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TISSUE AND SUBCELLULAR DISTRIBUTION OF MAMMALIAN ALDEHYDE-OXIDIZING CAPACITY*

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Abstract—Tissue and subcellular distribution of aldehyde-oxidizing capacity has been determined in several mammalian species, by the use of indole-3-acetaldehyde and nicotinamide adenine dinucleotide (NAD) as substrates. Activity, dependent upon added NAD, was found in liver, adrenal, intestine, kidney, ovary, testis, adipose tissue, uterus, heart, lung, brain, spleen, skeletal muscle, seminal vesicles, and bladder. Liver contained the greatest amount of activity. Female Sprague–Dawley rats had a higher level than male Sprague–Dawley rats of aldehyde-oxidizing capacity inliver and kidney.

The soluble and mitochondrial fractions of all rat tissues studied contained measurable amounts of the activity. The mitochrondrial and supernatant enzymes of rat liver appear to be different proteins, as determined by Sephadex G-200 column chromatography. In addition, it was confirmed that rat liver microsomes contain aldehyde-oxidizing capacity.

The results may be of importance in studies with such compounds as epinephrine, 5-hydroxytryptamine, choline, histamine, and ethanol, among others, since all of these substances can be converted, *in vivo*, to aldehydes that may undergo further oxidation.

VARIOUS compounds, such as ethanol, serotonin, tryptamine, norepinephrine, histamine, choline, and vitamin A,[1–4] are oxidized via aldehyde intermediates. However, comparatively little information is available concerning the tissue content or subcellular localization of the enzymes responsible for the oxidation of these aldehydes.

A nonspecific aldehyde dehydrogenase was first purified from liver acetone powder by Racker[5] and has been further studied by others.[6, 7] Most subsequent investigations have been limited to a few tissues or a few substrates. Thus, betaine aldehyde dehydrogenase is present in liver mitochondria and supernatant,[3] formaldehyde dehydrogenase was purified from beef liver[8] and found in rat liver mitochondria,[9] Tietz *et al.* reported the presence of an aldehyde dehydrogenase in liver microsomes,[10] and Büttner has found that aldehydes are oxidized in the presence of the supernatant fraction of liver and kidney as well as by the mitochondria of these tissues.[11] Kidney supernatant has long been used as a source of aldehyde dehydrogenase.[12] Walkenstein and Weinhouse have observed the oxidation of aldehydes by rat kidney and liver as well as by pigeon liver.[13] Maxwell and Topper have studied a steroid-sensitive aldehyde dehydrogenase from rabbit liver.[14] Brady and co-workers have evidence for both a NAD- and a NADP-dependent enzyme in brain tissue.[15, 16] As described in another paper, a

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NAD-dependent aldehyde dehydrogenase from brain mitochondria has been purified and studied in this laboratory.*

D-Glyceraldehyde-3-phosphate dehydrogenase[17] and succinic semialdehyde dehydrogenase[18] are relatively more specific and have been better characterized than the nebulous group of enzymes discussed above. Recently, an α-keto aldehyde dehydrogenase has been found in sheep liver.[19]

This report is concerned with the tissue and subcellular localization of aldehyde-oxidizing capacity of mammals. It also demonstrates that, as in the case of liver, most of this activity is attributable to NAD-linked dehydrogenases.[20]

METHODS

All chemicals were of high purity. Recrystallized indole-3-acetic acid, used as a standard, was found to be chromatographically homogeneous and to have the same melting point as that reported in the literature. In addition, ultraviolet and fluorescent spectra were used as a further check on the purity of the compound.

Indole-3-acetaldehyde bisulfite was obtained from Mann Biochemicals. Various lots were at least 90 per cent pure as determined by enzymatic assay.[7] After recrystallization by the method of Gray,[21] it was essentially pure. Free indole-3-acetaldehyde was obtained from its bisulfite compound by a modification of the method of Gray.[21] It was found by extraction of the aldehyde into ether from solutions of various pH values, that the compound was 50 per cent dissociated at pH 6·9 and completely dissociated at pH 9·6. Therefore, the pH of 1 volume of a 10^{-2} M solution of indole-3-acetaldehyde bisulfite was brought to pH 10·2–10·5 by addition of a few drops of saturated Na_2CO_3. This solution was immediately extracted five successive times with 1 volume of ether for each extraction. The ether extracts were combined and cooled to below 0° so that most of the water carried over was frozen out. The ether was then washed three successive times with 1 volume of water and the aqueous fractions discarded. The ether was removed under a stream of N_2, the oily aldehyde dissolved in N_2-deaerated water and used immediately. The aldehyde content was determined enzymatically.[7] At high concentrations of the free aldehyde, i.e. above 0·05 M, a white precipitate formed rapidly even at 0°. Such a precipitate was always found after the material had been frozen overnight.

Fluorometric assays were carried out with a Turner fluorometer, model 110. Occasional checks were made with an Aminco-Bowman spectrophotofluorometer. All visible and ultraviolet spectrophotometric measurements were made with a Gilford model 2000 attachment for the Beckman DU monochromator equipped to maintain a constant temperature of 25° in the cuvette compartment.

Tissues were homogenized in 0·25 M sucrose at 0° with a Teflon pestle and glass homogenizer. Mitochondria were prepared from liver, lung, testis, kidney, heart, and adipose tissue by the usual techniques[22] except that they were sedimented at 10,000 *g* for 10 min to minimize mitochondrial contamination of the supernatant fraction. Brain mitochondria were prepared in 0·25 M sucrose to the "crude" stage as previously described.[23] Microsomes were sedimented at 100,000 *g* for 1 hr. Sonic disruption of various fractions was carried out at 0° with a Branson model 125 sonifier at a setting of 3 which gave 3·5 A. A $\frac{3}{8}$-in. step horn was used. The sonic pressure was

* V. G. Erwin and R. A. Deitrich, *J. biol. Chem.* In press.

3 g, determined as recommended by the manufacturer. The time of sonic action was 3 min in 30-sec periods, interrupted by several minutes to allow cooling of the material.

Incubations were carried out in screw-top tubes at 30° for 10 min in 0·05 M K–Na phosphate buffer of pH 7·4, neutralized NAD and the bisulfite compound of indole-3-acetaldehyde or free indole-3-acetaldehyde present in the amounts indicated with each table or figure. The final volume was 1 ml. The pH of the incubation medium remained constant at 7·4 in all experiments. Controls routinely consisted of tubes stopped at zero time with all components added. Occasionally, additional controls were utilized that consisted of all components added except aldehyde; or added indole-3-acetic acid with and without added aldehyde. Experiments in which no NAD was added are discussed below.

In determining the rate of indole-3-acetaldehyde oxidation in various tissues from Sprague-Dawley rats, at least four concentrations of the aldehyde ranging from 0·4 to 9·6 mM were used and the data plotted by the conventional double reciprocal method to estimate the maximal velocities. The aldehyde concentration covered at least a fourfold concentration range for each experiment. The amount of NAD necessary for maximal activity was determined for each tissue. This amount ranged from 1 to 4 mM NAD.

Indole-3-acetic acid was determined by a modification of the procedure of Weissbach *et al.*[24] At the end of the incubation period, 0·2 ml of 10% $ZnSO_4$, and 0·1 ml of 1 N NaOH were added to the incubation mixture. Sufficient water to make the final volume 2·0 ml and approximately 5 ml of ethylene dichloride were added. The tubes were capped with Teflon-lined screw-caps and shaken for 3 min to remove unreacted indole-3-acetaldehyde. After centrifugation (2000 *g*, 20 min), a 1-ml aliquot of the upper aqueous layer was added to a mixture of 15 ml ethylene dichloride and 0·1 ml of 6 N HCl in a 50-ml glass-stoppered tube. After 3-min shaking, a 10-ml aliquot was transferred to 3 ml of 0·5 M K–Na phosphate buffer, pH 7·4, and shaken for 3 min. The fluorescence of the buffer layer was determined. Standards of indole-3-acetic acid were carried through the same procedure. Recovery was quantitative from solutions in the absence of tissue and 92 $\pm$ 8 per cent when tissue was present. No evidence of enzymatic destruction of indole-3-acetic acid was found.

Chloroform, as used in the original procedure of Weissbach *et al.*,[24] caused some quenching of the fluorescence of indole-3-acetic acid. Exposure of a chloroform-saturated solution of indole-3-acetic acid to intense u.v. light for 1 min resulted in the formation of new fluorescent compounds with maximal emission at 485 mμ. In the Turner fluorometer, a filter instrument, a tremendously increased fluorescence reading resulted. In the Aminco-Bowman spectrophotofluorometer only a decrease in the fluorescence at 360 mμ (uncorrected) is apparent along with the new peak of emission at 485 mμ. Although the measured fluorescence at 485 mμ is only a fraction of that at 360 mμ, the Turner fluorometer is much more sensitive at the longer wavelength. The reaction could be made quantitative and increase the sensitivity of the assay by about fourfold. All samples of chloroform commercially obtained, as well as freshly purified and redistilled solvent, gave this reaction. The reaction can also be observed to take place on chromatographic paper.

Indole-3-acetic acid was identified as the product of the enzymatic reaction by fluorescence spectra, u.v. spectra, behavior in the extraction procedure, and paper

chromatography in two solvent systems: 1-butanol:NH_4OH:H_2O (90:15:5) and 1-butanol:acetic acid:H_2O (4:1:1).

Protein was determined by the biuret method with bovine serum albumin as the standard. Succinic dehydrogenase was assayed by the method of Arrigoni and Singer.[25] Since indole-3-acetyl coenzyme A and indole-3-acetyl phosphate were possible products, the hydroxylamine assay for such compounds was carried out in some experiments.[26] No evidence for their presence was found.

A Sephadex* G-200 column was prepared as follows. Dry Sephadex G-200 was passed through a No. 200 sieve as recommended by Ackers.† The material remaining behind was suspended in a large volume of 0·05 M K–Na phosphate buffer, pH 7·4,

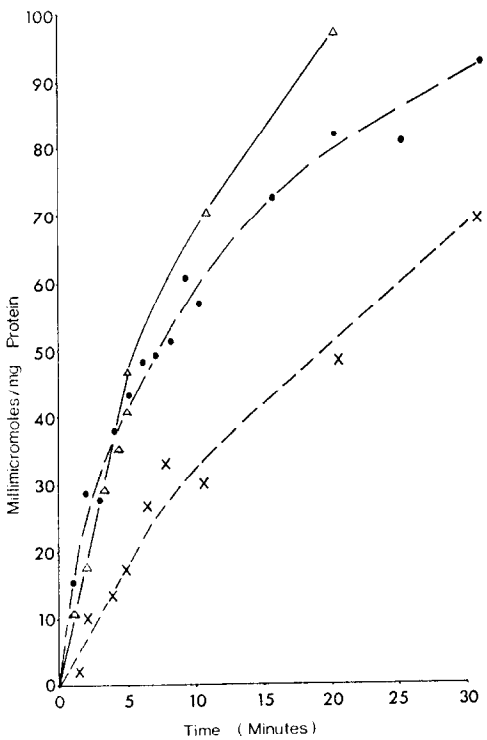

FIG. 1. Production of indole-3-acetic acid as a function of time.
Conditions: 2 × 10^{-3} M each NAD and indole-3-acetaldehyde bisulfite, 0·05 M K–Na PO_4, pH 7·4, 30°; ×— — —× rat liver mitochondria, ●— — —● rat liver homogenate, △———△ rat liver supernatant, prepared as described in the text.

and allowed to swell for several days. Fines were then decanted. The material was packed by gravity in a refrigerated column, 40 × 2·5 cm. The column was calibrated with the following proteins: beef serum albumin, hemoglobin, liver catalase, yeast alcohol dehydrogenase, glutamic dehydrogenase, and γ-globulin. Protein was located in the effluent by the absorption at 215 mμ or 280 mμ. The appropriate assay

* Pharmacia Fine Chemicals Inc., 501 Fifth Ave., New York, N.Y.

† Personal communication from Dr. G. Ackers, Johns Hopkins University School of Medicine.

was also carried out in the case of the enzymes. Rat liver mitochondrial aldehyde-oxidizing activity was obtained in a soluble form by sonic disruption as described above. Rat liver supernatant enzyme was obtained from a homogenate of 20 or 30 per cent by centrifugation at 100,000 *g* for 1 hr. The data were treated as described by Ackers.[27]

RESULTS

For those experiments in which it was important to obtain an estimate of the absolute rate of indole-3-acetic acid production, the free indole-3-acetaldehyde was used. In other experiments, where a relative figure was sufficient, the indole-3-acetaldehyde bisulfite was the substrate. Although the free aldehyde is relatively easy to prepare, it is unstable, as indicated by the rapid formation of a white precipitate and declining aldehyde concentration over a period of days. The rate of production of indole-3-acetic acid from the free aldehyde was generally greater than the rate of production of the acid from the aldehyde bisulfite. This was most apparent with the liver, where rates of production of the acid are severalfold higher when the free aldehyde was used.

As shown in Fig. 1, the reaction is linear with time for only a short period when liver homogenates are used. This was less apparent with other tissues except for brain, the curve for which closely resembles that for liver. Presence of nicotinamide had no effect, and the substrates present were in large amounts. Incubation of tissues with no substrates or either substrate alone for 10 min at 30°, followed by another 10-min incubation with the complete reaction mixture, always resulted in diminished activity. This would indicate that the enzyme system is very labile under these conditions. Similar results are obtained when the free aldehyde or the bisulfite compound was the substrate.

The production of indole-3-acetic acid by whole rat liver homogenates is essentially linear, with protein below 1·5 mg protein per tube, provided that sufficient concentrations of aldehyde are added. Other rat tissue homogenates studied also gave a linear relationship with protein added. In these cases, at least 5 mg protein per tube could be used. Similar results were obtained when either the free aldehyde or its bisulfite compound was the substrate.

Table 1 indicates the wide distribution of the enzyme in various tissues of the rat. It should be emphasized that the values of the first two columns are extrapolated maximal rates. However, since the enzyme is unstable and the reaction velocity begins to decline at 10 min, the rate would be somewhat greater if taken at a shorter time interval.

There is a demonstrable sex difference when kidney or liver is used. The variability from animal to animal and the difficulty in obtaining V_{max} values in whole homogenates results in the large standard deviations seen in Table 1. There was no sex difference apparent with any of the other tissues used.

Skeletal muscle, spleen, bladder, and seminal vesicles from the rat have been found to have small aldehyde-oxidizing ability. Although no enzymatic activity could be detected in serum or whole blood, this may be a reflection of the high blanks obtained with these materials. The ability to catalyze the oxidation of indole-3-acetaldehyde bisulfite to indole-3-acetic acid by NAD was also found in rabbit, monkey, and dog in liver, brain, lung, and kidneys; also in beef, sheep, and pig liver. The distribution of

TABLE 1. INDOLE-3-ACETALDEHYDE-OXIDIZING CAPACITY IN VARIOUS TISSUES OF THE RAT

Tissue	V_{max} (mμmoles/mg protein/10 min)							
	Male	S.D. or range	No. of expts.	Female	S.D. or range	No. of expts.	PO_4 stim.	NAD omitted
Liver*	292·0	±52·0	10	496·0	±109·0	8	2·35	0
Kidney†	41·0	±13·7	5	89·9	± 14·7	5	1·43	0·01
Heart	10·0	± 4·0	6	8·2	± 2·0	7	0·97	0
Small intestine	16·7	15·0–18·5	3	16·2	11·2–22·2	4	1·68	0·01
Gonads	30·2	21·1–43·3	3	20·2	15·4–20·8	4	1·40	0·02
Adipose	6·1	4·1–7·4	3	5·6	3·8–9·7	4	1·60	0
Uterus				43·8	21·0–58·8	4	1·10	
Lung	6·6	3·3–8·9	3	6·3	5·7–8·3	5	2·33	0
Brain	10·3	6·7–16·7	4	9·9	6·1–14·8	5	1·13	0·26
Adrenal	32·7	20·5–50·0	3	28·8	21·7–40·3	4		

Conditions. Tissues were homogenized at 0° in 0·25 M sucrose. Aliquots were incubated with 0·05 M K–Na phosphate buffer, pH 7·4, with an optimal amount of NAD as determined for each tissue, and at least four concentrations of indole-3-acetaldehyde. The data were plotted by conventional double reciprocal plots and V_{max} calculated as mμmoles of indole-3-acetic acid per mg protein per 10 min; PO_4 stim. = the ratio of activity in the presence of 0·25 M K–Na phosphate buffer, pH 7·4, to the activity in the presence of 0·005 M K–Na phosphate buffer, pH 7·4; NAD omitted = the ratio of activity in the absence of added NAD to the activity in the presence of added NAD after dialysis of 5 ml of the homogenate against 1 liter of 0·25 M sucrose for 3 hr with two changes of dialyzing fluid. The values for phosphate stimulation were carried out in the presence of 10^{-3} M indole-3-acetaldehyde bisulfite, 10^{-3} M NAD; those for NAD stimulation were carried out in the presence of 10^{-3} M indole-3-acetaldehyde bisulfite, 0·05 M K–Na phosphate, pH 7·4, and 10^{-3} M NAD where required.

* $< 0·05$ for difference between male and female.
† $< 0·02$ for difference between male and female.

the enzyme activity in these tissues closely parallels that found in the rat. Rabbit liver is the most active tissue and contains roughly three times the amount of activity found in rat liver. Most of the activity in rabbit liver is present in the soluble portion of the cell. Also reflected in Table 1 is the finding that high concentrations of phosphate slightly stimulate the activity in all tissue except heart, uterus, and possibly brain. This cannot be duplicated by NaCl or KCl in concentrations that result in the same ionic strength as the phosphate buffer employed. Use of arsenate in the same concentrations as phosphate results in almost identical rates. This indicates, in addition to the small amount of activity found in skeletal muscle which is known to contain large amounts of D-glyceraldehyde-3-phosphate dehydrogenase,[17] that the activity is probably not due to this enzyme. Also shown is the ratio of the rate in the absence of NAD to the rate in the presence of NAD. NADP caused some stimulation (8 per cent of that due to NAD with liver, 28 per cent with kidney, 65 per cent with gut, 10 per cent with lung, 35 per cent with heart and brain, and less than 2 per cent with fat and testes); however, the increase was never additive with that afforded by NAD alone. This may be due to the presence of a phosphatase capable of conversion of NADP to NAD,[28] or to a NADP-linked aldehyde dehydrogenase.

Table 2 gives the apparent K_m values for indole-3-acetaldehyde with liver, kidney,

TABLE 2. APPARENT K_m VALUES FOR INDOLE-3-ACETALDEHYDE WITH SEVERAL RAT TISSUES

Tissue	Apparent $K_m \times 10^3$		P*
	Male (molar)†	Female (molar)	
Liver	1·4 ± 0·4 (7)‡	4·5 ± 1·8 (7)	< 0·05
Kidney	4·7 ± 2·1 (5)	0·9 ± 0·2 (5)	< 0·05
Heart	0·8 ± 0·4 (5)	0·7 ± 0·2 (5)	

The experiments were carried out as detailed in Table 1. The apparent K_m values were calculated from the usual double reciprocal plots.

* P value for difference between the mean values for male and female.

† Standard deviation.

‡ Number of experiments.

and heart. These values refer to the system as a whole, which includes several enzymes that catalyze the oxidation of the aldehyde and should not be ascribed to any individual enzyme.

Substrate inhibition was observed above 5 mM concentrations of the free aldehyde with liver homogenates. Since some of the apparent K_m values are also of this order, extrapolation to V_{max} values is greater than is ideal.

Table 3 illustrates the distribution of indole-3-acetaldehyde-oxidizing capacity between the mitochondrial and supernatant fractions of several tissues. These values were obtained with 2 mM each NAD and indole-3-acetaldehyde bisulfite and 0·05 M K–Na phosphate buffer, pH 7·4. Essentially identical results were obtained when the free aldehyde was used in several experiments. The distribution of activity between supernatant and mitochondrial fractions of rat liver was not altered by preparation of

the mitochondria in 0·88 M sucrose. Also, most of the mitochondria could be sedimented at 2200 *g* for 10 min in 0·25 M sucrose. A comparison of the supernatant and mitochondrial enzyme is given in Table 4. These data indicate that the two enzyme systems are different.

TABLE 3. DISTRIBUTION OF INDOLE-3-ACETALDEHYDE-OXIDIZING CAPACITY BETWEEN MITOCHONDRIA AND THE SUPERNATANT FRACTION IN VARIOUS TISSUES OF THE RAT

Tissue	Aldehyde-oxidizing capacity			Succinic dehydrogenase		
	Supernatant (% act.)	Mitochondria (% act.)	(% rec.)	Supernatant (% act.)	Mitochondria (% act.)	(% rec.)
Liver (10)*	86·6	13·4	92·8	4·1	95·9	96·4
Kidney (4)	79·8	20·2	98·9	16·3	83·7	86·7
Testis (4)	46·7	53·2	93·7	0·5	99·5	100·0
Heart (4)	86·0	14·0	85·5	9·6	90·4	77·0
Brain (4)	60·4	39·6	77·8	4·5	95·5	100·0
Adipose (4)	86·5	13·5	86·3	58·2	41·8	48·0

Conditions. 2×10^{-3} M each NAD and indole-3-acetaldehyde bisulfite, 0·05 M K-Na PO_4, pH 7·4; 30°, 10 min. Mitochondria and supernatant prepared as described in the text. Percentage of activity (% act.) is calculated with *total recovered* activity as 100%. Per cent recovered (% rec.) is the percentage of the total amount of activity recovered from the whole homogenate after nuclei and cell debri had been removed.

* Number of experiments.

TABLE 4. COMPARISON OF RAT LIVER MITOCHONDRIAL AND SUPERNATANT ALDEHYDE-OXIDIZING CAPACITY

Procedure	Mitochondria	Supernatant
Increased phosphate concentration*	155% (3)† (146–172)‡	+352% (3) (318–416)
Stability: 1/2 life at 0°	13 days	3 days
1/2 life at −20°	21 days	11 days
Elution volume from 40 × 2·5 cm Sephadex G-200 column	111 ml (3) (101–120)	144 ml (3) (138–148)

Preparation of mitochondria and supernatant fractions carried out as described in the text. The assay was carried out as described in text and Table 3. Stability of the enzymes was determined at −20° with thawing of entire sample to obtain an aliquot. Sephadex columns were prepared and calibrated as described in text.

* Activity determined in presence of 0·005 M phosphate buffer and 0·25 M phosphate, buffer pH 7·4. Values are per cent of activity, taking the activity in the presence of 0·005 M phosphate as 100 per cent. NAD and indole-3-acetaldehyde bisulfite, 2 mM each, were present.

† Number of experiments.

‡ Range of values.

As shown in Table 5, the aldehyde-oxidizing activity associated with liver mitochondria cannot be released by repeated washing with 0·25 M sucrose, but can be released by sonication in 0·25 M sucrose or by freezing and thawing in 1·15% KCl. These results agree with a previous report by Glenn and Vanko.[3] Mitochondrial aldehyde dehydrogenase was partially purified as outlined by these workers. This

preparation catalyzes the reduction of NAD in the presence of formaldehyde, indole-3-acetaldehyde, and propionaldehyde.*

The presence of an aldehyde-oxidizing enzyme in the microsomal fraction of liver homogenates is also illustrated in Table 5. This activity is not removed by repeated 0·25 M sucrose washes, nor is it easily released by sonic disruption. Previously Tietz

TABLE 5. EFFECT OF VARIOUS PROCEDURES ON RAT LIVER MITOCHONDRIAL AND MICROSOMAL ALDEHYDE-OXIDIZING ACTIVITY

Procedure	Per cent of total recovered activity in sediment	
	Mitochondria (%)	Microsomes (%)
1st 0·25 M sucrose wash	75·3	85·0
2nd ,, ,, ,,	92·4	86·0
3rd ,, ,, ,,	99·0	100·0
4th ,, ,, ,,	100·0	
5th ,, ,, ,,	100·0	
Resuspension in water	77·3	
Freeze–thaw in 0·25 M sucrose	97·4	
Freeze–thaw in 1·15% KCl	37·8	
Sonically treated 3 min	3·3	44·6

Mitochondria were sedimented at 10,000 *g* for 10 min after each successive wash. Water-treated and frozen–thawed material was centrifuged at 45,000 *g* for 1 hr. Sonicated mitochondria were centrifuged at 196,000 *g* for 45 min. Microsomes were sedimented at 196,000 *g* for 45 min. The sonicated microsomes were sedimented at 196,000 *g* for 2 hr. Assay was carried out as described in the text and Table 3. Values are from a typical experiment.

et al. reported the presence of aldehyde dehydrogenase in liver microsomes.[10] Since most studies of the supernatant fraction were carried out on material that had been centrifuged at 10,000 *g* for 10 min, these preparations contained the microsomal enzyme as well as that of the supernatant. However, this microsomal activity amounts to only 3·7 per cent of the total aldehyde-oxidizing capacity of the homogenate and only 4·3 per cent of the total activity of the supernatant fraction. The microsomal fractions were essentially devoid of succinate dehydrogenase activity.

DISCUSSION

The choice of indole-3-acetaldehyde as a substrate to measure aldehyde-oxidizing capacity was based on several considerations: (1) a relatively rapid and sensitive technique is available for the assay of the product, indole-3-acetic acid, even in crude homogenates with little aldehyde-oxidizing capacity; (2) the compound is available commercially or may be easily prepared and is relatively stable as the bisulfite complex;[21] (3) the assay of the acid ensures inclusion of all aldehyde-oxidizing reactions in the determination, not just those that produce reduced NAD; (4) the assay can be used for pyridine-linked dehydrogenase in the presence of interfering enzymes such as NAD(P)H oxidases or alcohol dehydrogenase; (5) the aldehyde is a normal intermediate in one pathway of tryptamine metabolism.[24] Previously, Weissbach *et al.*[29] have used indole-3-acetaldehyde as a substrate for the steroid-sensitive aldehyde de-

* R. A. Deitrich and P. Philpott (unpublished experiments).

hydrogenase from rabbit liver. The chief disadvantages are the instability of the free aldehyde and the presence of substrate inhibition at high free aldehyde concentrations.

Most aldehyde dehydrogenases so far studied have a broad substrate specificity.[3, 5, 7] Only purified succinic semialdehyde dehydrogenase[18] and crystalline D-glyceraldehyde-3-phosphate dehydrogenase[17] have exhibited marked substrate specificity.*

All tissues examined had the ability to catalyze the oxidation of indole-3-acetaldehyde. The liver has the greatest amount of this activity. Lundquist *et al.*[31] have found that rat liver suspensions oxidize about 1·1 μmoles acetaldehyde per min/g wet weight at 37°. In order to compare our results with those of Lundquist and co-workers, it is necessary to treat our data on a wet weight basis and correct for the temperature difference. When this was done it was found that liver homogenates, prepared from male rat livers, are capable of carrying out the oxidation of about 10·5 μmoles of indole-3-acetaldehyde per min/g wet weight at 37°. Livers from female rats would be even more active. The data of Büttner[11] indicate about 7 μmoles acetaldehyde oxidized per min/g wet weight at 37° for liver homogenates prepared from male rats. Büttner used the CO_2 evolution method of Racker[5] instead of the direct assay of acetaldehyde, ethanol, and acetic acid used by Lundquist *et al.*[31] The apparent K_m values found for indole-3-acetaldehyde are much higher than most K_m values for various aldehydes with purified bovine dehydrogenase,[7] determined at pH 9·6.

It is probable that the bulk of the activity is due to NAD-linked dehydrogenases. This is best substantiated by the finding that dialysis renders the production of indole-3-acetic acid virtually dependent upon added NAD. The only exception noted was with brain homogenates. This may be a reflection of the difficulty of removing NAD, since aldehyde-oxidizing capacity has been purified from brain and was found to be attributable to an NAD-dependent dehydrogenase; no evidence was obtained for the presence of aldehyde oxidase activity.† Previously, Richert and Westerfield had stated that liver from molybdenum-deficient rats, with low xanthine oxidase and aldehyde oxidase levels, shows no decreased ability to oxidize acetaldehyde in the presence of NAD.[20] Our observations on this point are also in agreement with those of Lundquist *et al.*[31]

The greater aldehyde-oxidizing capacity found in liver and kidney from female rats is interesting, since Maxwell and Topper[14] have studied a steroid-sensitive aldehyde dehydrogenase from rabbit liver. Büttner[11] also reports a similar sex difference when acetaldehyde is used as the substrate with liver and kidney homogenates from Sprague–Dawley rats, but not from Wistar rats.

Several enzymes are present in both the supernatant and mitochondrial fractions of the cell.[32] The distribution of aldehyde dehydrogenase closely parallels that of malic dehydrogenase, which has also been reported to be present in the supernatant, mitochondrial, and microsomal fractions of tissue homogenates.[33–36] Evidence presented here, as well as previous substrate specificity determinations,[3] indicates that the enzymes in the supernatant and mitochondrial fractions are distinct proteins. Final decision on this point must await further purification and study of the enzymes.

Whether or not aldehydes, normally present in the body or introduced from the external environment, have any physiological effect is an important question. Renson

* Since submission of this paper for publication, Raison *et al.*[30] have reported that rabbit liver contains an aliphatic and an aromatic aldehyde dehydrogenase.

† V. G. Erwin and R. A. Deitrich, *J. biol. Chem.* In press.

et al.[2] have demonstrated that the aldehydes obtained by the oxidative deamination of 5-hydroxytryptamine, epinephrine, and tryptamine possess no activity on isolated intestinal segments. Also, Barondes and Field[37] have shown that, although aldehydes of this type do alter glucose metabolism in the pituitary gland, the effect is nonspecific and is not hormonal in nature. In studies carried out in this laboratory* it was concluded that 5-hydroxyindole-3-acetaldehyde did not participate in the blood pressure response of rabbits to injected serotonin. On the other hand, Akabane *et al.* [38, 39] have presented evidence that acetaldehyde has a marked effect on blood pressure, apparently by bringing about the release of epinephrine.

Although the relatively high level of aldehyde-oxidizing activity found in liver is probably sufficient to cope with endogenous aldehydes as well as large amounts of exogenous aldehydes, this may not be the case in other tissues, or when some inhibitory compound is present. The relatively low level of aldehyde-oxidizing capacity in such tissues as brain may be of pharmacological, if not physiological significance, since many compounds possess the ability to inhibit aldehyde oxidation.[7]

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