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Ethanolamine Metabolism in the Rat.* (31715)

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Investigations of the metabolism of ethanolamine-1,2-C^{14} in rats and pigeons have demonstrated its rapid oxidation to carbon dioxide(1,2). The results of similar studies with isotopically labelled ethanolamine have suggested a pathway involving initial oxidative deamination to glycolaldehyde(3,4). Enzymes for the conversion of ethanolamine to glycolaldehyde have been found in microorganisms(5) and plants(6,7), but have not been reported in mammalian systems. The oxidation of ethanolamine has been reported by a monoamine oxidase from human(8) and bovine(9) blood plasma, but the initial oxidation product was glyoxal in the latter system, which was the only one studied in detail (10).

Several enzyme systems have been elucidated which would allow for the oxidation of glycolaldehyde to carbon dioxide in mammalian tissues. Glycolaldehyde may be oxidized to glycolate and glyoxylate(11,12) and the glyoxylate decarboxylated(13). Upon transamination of glyoxylate to glycine(14, 15), the latter may be oxidized by a pyridoxal-dependent enzyme to carbon dioxide (15). Alternately, the glycine may be converted to serine(16) and the serine oxidized to carbon dioxide *via* pyruvate. Glycolaldehyde may also be converted to acetate (17) and subsequently oxidized through the citric acid cycle, or may be converted to sugars *via* a thiamine-dependent transketolase (18). The following study was undertaken to further elucidate the oxidative pathway of ethanolamine in the rat.

Materials and methods. Ethanolamine-1,2-C^{14} (New England Nuclear Co., Boston, Mass.) was purified by paper chromatography in 77% ethanol and was administered intraperitoneally. Wistar rats were employed throughout. Thiamine-deficient and pyridoxine-deficient diets and their respective control diets (General Biochemicals, Inc., Chagrin Falls, Ohio) were used in vitamin deficiency studies. All other rats were maintained on standard chow diets.

Respiratory CO_2 was collected for various time intervals in glass metabolic cages with the expired CO_2 being trapped in a series of bubble towers containing 20% sodium hydroxide. To facilitate the determination of radioactivity in a large number of samples, 3.0 ml aliquots of the alkaline trapping solutions were acidified in sealed flasks equipped with vials containing 0.2 ml hyamine hydroxide suspended from their stoppers. Complete transfer of the CO_2 to the hyamine occurred when the flasks were shaken at 37°C for 1 hour. The radioactivity in the hyamine was measured by the use of a Packard Tri-Carb liquid scintillation spectrometer, using a PPO:POPOP:toluene mixture.

To determine the distribution of radioactivity in various organs, the tissues were homogenized in 80% ethanol and boiled for 10 minutes. The centrifuged residue was washed successively with ether, 50% ethanol, and water. Radioactivity was measured in the combined washes by spotting 0.1 ml aliquots on aluminum planchets and counting with a Nuclear-Chicago gas flow ionization detector. The aliquots were sufficiently

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diluted to approach conditions of "infinite thinness," so no corrections for self-adsorption were made. The average counts per minute for 3 planchets were compared to a carbon-14 reference standard for calculation of microcuries and per cent of administered dose.

The ethanol extracts of tissues were separated into 3 fractions by a modification of the procedure of Cossins and Beevers(19). The ethanol extracts were concentrated to dryness and extracted 3 times with ether. The combined ether extracts comprised the "lipid" fraction. The ether insoluble residue was redissolved in water and allowed to pass through a column of Dowex AG-50-X8 (H^+ form). The fraction passing through the column with water comprised the "organic acid and sugar" fraction. The "amino acid" fraction was then eluted from the resin with 6 N hydrochloric acid.

The spectrophotometric assay developed by Dische and Borenfreund was used for detection of glycolaldehyde(20). Aliquots (2.0 ml) of the samples to be assayed were mixed with 0.4 ml of 50% trichloroacetic acid and 4.8 ml of diphenylamine solution (1% in glacial acetic acid), shaken thoroughly and placed in boiling water for 30 minutes. The optical density was determined at 660 and 580 mμ on a spectrophotometer. The difference between the optical densities at these wave-lengths is proportional to the glycolaldehyde concentration. A standard curve was prepared using a liver homogenate as control. The optical density difference was proportional to the added glycolaldehyde at concentrations from 1 to 15 μg per milliliter. This method does not distinguish between glycolaldehyde and glyoxal.

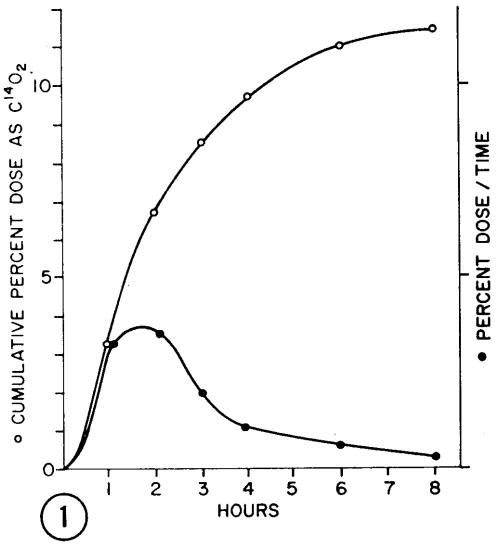
Homogenates (20 % w/v) of rat liver were prepared in 0.25 M sucrose-0.001 M EDTA (ethylenediaminetetraacetate), pH 7.0. A total particulate fraction(21) was prepared by centrifuging the homogenate at 105,000 × *g* for 60 minutes, resuspending the precipitate in sucrose-EDTA, recentrifuging, and finally resuspending the precipitate in the original volume of sucrose-EDTA. Subcellular fractionation was achieved by centrifuging a homogenate at 1200 × *g* for 10 minutes. The resuspended precipitate constituted the "nuclear fraction." The supernate was centrifuged at 22,000 × *g* for 10 minutes. The resuspended precipitate was the "mitochondrial fraction." The supernate was centrifuged at 105,000 × *g* for 60 minutes to separate the "microsomal fraction" and the "supernatant fraction."

Results. The time course of expiration of $C^{14}O_2$ following intraperitoneal injection of 25 microcuries (0.52 μmole) of ethanolamine-1,2-C^{14} is shown in Fig. 1. The total expired $C^{14}O_2$ in 8 hours was 11.5% of the injected dose. Maximum excretion was observed from 1 to 2 hours following administration.

Pyridoxine-deficient rats showed an impaired ability to rapidly oxidize ethanolamine to CO_2 (Fig. 2). Significantly less $C^{14}O_2$ was expired in 6 hours by the pyridoxine-deficient animals than by the controls, and the rapid excretion rate between 1 and 2 hours was markedly depressed. Rats on the thiamine-deficient diets showed no significant impairment of $C^{14}O_2$ expiration (Fig. 3). Neither the total $C^{14}O_2$ excretion nor the rapid initial rate of excretion was significantly affected. In these studies, groups of male Wistar rats were placed on thiamine-deficient diets for 27 days or pyridoxine-deficient diets for 34 days. Control rats for each group were maintained on deficient diets supplemented with the respective vitamin. The body weights of the thiamine-deficient rats either decreased or were unchanged during the 27-day diet period. The body weights of the pyridoxine controls doubled during the 34-day period, while the pyridoxine-deficient rats gained only half of their initial weight. At the end of the diet periods the rats were injected intraperitoneally with 10 microcuries (0.20 μmole) of ethanolamine-1,2-C^{14} and expired $C^{14}O_2$ was collected at intervals for 6 hours.

The distribution of radioactivity in rat tissues was determined 8 hours after the administration of ethanolamine-1,2-C^{14} (25 μc). The specific radioactivity (per cent of C^{14}/g wet tissue) and the per cent of the injected dose was determined for several tissues and organs (Table I). Approximately 50% of the injected radioactivity was found in the liver. The spleen, kidneys, and small in-

testine contained significant amounts of radioactivity. Heart, brain and diaphragm contained traces of radioactivity amounting to only 1% of the injected dose. The highest specific radioactivity was found to occur in the liver. The radioactivity in these tissues was found to be distributed in "lipid," "amino acid," and "organic acid and sugar" fractions



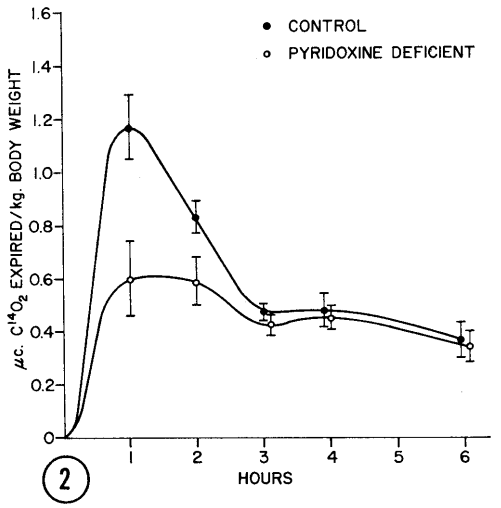


FIG. 1. Expiration of $C^{14}O_2$ by rats injected intraperitoneally with 25 μc ethanolamine-1,2-C^{14} (50 μc/μm).

FIG. 2. Expiration of $C^{14}O_2$ by pyridoxine-deficient and control rats after intraperitoneal injection of 10 μc ethanolamine-1,2-C^{14} (spec. act 10 μc/μm). Values represent the mean for 5 rats ± standard error.

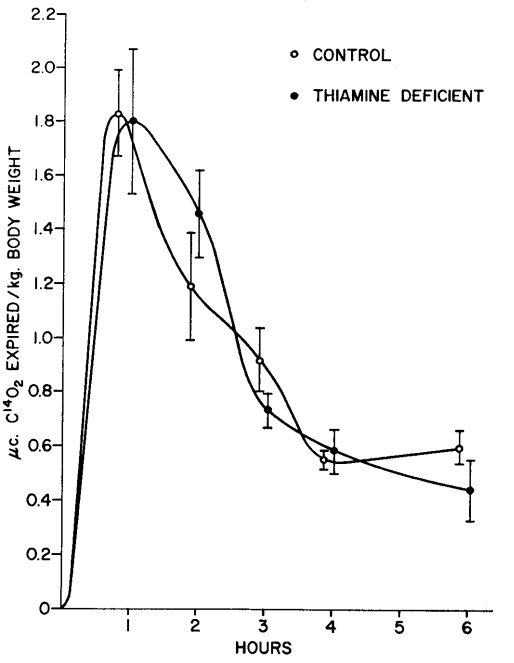


FIG. 3. Expiration of $C^{14}O_2$ by thiamine-deficient and control rats after intraperitoneal injection of 10 μc ethanolamine-1,2-C^{14} (spec. act. 10 μc/μm). Values represent the mean for 5 rats ± standard error.

(Table I). Nearly all the radioactivity in the liver was found in the lipid fraction. The other tissues also contained highly radioactive lipid fractions.

Slices and homogenates of liver, spleen, brain, kidney, heart, diaphragm and intestine were incubated in a closed system with ethanolamine-1,2-C^{14} (5 μc) for 6 hours at 37°C in 0.1 M sodium phosphate buffer, pH 7.4. No significant quantities of $C^{14}O_2$ were detected in these samples after acidification. The addition of various combinations of ATP, Mg^{++}, pyridoxal phosphate, α-ketoglutarate, pyruvate, and thiamine pyrophosphate failed to stimulate $C^{14}O_2$ formation by rat liver slices incubated with ethanolamine-1,2-C^{14}. Tissue slices did, however, utilize the ethanolamine, as measured by its disappearance from the media. Liver utilized 39%, spleen 25%, diaphragm 15%, kidney 15%, brain 10%, heart 10% and intestine 2% during a 6-hour incubation. Fractionation of the radioactivity in the washed tissue slices showed that all tissues studied converted a significant amount

TABLE I. Distribution of Radioactivity in Extracts of Rat Tissues 8 Hours After Administration of Ethanolamine-1,2-C^{14} (25 μc).*

Tissue	% C^{14}/g tissue	% of dose in organ	% in fractions†		
			"Amino acid"	"Organic acid and sugar"	"Lipid"
Liver	9.12	49.2	6.5	1.1	91.2
Spleen	2.80	1.55	66.7	2.6	29.9
Brain	2.04	2.20	40.0	.8	53.6
Kidney	0.49	0.38	28.5	7.1	59.5
Heart	0.30	0.41	32.8	2.9	58.8
Diaphragm	0.68	0.34	37.7	.0	60.3
Intestine (.6 g segment)	2.73	—	91.7	11.8	2.4

* Specific activity: 50 μc/μm.
† Extracts were fractionated by the method of Cossins and Beevers(19).

of ethanolamine to lipids, and, more limitedly, to other compounds (Table II).

Glycolaldehyde was not identified as an intermediate in ethanolamine oxidation by rat liver tissue slices, homogenates, or particulate systems either by direct assay or by isotope trapping procedures. Similarly, the direct oxidation of ethanolamine by rat liver mitochondrial amine oxidase could not be demonstrated. No oxidation of ethanolamine could be detected in rat blood plasma preparations by O_2 utilization, glycolaldehyde or glyoxal formation, or isotope trapping procedures, although its oxidation has been reported in human and bovine blood plasma (8,9).

Discussion. Ethanolamine-1,2-C^{14} was readily oxidized to respiratory $C^{14}O_2$ in the intact rat. The maximum rate of excretion was observed between 1 and 2 hours after administration indicating a rapid oxidation of the ethanolamine. The rate of excretion then dropped suggesting either a dilution of the compound by endogenous pools of ethanolamine or the conversion of the ethanolamine into compounds not readily catabolized.

TABLE II. Fractionation of Radioactivity in Extracts of Tissue Slices Incubated* with Ethanolamine-1,2-C^{14}.†

Tissue	% of radioactivity in fractions‡		
	"Amino acid"	"Organic acid and sugar"	"Lipid"
Liver	65.9	22.6	11.6
Kidney	98.2	.7	1.1
Brain	97.0	.5	2.5
Spleen	97.4	1.6	1.1
Heart	92.2	2.9	4.9
Diaphragm	97.0	.9	2.1
Intestine	99.0	.7	.3

* Tissue slices were incubated with 5 μc ethanolamine-1,2-C^{14} as described in the text.
† Specific activity: 50 μc/μm.
‡ Tissue extracts were fractionated by the method of Cossins and Beevers(19).

The oxidation of ethanolamine may occur by pathways involving several pyridoxine or thiamine-dependent enzymes. The interconversion of serine and glycine(16) and the oxidative decarboxylation of glycine(22,23) involve pyridoxal phosphate. The formation of an "active glycolaldehyde" appears to involve thiamine pyrophosphate(24). Also, the postulated conversion of ethanolamine to glycolaldehyde may be a transamination or deamination; both reactions usually involve pyridoxal phosphate. Thus, depending on the operative metabolic pathway, rats with a pyridoxine or thiamine deficiency may have an altered rate of oxidation of ethanolamine to CO_2. Thiamine-deficient rats showed no significant differences in the rate of excretion of respiratory $C^{14}O_2$. Pyridoxine-deficient rats, however, showed a decreased rate of excretion of respiratory CO_2. This decreased capacity to form carbon dioxide implicates a pyridoxine-dependent enzyme system in the pathway for the oxidation of ethanolamine. The observed inhibition could occur at either an initial deamination step, or later in the pathway at the decarboxylation or transamination step.

Urea-C^{14} was one of the labelled products from ethanolamine-1,2-C^{14} in the rat(1). Since the synthesis of urea occurs predominantly in the liver and the only carbon atom in the compound is derived from CO_2, it seems reasonable that the ethanolamine catabolism might take place in the liver. Ex-

tensive $C^{14}O_2$ formation was observed from ethanolamine-1,2-C^{14} when administered to isolated perfused rat liver (unpublished observation). However, although the slices of liver and several other tissues showed extensive uptake and metabolism of ethanolamine, no significant $C^{14}O_2$ was formed. On the other hand, glycolaldehyde, the postulated initial product of ethanolamine, is readily oxidized to carbon dioxide by rat liver slices and tissue homogenates(3). Either the oxidation of ethanolamine does not proceed *via* glycolaldehyde, or the required enzyme system is not active in tissue slices and homogenates. This would tend to eliminate the involvement of a simple transaminase or amine oxidase, since enzymes of these types are usually stable in tissue slice preparations. Our inability to demonstrate ethanolamine oxidation or glycolaldehyde formation would agree with this conclusion, although ethanolamine oxidation in plant tissues involves a monoamine oxidase (25).

The main metabolic pathway for ethanolamine in the intact rat, tissue slices, and tissue homogenates is incorporation into the phospholipid fraction. After 8 hours, 54% of the ethanolamine administered to an intact rat was found in the liver, spleen, kidneys, heart, brain and diaphragm, and 11.5% was accounted for as $C^{14}O_2$. Approximately 85% of tissue radioactivity accounted for was in the lipid fraction. Incorporation of ethanolamine into lipids occurred in individual tissue slices, confirming the ubiquitous nature of the ethanolamine incorporating system(26). The liver was the most effective tissue for incorporating ethanolamine into phospholipids. Heart and brain tissues were found to be the next most effective. The pathway of incorporation presumably involves the exchange of ethanolamine with phosphatidylserine forming phosphatidylethanolamine and serine (27).

The incorporation of ethanolamine-1,2-C^{14} into organic acids and carbohydrates is significant, but much less than that of lipid incorporation. The extent of incorporation into the amino acid fractions is difficult to interpret since the nonmetabolized ethanolamine would be present in this fraction. It does, however, indicate that liver is the most significant tissue in the rat with respect to ethanolamine metabolism. The specific identification of the amino acid, organic acid, and carbohydrate intermediates of ethanolamine and their significance are under investigation.

Summary. Ethanolamine-1,2-C^{14} was readily metabolized by the rat, rat tissue slices, and rat tissue homogenates. Over 50% of the administered ethanolamine-1,2-C^{14} was incorporated into the lipid fraction and 11% was oxidized to $C^{14}O_2$ by the intact rat in an 8-hour period. Ethanolamine oxidation was decreased by vitamin B_6 deficiency but not by thiamine deficiency. Glycolaldehyde was not detected as an intermediate in ethanolamine oxidation in rat tissue preparations.

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Effect of 2-Mercaptoethanol on Rabbit Pancreas Isoantibodies.* (31716)

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Previous reports(1,2) have described the production of isoantibodies to rabbit pancreas. These antibodies, which were produced by rabbits following intradermal injections of a simple saline extract of rabbit pancreas incorporated into complete Freund adjuvant, have been found to be both species- and organ-specific. They reacted with some but not all of a random selection of rabbit pancreas extracts, but never reacted with the pancreas extract of the antibody-producing rabbit itself. Later, the occasional appearance of autoantibodies to pancreas was noted in some rabbits receiving trichloroacetic acid precipitated pancreas extract in Freund adjuvant(3). No reaction was seen when normal rabbit serum was tested with pancreas extract.

Kidd and Friedwald(4) have described a naturally-occurring, heat-labile antibody that is present in normal rabbit sera and which reacts with extracts of many organs of normal rabbits (including the rabbit's own organs). Moreover, following immunization with pancreas extract, an increase in the titer of the reaction with autologous organ extracts such as gastrointestinal mucosa was observed(2). The present report describes some unusual immunochemical properties of these natural and induced rabbit antibodies to rabbit organ extracts.

Materials and methods. Preparation of antisera. Antisera were obtained by injecting rabbits with a saline extract of pooled rabbit pancreas extract which had been incorporated into an equal volume of complete Freund adjuvant. A total of 1 ml of the emulsion was injected intradermally at 3 week intervals, alternating between the 4 foot pads and the back of the neck. Trial bleedings from the central artery of the ear were taken just prior to each immunization. The final bleeding was taken from the heart. Antiserum to bovine serum albumin (BSA) was prepared following the same injection schedule.

Sucrose density gradient ultracentrifugation. Starting with 40% sucrose in phosphate buffered saline (pH 7.2) and following with 30%, 20%, and 10% solutions, 1.1 ml samples of each solution were layered into a 5 ml cellulose centrifuge tube. After equilibration for 2 hours at 4°C, 0.5 ml of serum in 10% sucrose solution was added. A few drops of brom phenol blue were added to the sucrose-serum mixture as a marker of albumin. After ultracentrifugation at 125,000 *g* for 18 hours in the Spinco model L ultracentrifuge using the SW-39 swinging bucket rotor, a hole was punched in the bottom of the centrifuge tube with a #25 gauge needle and the fractions were collected into tubes; 7 drops were allowed in each fraction.

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