation of FA and AA were determined at 340 nm using a thermostatted (25°) cuvette. Solutions contained 0.1 M sodium pyrophosphate buffer (pH 8.0), 0.67 mM NAD^+, and 1 mM pyrazole [1]. Total FA concentrations were varied from 10 μM to 2.4 mM. Total AA concentrations were varied from 64 μM to 45 mM.

At each concentration of FA, the rate was measured in the presence and in the absence of GSH. Formaldehyde and GSH react reversibly to form the adduct, *S*-hydroxymethylglutathione, the dissociation constant of which is reported to be 1.5 mM under the assay conditions [1]. This adduct appears to be the true substrate of FDH [1]. Total GSH concentrations in the cuvette were adjusted in order to maintain the free GSH concentration constant at 1.5 mM.

Initial rates were calculated from the increase in absorbance resulting from the addition to the cuvette of a selected volume (usually 25 μl) of the centrifuged mucosal suspension. The absorbance increased linearly with time for at least the initial 3 min. Specific activities [$nmoles \cdot min^{-1} \cdot (mg\ protein)^{-1}$] were calculated using a molar absorptivity of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$ for NADH; protein concentrations were determined by the method of Peterson [15]. Rates of NADH formation were measured in the absence of added FA or AA, and these rates were subtracted from the apparent values measured in the presence of the substrates.

Estimates of the Michaelis–Menten constants, V_{max} and K_m, for the enzymes catalyzing the oxidation of FA and AA were obtained using the nonlinear least-squares computer program, NONLIN [17, 18]. Initial estimates were derived from Hofstee [19]

* R. R. Miksch, *Formaldehyde in Air: A Revised NIOSH Procedure.* Lawrence Berkeley Laboratory, Berkeley, CA (1980).

plots. These estimates were refined by nonlinear regression analysis of the initial velocity on the substrate concentration.

Analysis of NPSH concentrations in the respiratory mucosa. Nonprotein sulfhydryl concentrations were determined in the respiratory mucosa of unexposed rats and of rats exposed to 15 ppm of FA, 6 hr/day, for 9 days. The analyses were performed on freshly collected samples of tissue immediately after termination of the final exposure. With the exception of the final spectrophotometric measurement, all procedures were carried out at 4°.

The NPSH concentrations were analyzed in samples of mucosa from individual rats, using a modification of the method of Sedlak and Lindsay [20]. Respiratory mucosa from each animal was suspended in tared centrifuge tubes that contained 0.6 ml of 0.02 M EDTA. The samples were weighed and homogenized, and 0.47 ml was removed and mixed with 0.067 ml of cold 50% trichloroacetic acid (TCA). Precipitated macromolecules were removed by a 5-min centrifugation at approximately 9000 *g*. A 0.4-ml aliquot from the supernatant fraction was transferred to a semimicro cuvette that contained 1.1 ml of 0.5 M Tris buffer and 1 mM EDTA (pH 8.9). DTNB (50 μl) was added from a stock solution in methanol, and the absorbance was read immediately at 412 nm.

Analysis of NPSH concentrations in the liver. Weighed whole livers from four unexposed rats were homogenized at 0° in 20 ml of 0.02 M EDTA. An aliquot of the homogenate was diluted with a 4-fold excess of EDTA, and 0.875 ml of this suspension was mixed with 0.125 ml of 50% TCA. The precipitate was removed by centrifugation. A 0.1-ml aliquot of the supernatant fraction was transferred to a cuvette containing 1.4 ml of Tris/EDTA buffer, and 50 μl of DTNB was added. The absorbance was read immediately at 412 nm.

The concentrations of NPSH groups in the tissue samples were calculated using a molar absorptivity of 13.6×10^3 M^{-1} cm^{-1} for 2-nitro-5-mercaptobenzoate, which was determined under the reaction conditions using a GSH solution of known concentration. The calculated molar absorptivity is in agreement with the value reported by Sedlak and Lindsay [20].

RESULTS

Oxidation of FA by nasal mucosal homogenates. The specific activities for the NAD^+-dependent oxidation of FA by homogenates of the nasal mucosa of unexposed rats and of rats exposed to FA (15 ppm, 6 hr/day, 10 days) were calculated over the range of substrate (FA or *S*-hydroxymethylglutathione) concentrations. In the absence of GSH, the substrate concentration was considered to be equal to the total concentration of FA in the cuvette. In the presence of 1.5 mM free GSH, however, the concentrations of the adduct, *S*-hydroxymethylglutathione, and of FA were assumed to be equal to one-half of the total FA in the cuvette, in accordance with the fact that the dissociation constant of the adduct is 1.5 mM [1].

The specific activities for catalysis of FA oxidation by respiratory and olfactory mucosal homogenates from unexposed rats are depicted in Fig. 1 in the form of Hofstee [19] plots. In the absence of GSH, the plots appeared to be linear (open circles), implying that FA oxidation was catalyzed primarily or exclusively by a single enzyme under the reaction conditions. As this enzyme did not require GSH, it is presumably an isozyme of AldDH. In the presence of 1.5 mM free GSH, however, the Hofstee plots were curved (filled circles), suggesting the presence of at least one additional enzyme that is capable of catalyzing the oxidation of FA in the respiratory and olfactory mucosa of the rat. This enzyme activity was only expressed in the presence of GSH and, hence is presumably due to FDH. Very similar plots to those shown in Fig. 1 were obtained when the tissue was obtained from rats exposed to FA (15 ppm, 6 hr/day, 10 days).

The lines drawn in the figure were calculated by computer, assuming that conventional Michaelis–Menten kinetics are applicable to the enzymes involved. Table 1 summarizes the computer-estimated values of V_{max} and K_m for FDH and AldDH, based on the assumption that only two enzymes are catalyzing the NAD^+-dependent oxidation of FA under the reaction conditions. The specific activities of FDH and of AldDH in unexposed rats and in rats exposed

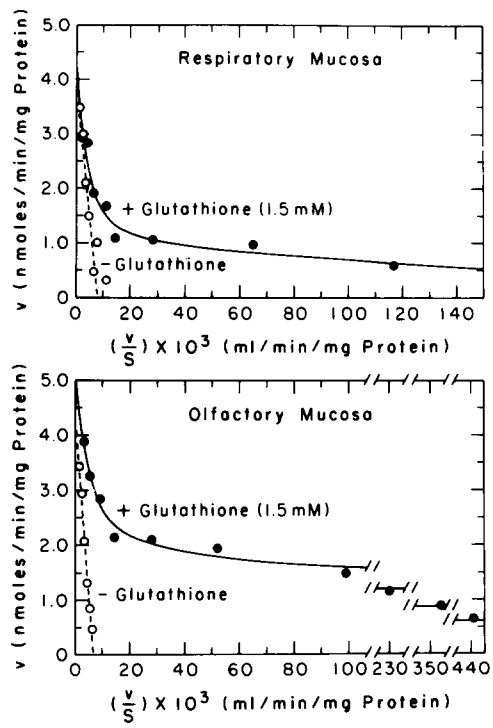

Fig. 1. Plots of the specific activity vs the ratio of the specific activity to the substrate concentration for the oxidation of formaldehyde by NAD^+ catalyzed by homogenates of the respiratory and olfactory mucosa from control male Fischer-344 rats. In the absence of GSH, the substrate concentration was assumed to be the total FA concentration. In the presence of 1.5 mM free GSH, the substrate concentration was assumed to be half the total FA concentration (see text). Lines were calculated by computer assuming the overall rate to be described by one Michaelis–Menten term in the absence of GSH and two in its presence.

Table 1. Estimated kinetic constants for the NAD^+-dependent oxidation of *S*-hydroxymethylglutathione or formaldehyde to *S*-formylglutathione or formate catalyzed by homogenates of respiratory and olfactory mucosa from Fischer-344 rats*

Enzyme	Tissue†	V_{max}‡ [nmoles·min^{-1}·(mg protein)$^{-1}$]	K_m (μM)
		Control	
FDH	Respiratory mucosa	0.90 ± 0.24	2.6 ± 2.6
	Olfactory mucosa	1.77 ± 0.12	2.6 ± 0.5
AldDH	Respiratory mucosa	4.07 ± 0.35	481 ± 88
	Olfactory mucosa	4.39 ± 0.14	647 ± 43
		Exposed§	
FDH	Respiratory mucosa	0.85 ± 0.08	2.0 ± 1.0
	Olfactory mucosa	1.84 ± 0.18	4.0 ± 0.8
AldDH	Respiratory mucosa	2.86 ± 0.10	445 ± 33
	Olfactory mucosa	3.96 ± 0.06	533 ± 17

* Assay conditions: 0.1 M sodium pyrophosphate (pH 8.0) containing 0.67 mM NAD^+ and 1 mM pyrazole, 25°; activity of FDH was measured in the presence of 1.5 mM free GSH.

† Mucosal samples from eight rats were combined for the measurement of the enzyme activities.

‡ Estimates shown are mean ± S.D., calculated by nonlinear regression analysis of the initial velocity on the substrate concentration; df = 4.

§ Rats were exposed to 15 ppm of formaldehyde (6 hr/day, 10 days).

repeatedly to 15 ppm of FA were approximately the same. The specific activity and apparent K_m of FDH were also determined in the soluble fraction of a rat liver homogenate: values obtained in liver were V_{max}: 2.0 ± 0.3 nmoles·min^{-1}·(mg protein)$^{-1}$ and K_m: 4.5 ± 1.9 μm, showing that the specific activities of FDH in homogenates of the nasal mucosa and of liver were similar in magnitude. However, due to the fact that the liver supernatant fraction did not contain microsomes, the specific activities of FDH calculated for the nasal mucosa and liver are not exactly comparable. The specific activity and apparent K_m of FDH in liver are in satisfactory agreement with values reported by Goodman and Tephly [16] using Holtzmann rats. Koivusalo *et al.* [4] have reported that the V_{max} and K_m of highly purified FDH

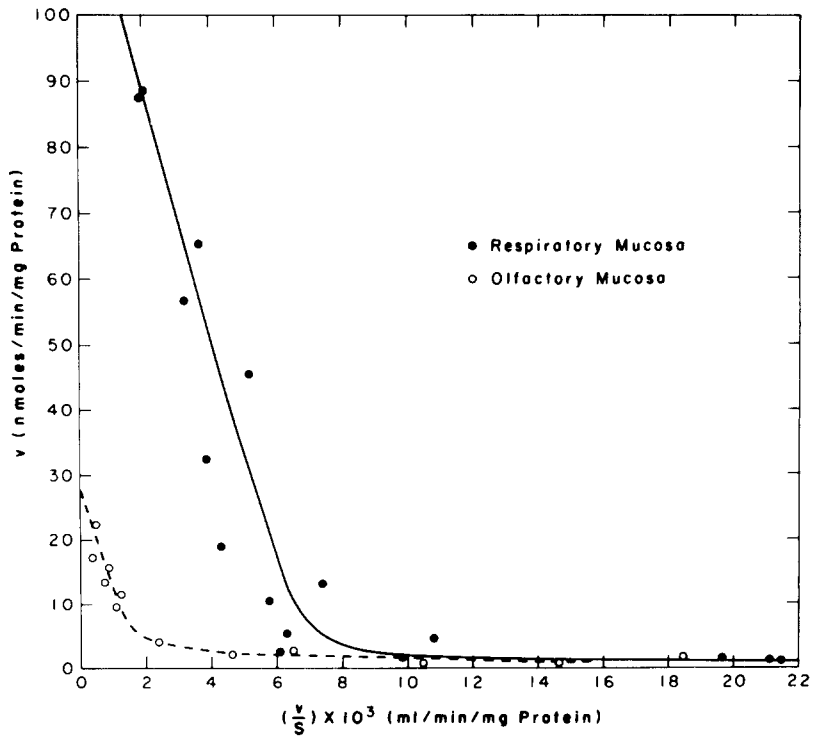

Fig. 2. Plots of the specific activity vs the ratio of the specific activity to the substrate concentration for the oxidation of acetaldehyde by NAD^+ catalyzed by homogenates of the respiratory and olfactory mucosa from control male Fischer-344 rats. Lines were calculated by computer, assuming the overall rate to be described by two Michaelis–Menten terms.

from rat liver are 2.70 μmoles · min^{-1} · (mg protein)$^{-1}$ and 0.92 μM respectively.

The most notable difference between FDH and AldDH shown in Table 1 is the much smaller apparent K_m of the former enzyme with respect to its substrate, *S*-hydroxymethylglutathione, than of the latter enzyme with respect to its substrate, formaldehyde. These data suggest that, at low concentrations of FA, nasal mucosal FDH should be much more effective than AldDH as a catalyst of FA oxidation, provided the concentration GSH is high enough to ensure that the substrate of FDH is formed. The specific activity of FDH in the olfactory mucosa was approximately twice that in the respiratory mucosa, but the specific activity of AldDH was similar in both tissue samples.

Oxidation of AA by nasal mucosal homogenates. The specific activities for catalysis of AA oxidation are depicted in Fig. 2. The oxidation of AA was apparently catalyzed by at least two enzymes in both tissues. One of the two isozymes (denoted by I in Table 2) has a large K_m with respect to acetaldehyde. The K_m of the other isozyme (denoted by II in Table 2) could not be precisely determined in the crude homogenates. Although the kinetic constants for this isozyme are not known with certainty, its existence in the masal mucosa appears to be well-established by the studies at low AA concentrations. Multiple forms of AldDH have been identified previously in rat liver, and the isozymes of AldDH are known to differ with respect to their substrate specificities [3]. The present observations suggest that the nasal mucosa may contain a multiplicity of isozymes of AldDH.

The specific activity of the higher K_m isozyme of AldDH in the respiratory mucosa was approximately five to eight times greater than that in the olfactory mucosa (Table 2). The specific activity of the lower K_m isozyme of AldDH with respect to AA oxidation appeared to be approximately the same in both tissues. Exposure to 1500 ppm of AA (6 hr/day, 5 days) did not induce a substantial change in the activities of the isozymes of AldDH (Table 2).

Concentrations of NPSH in the respiratory mucosa. Inasmuch as reaction of GSH with FA is essential for oxidation of FA by FDH, it was of interest to determine the concentration of NPSH groups in the respiratory mucosa and to investigate the effects of exposure to FA on the NPSH concentration. The NPSH concentrations in the respiratory mucosa of control and exposed (15 ppm, 6 hr/day, 9 days) rats were 2.60 ± 0.47 μmoles/g and 2.99 ± 0.23 μmoles/g respectively. (Values are mean ± S.D. for four rats per group, analyzed individually.) The difference between the groups was not statistically significant ($0.25 > P > 0.1$; two-tailed *t*-test). These concentrations are approximately half of that found in the livers of unexposed rats: 5.77 ± 0.34 μmoles/g. The concentration of NPSH in liver is in agreement with the concentration of GSH reported by Chasseaud [21].

DISCUSSION

Homogenates of the respiratory and olfactory mucosa have similar specific activities of FDH and of an isozyme of AldDH that catalyzes the oxidation of FA. The K_m of the latter enzyme with respect to FA is much larger than that of FDH with respect to *S*-hydroxymethylglutathione. These data are not directly interpretable in terms of *in vivo* K_m values. However, due to the low concentration of FA that exists normally in tissues (i.e. between 0.05 and 0.5 μmole/g [22]), most of which is reversibly-bound to cellular nucleophiles, it is very unlikely that the concentration of FA that is available for oxidation by AldDH would approach the apparent K_m of the enzyme unless AldDH and the sources of endogenous FA were tightly compartmentalized. Notwithstanding this possibility, the results of the kinetic studies and the analyses of NPSH in the nasal mucosa indicate that the NAD$^+$-dependent oxidation of FA in this tissue is mainly catalyzed by FDH.

Although the oxidation of FA occurred at approximately the same rates in respiratory and olfactory mucosal homogenates, the oxidation of AA occurred

Table 2. Estimated kinetic constants for the NAD$^+$-dependent oxidation of acetaldehyde to acetate catalyzed by homogenates of respiratory and olfactory mucosa from Fischer-344 rats*

AldDH isozyme	Tissue†	V_{max}‡ [nmoles · min^{-1} · (mg protein)$^{-1}$]	K_m‡ (mM)
		Control	
I	Respiratory mucosa	128 ± 10	20 ± 3
	Olfactory mucosa	28 ± 4	22 ± 7
II	Respiratory mucosa	0.8 ± 0.9	$3 \times 10^{-4} \pm 9 \times 10^{-2}$
	Olfactory mucosa	2.2 ± 0.6	$1 \times 10^{-1} \pm 5 \times 10^{-2}$
		Exposed§	
I	Respiratory mucosa	140 ± 7	21 ± 2
	Olfactory mucosa	17.8 ± 0.6	14 ± 1
II	Respiratory mucosa	1.5 ± 1.1	$1 \times 10^{-1} \pm 2 \times 10^{-1}$
	Olfactory mucosa	1.3 ± 0.2	$7 \times 10^{-2} \pm 3 \times 10^{-2}$

* Assay conditions: 0.1 M sodium pyrophosphate (pH 8.0) containing 0.67 mM NAD$^+$ and 1 mM pyrazole, 25°.

† Mucosal samples from eight rats were combined for the measurement of the enzyme activities.

‡ Estimates shown are mean ± S.D., calculated by nonlinear regression analysis of the initial velocity on the substrate concentration; df = 13.

§ Rats were exposed to 1500 ppm of acetaldehyde (6 hr/day, 5 days).

considerably more rapidly in homogenates of the former than of the latter tissue. The difference in specific activities of the two tissues is likely due to a high K_m isozyme of AldDH, the specific activity of which is greater in the respiratory than in the olfactory mucosa. Another isozyme of AldDH is also present in the nasal mucosa, but the specific activity of this isozyme was approximately the same in both tissue preparations. The lower K_m isozyme of AldDH with respect to AA oxidation may be the same enzyme as that which catalyzes the oxidation of FA. However, proof of this hypothesis will require kinetic studies using the pure isozyme.

These studies have shown that FDH and at least two isozymes of AldDH are present in the respiratory and olfactory mucosa of the rat. In addition to their normal roles in intermediary metabolism, these enzymes may also function as defense mechanisms helping to minimize or prevent toxic injury to masal tissues resulting from exposure to airborne aldehydes or to aldehyde precursors [12, 13]. The role of FDH and AldDH as detoxifying enzymes during *in vivo* exposures to FA and AA will be the subject of a future investigation.

REFERENCES

1. L. Uotila and M. Koivusalo, *J. biol. Chem.* **249**, 7653 (1974).
2. R. A. Deitrich, *Biochem. Pharmac.* **15**, 1911 (1966).
3. H. Weiner, in *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. H. Weiner and B. Wermuth), pp. 1–9. Alan R. Liss, New York (1982).
4. M. Koivusalo, T. Koivula and L. Uotila, in *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. H. Weiner and B. Wermuth), pp. 155–68. Alan R. Liss, New York (1982)
5. W. D. Kerns, K. L. Pavkov, D. J. Donofrio, M. M. Connell, R. I. Mitchell, E. J. Gralla and J. A. Swenberg, *Cancer Res.* **43**, 4382 (1983).
6. V. J. Feron, A. Kruysse and R. A. Woutersen, *Eur. J. Cancer clin. Oncol.* **18**, 13 (1982).
7. H. d'A. Heck, T. Y. Chin and M. Casanova-Schmitz, in *Formaldehyde Toxicity* (Ed. J. E. Gibson), pp. 26–37. Hemisphere Publishing Corp., Washington, DC (1983).
8. R. M. David and H. d'A. Heck, *Toxicologist* **3**, A331 (1983).
9. J. A. Swenberg, E. A. Gross, J. Martin and J. A. Popp, in *Formaldehyde Toxicity* (Ed. J. E. Gibson), pp. 132–47. Hemisphere Publishing Corp., Washington, DC (1983).
10. L. M. Appelman, R. A. Woutersen and V. J. Feron, *Toxicology* **23**, 293 (1982).
11. R. E. Albert, A. R. Sellakumar, S. Laskin, M. Kuschner, N. Nelson and C. A. Snyder, *J. natn. Cancer Inst.* **68**, 597 (1982).
12. J. Ashby and P. A. Lefevre, *Carcinogenesis* **3**, 1273 (1982).
13. A. R. Dahl and W. M. Hadley, *Toxic. appl. Pharmac.* **67**, 200 (1983).
14. C. S. Barrow and W. H. Steinhagen, *Fund. appl. Toxic.* **2**, 33 (1982).
15. G. L. Peterson, *Analyt. Biochem.* **83**, 346 (1977).
16. J. I. Goodman and T. R. Tephly, *Biochim. biophys. Acta* **252**, 489 (1971).
17. C. M. Metzler, G. L. Elfring and A. F. McEwen, *Biometrics* **30**, 562 (1974).
18. C. M. Metzler, G. L. Elfring and A. J. McEwen, *A User's Manual for NONLIN and Associated Programs.* Upjohn Co., Kalamazoo, MI (1976).
19. B. H. J. Hofstee, *Science* **136**, 329 (1952).
20. J. Sedlak and R. H. Lindsay, *Analyt. Biochem.* **25**, 192 (1968).
21. L. F. Chasseaud, in *Glutathione: Metabolism and Function* (Eds. I. M. Arias and W. B. Jakoby), pp. 77–114. Raven Press, New York (1976).
22. H. d'A. Heck, E. L. White and M. Casanova-Schmitz, *Biomed. Mass Spectrom.* **9**, 347 (1982).