Formation and Excretion of Pyrrole-2-carboxylic Acid

WHOLE ANIMAL AND ENZYME STUDIES IN THE RAT*

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SUMMARY

A corrected method for the measurement of pyrrole-2-carboxylate in rat urine was used in studies of its excretion under various experimental conditions. The findings implicated administered hydroxy-L-proline as a relatively efficient source of urinary pyrrole-2-carboxylate and tended to exclude administered l-proline as a significant direct source. Removal of aerobic gut flora had no influence on the excretion of pyrrole-2-carboxylate either endogenously or following hydroxy-L-proline administration.

Related studies showed that rat kidney L-amino acid oxidase catalyzes oxidation of hydroxy-L-proline to Δ^-pyrroline-4-hydroxy-2-carboxylate, which is converted to pyrrole-2-carboxylate on acidification of reaction mixtures. All findings were consistent with hydroxy-L-proline as the source of endogenous pyrrole-2-carboxylate excretion.

Excretion patterns and labeling patterns were compared after administration of pyrrole-2-carboxylate or of hydroxy-L-proline epimers. From these data, the true excretion product of hydroxy-L-proline oxidation by L-amino acid oxidase appeared to be the unstable oxidation product, Δ^-pyrroline-4-hydroxy-2-carboxylate, which is converted to pyrrole-2-carboxylate in urine.

The capacity of homogenates of guinea pig kidney and human kidney to carry out oxidation of hydroxy-L-proline to pyrrole-2-carboxylate was much less than that of rat kidney, consistent with the lower levels of urinary pyrrole-2-carboxylate in these species.

Experiments designed to examine the modest increase of pyrrole-2-carboxylate excretion after proline loads led to new observations on tissue levels of hydroxy-L-proline following proline administration and on the inhibition by L-proline of hydroxy-L-proline oxidase.

Pyrrole-2-carboxylic acid, as a compound of biological interest, was isolated after chemical degradation of sialic acids (3) and was subsequently identified as an acid-catalyzed dehydration product of Δ^-pyrroline-4-hydroxy-2-carboxylate (4). The latter compound is formed by the oxidation of L-isomer of hydroxyproline, catalyzed by mammalian L-amino acid oxidase (4) or by a more specific allohydroxy-D-proline oxidase of Pseudomonas (5). That pyrrole carboxylate can be derived from L-amino acid oxidase action on hydroxyproline provided an explanation for the appearance of pyrrole carboxylate in rat or human urine after administration of the L isomers of hydroxyproline (6).

From a later observation in rats (7), that radioactive L-proline also labeled urinary pyrrole carboxylate, the pathway, L-proline → hydroxy-L-proline → pyrrole carboxylate, was inferred (7). However, no metabolic reaction was then known in animal tissues to account for the conversion of hydroxy-L-proline to the cyclic ketimine, Δ^-pyrroline-4-hydroxy-2-carboxylate. In animal tissues, rather, the principal fate of hydroxy-L-proline has been defined as oxidation to the cyclic aldime, Δ^-pyrroline-3-hydroxy-5-carboxylate (8), and subsequent reactions of this intermediate (9, 10). Hypothetical explanations for pyrrole carboxylate formation from L-proline also included the possibility that L-proline itself might be the source, via a direct pathway independent of hydroxyproline, or that hydroxy-L-proline formed from L-proline could be converted to pyrrole carboxylate either by an undefined reaction in animal tissues, or by a route involving the intestinal flora (9).

In a recent brief publication (11), we reported that in rats pyrrole carboxylate is labeled more directly from hydroxy-L-proline than from L-proline, and that a demonstrable enzymatic basis for this reaction was the oxidation of hydroxy-L-proline to the cyclic ketimine by mammalian L-amino acid oxidase of kidney; the latter finding was independently confirmed in a subsequent report (12). Our report (11) also presented data for the urinary excretion of pyrrole carboxylate by man; our values were in close agreement with less extensive studies published earlier (13, 14).

Subsequent to that publication, we learned that our measurements of pyrrole carboxylate levels in both rat and human urine were in error because they included nonspecific compounds that had not been removed entirely either by our procedure, or by those procedures employed by other laboratories (13, 14). An account of the methodologic problems and of corrected values...
for human urinary pyrrole carboxylate is published separately (15).

The present paper provides detailed data, using the corrected method, on the pattern of excretion of free pyrrole carboxylate under various experimental circumstances. While qualitatively the broad conclusions based on the less specific method (11) remained valid, the selective role of hydroxy-L-proline as a source of pyrrole carboxylate has emerged more clearly. In addition, kinetic data are provided for the oxidation of hydroxy-L-proline to the cyclic ketimine by tissue homogenates in several species and by the homogeneous L-amino acid oxidase of rat kidney.

Two new metabolic inferences concerning pyrrole carboxylate have emerged from the work reported here. One is the conclusion that urinary pyrrole carboxylate of endogenous origin, or that labeled from administered proline or hydroxyproline, is probably formed from the unstable pyrrole precursor in the urine rather than in the tissues of the animal. In contrast, administered pyrrole carboxylate is extensively conjugated and otherwise metabolically altered. Another inference is that alloxohydroxy-D-proline, (or its pyrroline oxidation product or both) is metabolized by the whole animal via an unknown route in addition to that forming pyrrole carboxylate.

Experiments designed to investigate the role of proline as a pyrrole carboxylate precursor have provided new information on the effect of a proline load on free hydroxyproline of rat liver and on the competition by proline for the oxidation of hydroxy-L-proline via the major pathway through \( \Delta^1 \)-pyrroline-3-hydroxy-5-carboxylate.

**EXPERIMENTAL PROCEDURE**

**Materials**

*Animals and Tissues*—Male Wistar rats (75 to 125 g) and albino guinea pigs (200 to 400 g) were purchased from Bar F Enterprises, Perry Hall, Md. Frozen rat kidneys and guinea pig kidneys were purchased from Pel-Freez Biologicals, Rogers, Ark., and were kept at \(-15^\circ\) C until use. Samples of human kidney were obtained at autopsy, with the assistance of Dr. Melvin Reuber of the Department of Pathology, this institution, and were stored at \(-15^\circ\) C prior to use. Human kidney samples, from males who died shortly after trauma, were obtained within a few hours postmortem.

*Special Compounds*—Hog kidney L-amino acid oxidase, beef liver catalase, hydroxy-L-proline, alloxohydroxy-D-proline, L-proline (hydroxyproline-free), pyrrole-2-carboxylate, 4,5-dimethylamino-3-carboxybenzaldehyde, and certain radioactive compounds (hydroxy-L-[\( {\text{U}} \)-\( ^{14} \)C]proline, hydroxy-L-[\( \text{H} \)-\( ^{14} \)C]proline, L-[\( \text{U} \)-\( ^{14} \)C]proline, and \( \Delta^1 \)-pyrroline-3-hydroxy-5-carboxylate) were prepared and tested for purity as described separately (15). The more specific method (Method 2) involved the further fractionation of the Dowex 50 column by passage through an amino acid analyzer resin (Durrum DC-1, Durrum Chemical Co.); only one of the several peaks eluted proved to be free pyrrole carboxylate and it was measured after separation from the other components. Details of this method are presented and illustrated separately (15).

When unlabeled pyrrole carboxylate was measured, radioactive pyrrole carboxylate was added to the urine initially, and its recovery in the final fraction permitted correction for losses of pyrrole carboxylate during purification. When radioactive pyrrole carboxylate was measured, carrier pyrrole carboxylate (usually 5 mg) was added to the urine initially, and its recovery in the final fraction similarly permitted correction for losses of radioactivity in pyrrole carboxylate. When specific activity values were desired, no additions were made and the peak of radioactivity corresponding to pyrrole carboxylate (illustrated in Figs. 2 and 3) was collected from the amino acid analyzer column.

**Alkalinitization of Urine and NaBH\(_4\) Treatment*—Rats were offered water which contained 2% sucrose and 2% Na\(_2\)CO\(_3\); this produced a daily weight gain of 5 to 7 g in a voluminous urine of pH 8 to 9. To reduce possible losses of pyrrole carboxylate precursors, urine was collected only during the day at intervals of 2 to 3 hours. The alkaline urine was divided in half; each half was brought to 0.01 M NaOH and one was treated with NaBH\(_4\) (final concentration, 0.1 M) and kept at room temperature for 20 min. Each half then was acidified with 6 N HCl (final pH 3 to 4). After addition of [\( \text{U} \)-\( ^{14} \)C]pyrrole carboxylate (4 X 10\(^6\) cpm) to each sample, pyrrole carboxylate was isolated by Method 2.

**Measurement of Urinary Free Pyrrole and Hydroxyproline**—After extraction of urine to remove pyrrole carboxylate, the aqueous phase was applied to a small column of Dowex 50-H\(^+\); the column was washed with water and eluted with 0.3 M NaCl for the separate elution of proline and hydroxyproline (2). If radioactively labeled, proline and hydroxyproline were detected by counting; unlabeled hydroxyproline was detected in eluates after adding tritiated hydroxy-L-proline to the initial sample. Each compound was measured with the amino acid analyzer as described separately (15). For further purification, appropriate fractions from the amino acid analyzer column were desalted (Dowex 50-H\(^+\) column) and, after concentration, were run on paper electrophoresis at pH 2 and a. Hydroxyproline in purified fractions was measured by a minor modification of the Neuman-Logan method (20); proline was measured by an acid ninhydrin method (Procedure R (21)). Losses on purification of unlabeled proline or hydroxyproline were corrected by the recovery of the corresponding radioactive compound added to the initial urine sample.

**Antibiotic Treatment and Fecal Bacterial Counts**—Male Wistar rats were treated by twice-daily oral administration (stomach tube) of a 1-ml suspension containing 30 mg of neomycin sulfate.
75 mg of phthalylsulfathiazole, 25 mg of chlortetracycline, and 25 mg of chloramphenicol. Fecal bacterial counts were followed by plating fresh weighed fecal samples, emulsified in sterile 0.9% NaCl solution (saline). Aliquots (0.1 ml) of suitable dilutions were mixed with 2.0 ml of 0.68% MacConkey’s agar at 50° and immediately poured over plates of 1.35% MacConkey’s agar. Colonies were counted after the plates were incubated 24 hours at 37°. In a number of experiments, antibiotic treatment lowered the fecal bacterial count from over 10^9 colonies per g of feces (wet weight) to no detectable colonies (less than 300 per g), after 2 days of drug treatment.

Measurement of Free Amino Acids in Rat Liver—Rats were killed with ether; livers were removed, rinsed with water, blotted, and weighed. About one-third was minced and homogenized (Sorval Omni-Mixer, 1 min at 6000 rpm) in 10% trichloroacetic acid (2 ml per g) at 4°. After centrifugation at 25,000 × g for 30 min, the supernatant solution was removed and treated like the aqueous residue of urine described above. Radioactive hydroxy-L-proline or L-proline was added to the trichloroacetic acid supernatant solutions to permit correction for losses.

Preparation and Assay of Hydroxyproline Oxidase—The particular fraction of rat kidney homogenate that catalyzes conversion of hydroxy-L-proline to Δ1-pyrroline-3-hydroxy-5-carboxylate (hydropyrroline oxidase) was prepared essentially as described earlier (8). The twice-washed pellet was resuspended in 0.05 M sodium phosphate, pH 7.5. Incubations were carried out at 37° with hydroxy-L-proline (4 to 25 mM) and 0.002 M EDPTA in 0.05 M sodium phosphate, pH 7.5, in an air atmosphere with shaking for several hours (final volume, 3 to 4 ml). The reaction was stopped by adding 0.8 ml of 10% trichloroacetic acid followed by centrifugation at 100,000 × g for 15 min. The supernatant solution was used for the measurement of total Δ1-pyrroline (see below) and for the specific measurement of Δ1-pyrroline-3-hydroxy-5-carboxylate with the amino acid analyzer (2).

Δ1-Pyrrolines were measured after treatment with o-aminobenzaldehyde by a modification (17) of a method described earlier (22). Isolation of Δ1-pyrroline-3-hydroxy-5-carboxylate from the amino acid analyzer column indicated a color yield of 0.5 A unit per μmol at 444 nm (2); the comparable color yield for Δ1-pyrroline-3-hydroxy-5-carboxylate at 444 nm is 0.88 A unit per μmol (17).

Measurement of Pyrrole Carboxylate in Tissue Homogenates—Homogenates of liver or kidney were prepared by mincing tissue with scissors and homogenizing the mince in 4 volumes of 0.25 M sucrose in a Sorvall Omni-Mixer (6000 rpm, 1 min, 4°). Aliquots of the homogenate were incubated with 0.5 μmol of allohydroxy-n-proline or 200 to 500 μmol of hydroxy-L-proline in 0.05 M sodium phosphate, pH 7.5, with 50 units of added catalase in a final volume of 2 ml. Incubations were carried out at 37° in an air atmosphere for 2 to 5 hours. Incubations were also carried out with 0.02 and 0.04 μmol of pyrrole carboxylate under the same conditions and tissue blanks were run containing substrate, but with trichloroacetic acid added at zero time. The reaction was stopped by adding 1 ml of 10% trichloroacetic acid and removing the precipitate by centrifuging at 100,000 × g for 15 min. Suitable aliquots of the supernatant solution were brought to 2 ml with water, treated with 1 ml of p-dimethylaminobenzaldehyde solution (5% in 7 N propyl alcohol) and 2 ml of 3.6 N HSO4; the heating step and absorbance measurement were carried out for pyrrole carboxylate assays in urine (15).

Purification and Assay of Amino Acid Oxidase—The enzyme was purified from rat kidney as described earlier (23), except that the crystallization step was not attempted. The enzyme recovered after the final gel filtration step appeared to be about 50% pure by polyacrylamide gel electrophoresis; enzyme activity, confined to the major protein band, was eluted from the gel and was considered to represent homogeneous enzyme.

The enzyme was assayed with L-leucine as substrate under the conditions described by Nakao and Danowski (24). The α-ketosuccinopropionate formed was measured as the 2,4-dinitrophenylhydrazone by a modification of the method of Friedemann and Haugen (25). Assays of enzyme fractions with hydroxy-L-proline were carried out as described for pyrrole carboxylate measurement in tissue homogenates. In assays of enzyme fractions with L-proline as substrate, incubations were carried out as described for L-leucine. The expected product, Δ1-pyrroline-2-carboxylate, was measured with o-aminobenzaldehyde as described for the other Δ1-pyrrolines, the quantity of product was calculated from the absorbance using the color yield of Δ1-pyrroline-2-carboxylate, calibrated as described above.

Other Procedures—Polyacrylamide gel electrophoresis was carried out with 7% gels in pH 0.5 glycine buffer in a Carlaw apparatus; gels were fixed with 12.5% trichloroacetic acid and stained with Coomassie blue. For elution of enzyme, gels were sliced into 1-mm disks, these were extracted by shaking with 0.6 ml of 0.006 M sodium phosphate, pH 7.9, for 15 hours at 4°. Added L-α-amino acid oxidase activity (L-leucine or hydroxy-L-proline as substrate) was not inhibited in the presence of the gel slices. About 30% of the activity applied to the gels was recovered by elution in this manner.

Protein and Radioactivity Assays—Protein was estimated by the method of Warburg and Christian (26) with crystalline beef serum albumin as standard. Radioactivity was measured as noted elsewhere (15).

RESULTS

Studies with Intact Rats

Excretion of Pyrrole Carboxylate from Endogenous Sources—Table I shows values obtained by Method 2 for the daily excretion of pyrrole carboxylate in several groups of rats. The mean value, 0.012 μmol/24 hours/100 g, is about 3-fold less than that earlier reported using a less specific method (11).

Effect of a Hydroxyproline or Proline Load—The data shown in Fig. 1, obtained by the revised pyrrole carboxylate method, indicate more than a 30-fold increase in pyrrole carboxylate excretion after large hydroxy-L-proline loads. Similarly administered L-proline produced at most a 3-fold increase in pyrrole carboxylate excretion. It is of particular interest that, of the several pyrrole-reactive components eluted from the amino acid analyzer column in the more specific method (15), only the true pyrrole carboxylate peak was increased by hydroxyproline or proline administration, implying that the other quasi-pyrrole carboxylate peaks were metabolically unrelated to pyrrole carboxylate. It therefore was apparent that at the higher levels of true pyrrole carboxylate measured in this kind of experiment, Method 1 and Method 2 would give very similar values.

Conversion of Radioactive Hydroxy-L-proline and L-Proline to Pyrrole Carboxylate—Earlier data based on the less specific method for pyrrole carboxylate isolation indicated that administration to rats of either radioactive L-proline or hydroxy-L-proline resulted in excretion of labeled pyrrole carboxylate; radioactivity in pyrrole carboxylate represented an approximately 10-fold greater fraction of the dose of hydroxyproline than of proline. Repetition of these experiments with the more discriminating method revealed the presence of labeled impurities which separated from pyrrole carboxylate on the amino acid analyzer column (Fig. 2). Such labeled impurities were much greater after administering radioactive proline than after radioactive hydroxy-

| Table I | Urinary pyrrole carboxylate in untreated rats |
|---------|----------------------------------|
| Groupa | Pyrrole carboxylate μmol/24 hr/100 g body wt b |
| 1 (6)  | 0.011 |
| 2 (6)  | 0.012 |
| 3 (4)  | 0.012 |
| 4 (8)  | 0.010 |
| 5 (8)  | 0.017 |

a Number of rats in each group is shown in parentheses.

b For the entire group of 32 rats, the mean and standard error for this value was 0.012 ± 0.002.
similar results. The ordinate shows 10-fold increase over the endogenous level. Urine was collected for 24 hours and assayed for pyrrole carboxylate by Method 1 (high values) or by Method 2: as discussed in the text, at high values of pyrrole carboxylate Methods 1 and 2 give similar results. The ordinate shows 10-fold increase over the endogenous level. Urine was collected for 24 hours and assayed for pyrrole carboxylate (PCA) excretion (0.019 μmol/hr/100 g, Table I); the abscissa shows proline or hydroxyproline administered per 100 g body weight. A control experiment excluded the possibility that excreted hydroxyproline might yield significant increases in pyrrole carboxylate by conversion in the urine. When urine from eight rats was collected for 24 hours in the presence of 2 mmol of hydroxy-L-proline (the expected excretion ratio for a load of 2.5 mmol per 100 g), the urinary pyrrole carboxylate was elevated only 30% over the basal level.

Fig. 1. Increased excretion of pyrrole carboxylate (PCA) following hydroxy-L-proline or proline loads. L-Proline (C) or hydroxy-L-proline (\( \bullet \)) was administered subcutaneously in four to five divided doses over 5 hours to groups of rats (4-6) for each dose level. Urine was collected for 24 hours and assayed for pyrrole carboxylate by Method 1 (high values) or by Method 2: as discussed in the text, at high values of pyrrole carboxylate Methods 1 and 2 give similar results. The ordinate shows 10-fold increase over the endogenous level. Urine was collected for 24 hours and assayed for pyrrole carboxylate (PCA) excretion (0.019 μmol/hr/100 g, Table I); the abscissa shows proline or hydroxyproline administered per 100 g body weight. A control experiment excluded the possibility that excreted hydroxyproline might yield significant increases in pyrrole carboxylate by conversion in the urine. When urine from eight rats was collected for 24 hours in the presence of 2 mmol of hydroxy-L-proline (the expected excretion ratio for a load of 2.5 mmol per 100 g), the urinary pyrrole carboxylate was elevated only 30% over the basal level.

L-proline (Fig. 2). The corrected recovery of radioactive pyrrole carboxylate after L-proline or hydroxy-L-proline administration is shown in Table II. The per cent of administered radioactivity recovered in true pyrrole carboxylate from hydroxy-L-proline was 80- to 200-fold greater than that from proline. The specific activity of pyrrole carboxylate in urine seemed significantly higher (3- to 4-fold) than that of free hydroxyproline in urine, whether proline or hydroxyproline was the radioactive compound administered (Table II). These values correct the much greater apparent discrepancy in specific activities, after administration of radioactive proline, reported on the basis of the less specific method for pyrrole carboxylate (1).

Fig. 2. Elution pattern of urinary radioactivity and carrier pyrrole carboxylate after administration of radioactive L-proline or radioactive hydroxy-L-proline. A, administration of 5.9 \( \times 10^8 \) dpm of hydroxy-L-[\( \text{U-}^{14}\text{C}\)]-proline to eight rats. The sample, isolated by Method 1, containing 2.3 \( \times 10^6 \) dpm and 0.5 pmol of added carrier pyrrole carboxylate, was eluted from the DC-1 resin column as described earlier (15). B, administration of 5.5 \( \times 10^8 \) dpm of L-[\( \text{U-}^{14}\text{C}\)]-proline to eight rats. The sample, isolated by Method 1, containing 6.2 \( \times 10^6 \) dpm and 0.5 pmol of carrier pyrrole carboxylate was treated like that in A. A \( \text{dc} \) (O- - -O) refers to the color developed with p-dimethylaminobenzaldehyde in 0.5-ml aliquots. Radioactivity is counts per min (● — ●) per 0.1 ml of the column eluate.

Pyrrole Carboxylate Excretion after Pyrrole Carboxylate Administration—Following intraperitoneal administration to rats of carboxyl-labeled \(^{14}\text{C}\)pyrrole carboxylate (10 mg), Letellier and Bouthillier earlier reported that one-quarter to one-third of urine radioactivity was free pyrrole carboxylate, a large fraction being distributed between the glycine and glucuronic acid conjugates of pyrrole carboxylate (27). In our experiments, using Method 2, administration of 3 μmol of unlabeled pyrrole carboxylate to each of six rats resulted in the recovery of only 6.7% of the dose as free pyrrole carboxylate with no accompanying increase in the pyrrole-reactive components which separate from true pyrrole carboxylate on the amino acid analyzer column (15). [\( 2-^{14}\text{C}\)]Pyrrole carboxylate (2.5 \( \times 10^4 \) dpm/rat) was administered intraperitoneally in three separate trials involving groups of 6 to 14 animals. The fraction of the dose excreted in the first 24-hour period ranged between 71 and 85%. Of the total radioactivity in urine, 88% (in each of the three trials) was retained in the aqueous phase after ether extraction. Essentially all of the radioactivity in the ether extract was recovered from the Dowex 50 column effluent (Method 1) and on further fractionation by the amino acid analyzer method (Method 2), was distributed between two peaks shown in Fig. 3A. The peak of free pyrrole carboxylate represented 4 to 5% of the administered dose, in agreement with results after administering the unlabeled compound.

Products of Allohydroxy-L-proline Metabolism—\( \Delta^1 \)Pyrrole 4-hydroxy-2-carboxylate is the only known direct product of the mammalian metabolism of the \( \text{p} \) epimers of hydroxyproline (4). To investigate the possibility that the pyrroline product is consumed in reactions other than that producing pyrrole carboxylate, both radioactive and unlabeled allohydroxy-\( \text{p} \)-proline, in separate experiments, were injected subcutaneously in rats. After administering 20 μmol of unlabeled allohydroxy-\( \text{p} \)-proline to each of six rats, urine was collected for 24 hours and the free pyrrole carboxylate and urinary hydroxyproline were measured. The total hydroxyproline excreted was 90 μmol, or 75% of the total amount administered. Free pyrrole carboxylate accounted for only 4.5 μmol, so that the 25 μmol unaccounted for could have been conjugated or metabolically altered pyrrole carboxylate.

To investigate this possibility, eight rats were injected subcutaneously with a total of 2.7 \( \times 10^8 \) dpm of allohydroxy-\( \text{p} \)-[\( \text{U-}^{14}\text{C}\)]-proline. Urine collected during the next 24 hours contained 51% of the administered radioactivity. Of total urine radioactivity, the ether extract contained 10%, all of which appeared to be in the pyrrole carboxylate peak (Fig. 3B). Examination of the aqueous phase following ether extraction indicated that essentially all the radioactivity was in residual allohydroxy-\( \text{p} \)-proline; hydroxy-L-proline accounted for less than 1% of the aqueous radioactivity. A small fraction of radioactivity in the aqueous phase
Radioactivity in Rat Urine Pyrrole Carboxylate after Administration of Radioactive L-Proline or Radioactive Hydroxy-L-Proline

Method 1 refers to the earlier method for isolating pyrrole carboxylate (11). Method 2 is based on measurement of radioactivity of the free pyrrole carboxylate peak (Fig. 2) after chromatographic purification.

| Compound administered                  | Radioactivity in urinary pyrrole carboxylate | Specific activity of urinary pyrrole carboxylate (Method 2) | Specific activity of urinary hydroxyproline |
|----------------------------------------|---------------------------------------------|-----------------------------------------------------------|------------------------------------------|
| L-[U-14C]Proline (8)*, 5.5 x 10^6 dpm  | 1.2 x 10^6                                 | 0.4 x 10^6                                                | 2.0 x 10^6                                |
| Hydroxy-L-[G-3H]Proline (5), 5.9 x 10^6 dpm  | 8.7 x 10^6                                 | 3.5 x 10^6                                                | 1.5 x 10^6                                |
| Hydroxy-L-[G-3H]Proline (6) 3.6 x 10^6 dpm | 1.1 x 10^4                                 | 2.6 x 10^4                                                | 1.5 x 10^4                                |

* Number of rats in each experiment is shown in parentheses.

Excretion of Pyrrole Carboxylate in Alkalized Urine—The findings above suggested that pyrrole carboxylate formed in vivo from hydroxy-L-proline or allohydroxy-D-proline, via Δ1-pyrroline-4-carboxylate, might arise by dehydration of the excreted pyrrrole compound in the urine rather than in the tissues of the animal; this would explain the extensive formation of pyrrole carboxylate derivatives when pyrrole carboxylate itself was administered to the rat, and their absence from the urine when pyrrole carboxylate was formed in vivo from a metabolic precursor. The relative stability of the pyrrole compound in alkaline solution suggested an experiment in which rat urine was made alkaline by NaHCO₃ ingestion. If a significant fraction of pyrrole carboxylate was formed in the urine, then this process might be sufficiently slowed in alkaline urine to permit accumulation of the pyrrole compound itself, and its detection as hydroxyproline after NaBH₄ reduction. In one such experiment, urine was collected every 2.5 hours for 58 hours from six rats; the urine pH varied between 7 and 8. At each 2.5-hour interval, 80% of that in the urine) could have been unextracted pyrrole carboxylate or tritiated water, but there was no significant radioactivity attributable to water-soluble pyrrole carboxylate conjugates. In contrast, after administration of radioactive pyrrole carboxylate, most of the urine radioactivity was not ether-extractable (presumably conjugates of pyrrole carboxylate), and the ether extract contained a prominent peak of radioactivity eluting before pyrrole carboxylate (Fig. 3A). These findings suggested that after pyrrole carboxylate administration, urinary pyrrole carboxylate is largely present as conjugates and other metabolites but that urinary pyrrole carboxylate formed from administered allohydroxy-D-proline is almost entirely free pyrrole carboxylate. The data noted above also imply a significant conversion of hydroxy-D-proline to products other than pyrrole carboxylate.

Effect of Antibiotic Treatment—The possibility has been noted (9) that hydroxy-L-proline might be converted to pyrrole carboxylate by way of preliminary epimerization to allohydroxy-D-proline in the rat intestine. In initial experiments outlined earlier (11), pyrrole carboxylate excretion after a large hydroxy-L-proline load was uninfluenced by essentially complete removal of fecal bacteria, as tested by aerobic colony formation. The high level of pyrrole carboxylate excreted in response to a hydroxy-L-proline load (average of 0.53 μmol/100 g in a control period; average of 0.60 μmol/100 g after gut sterilization) obviated the need for any antibiotic pretreatment before pyrrole carboxylate administration.
objection that pyrrole carboxylate was measured by Method 1; at this high level of pyrrole carboxylate, Method 1 and Method 2 gave equivalent results (see above). However, in a further test of the influence of antibiotic treatment on endogenous pyrrole carboxylate excretion, Method 1 also was used initially. Although there was no apparent effect of antibiotic treatment on endogenous pyrrole carboxylate excretion, the nonspecificity of the method for pyrrole carboxylate might have concealed real differences. Accordingly, the experiment was repeated with six rats, which were injected intraperitoneally with hydroxy-L-[3H]-proline (3.3 × 10^6 dpm as a total dose), both before and 3 days after beginning antibiotic treatment, when feces appeared sterile. Radioactivity in pyrrole carboxylate in both the pre- and post-treatment periods represented 0.05% of the dose, indicating no demonstrable effect of antibiotic treatment.

Studies with Tissue Preparations

Pyrrole Carboxylate Formation in Homogenates—In preliminary incubations of rat kidney and rat liver homogenates with L-proline or hydroxy-L-proline, hydroxy-L-proline appeared to be a better source of pyrrole carboxylate; at 0.2 m hydroxy-L-proline, 500 to 900 nmol of apparent pyrrole carboxylate were formed per g wet weight of kidney per hour. The reaction mixture, treated with p-dimethylaminobenzaldehyde, formed a colored product whose spectrum between 450 and 600 nm was identical with that produced by authentic pyrrole carboxylate. Liver homogenates had about 25% of the activity of kidney homogenates. In similar incubations with L-proline the formation of pyrrole carboxylate was not detectable. From the sensitivity of the colorimetric assay, less than 3 nmol of true pyrrole carboxylate per g of tissue per hour could have been formed from proline.

Stability of Pyrrole Carboxylate and of the Pyrrole Precursor of Pyrrole Carboxylate—The conversion of hydroxy-L-proline to pyrrole carboxylate in kidney homogenates implied the intermediate formation of N1-pyrrole-4-hydroxy-2-carboxylate, a known source of pyrrole carboxylate and a plausible oxidation product of hydroxy-L-proline. Pyrrole carboxylate itself was shown to be stable in kidney homogenates by direct addition and incubation under the same conditions. It was also necessary to test the stability of N1-pyrrole-4-hydroxy-2-carboxylate, since pyrrole carboxylate might be only one among other products of this intermediate. Because hydroxy-L-proline is predominantly oxidized to N1-pyrrole-3-hydroxy-5-carboxylate in kidney preparations (9, 10), allohydroxy-d-proline was used as a more efficient precursor of the ketimine pyrroline product, via d-amino acid oxidase catalysis. Accordingly, 1 μmol of this substrate was incubated with 0.5 ml of rat kidney homogenate under the conditions noted above. After 3 hours at 37°, assays showed the formation of 0.45 μmol of pyrrole carboxylate and the presence of 0.62 μmol of allo-hydroxy-d-proline.

Elimination of d-Amino Acid Oxidase as the Major Basis for Homogenate Activity—Pyrrole carboxylate formation from hydroxy-L-proline might have resulted from d-amino acid oxidase action on a contaminating d-hydroxyproline epimer. This was ruled out on two grounds. First, the hydroxy-L-proline used was shown by tests with crystalline d-amino acid oxidase (19) to contain less than 1 part of a d epimer in 200,000 parts of the L isomer, while the pyrrole carboxylate formed sometimes exceeded 1 part per thousand of the hydroxy-L-proline. Furthermore, most of the pyrrole carboxylate-forming activity in the homogenate was recovered in the supernatant fraction after centrifugation at 25,000 × g for 2 hours, while most of the d-amino acid oxidase activity (measured by pyrrole carboxylate formation from allohydroxy-

| Table III |
| --- |
| **Purification of rat kidney L-amino acid oxidase** |
| Purification and assay methods are referred to in the text. |

| Fraction | Total protein | Total units | -Fold purification | Ratio of units of L-leucine to hydroxy-L-proline |
| --- | --- | --- | --- | --- |
| 1. Homogenate | 10,170 | 2800 | 1.0 | 5.0 |
| 2. Supernatant, 700 × g | 10,380 | 2880 | 1.4 | 3.7 |
| 3. Supernatant, 54,000 × g | 3,840 | 1330 | 2.5 | 5.4 |
| 4. (NH₄)₂SO₄, 0.33-0.55 fraction | 890 | 730 | 5.7 | 10.0 |
| 5. First DEAE | 230 | 520 | 16 | 4.7 |
| 6. (NH₄)₂SO₄, 0.45 plus 0.52 fractions | 70 | 360 | 33 | 5.2 |
| 7. Second DEAE | 18 | 130 | 50 | 3.4 |
| 8. Sephadex G-200 | 6 | 67 | 81 | 4.0 |
| 9. Polyacrylamide gel eluate | — | — | — | — |

* With L-leucine as substrate.
* Polyacrylamide gel electrophoresis yielded three bands; the major band, estimated at 45% of total protein by densitometry, corresponded to the enzyme activity. Approximately 30% of both the L-leucine and hydroxy-L-proline-oxidizing activity applied to the gel was recovered from this region. The -fold purification is based on the estimated removal of non-enzyme protein.
catalyzed by L-amino acid oxidase, since this step would remove similar to those shown for the purified enzyme.

The enzyme was Fraction 8 (Table III).

The data of Table V show that a large subcutaneously administered 14 g of rat kidney) and hydroxy-L-proline, l-proline, or glycine as shown, in a final volume of 3 ml. The final pH was adjusted to 7.0 where necessary. Incubation was carried out at 37°C in air for 2 hours. The reaction was stopped by adding 0.8 ml of 10% trichloroacetic acid and the supernatant solutions were analyzed for hydroxyproline and Δ-5-pyrroline-3-hydroxy-5-carboxylate as noted under "Methods." Total Δ-5-pyrroline refers to the assay with o-aminobenzaldehyde; micromoles were calculated on the basis of the Δ-5-pyrroline-5-carboxylate standard.

Inhibition of Hydroxy-L-proline Oxidase by L-Proline—One possible explanation for the slight stimulation of pyrroline carboxylate excretion by administration of proline loads (Fig. 1) is selective inhibition by proline of the major pathway of hydroxy-L-proline oxidation, i.e. oxidation to Δ-5-pyrroline-3-hydroxy-5-carboxylate (9, 10). To investigate this, the effect of proline on tissue levels of hydroxy-L-proline was examined; in addition, direct inhibition by proline of crude hydroxy-L-proline oxidase was tested.

The data of Table V show that a large subcutaneously administered L-proline load decreased the free hydroxy-L-proline in liver. Direct tests showed that, at high concentrations, L-proline markedly inhibited formation of the major pyrroline oxidation product of hydroxy-L-proline (Table VI). Δ-5-Pyrroline-3-hydroxy-5-carboxylate proved stable after addition to these incubation mixtures, as also may be inferred from the stoichiometric balance between added hydroxy-L-proline, hydroxy-L-proline remaining, and Δ-5-pyrroline-hydroxy-5-carboxylate formed (Table VI).
**Pyrrole Carboxylate Formation and Excretion in Guinea Pigs**

The reported absence of L-amino acid oxidase from guinea pig kidney (29) prompted an examination of guinea pig urine for its content of pyrrole carboxylate. After subcutaneous administration of hydroxy-L-[5-3H]proline to male albino guinea pigs, excretion of radioactive pyrrole carboxylate (Method 1) was only one-fifth to one-tenth the comparably measured value in the rat, not correcting for the body weight differences. Endogenous excretion of unlabeled pyrrole carboxylate (Method 1) was only about one-fourth that measured comparably in rat urine (11). Because Method 1 later was shown to overestimate true pyrrole carboxylate both in rat urine and human urine, even these low values for guinea pig urine may be falsely high, and from our present data we cannot be certain that any pyrrole carboxylate is normally excreted in guinea pig urine.

In agreement with early assays (29), we were unable to demonstrate unequivocal L-amino acid oxidase in homogenates of guinea pig kidney, either with L-leucine or hydroxy-L-proline as substrate; pyrrole carboxylate formation from hydroxy-L-proline was no greater than 5% that of rat kidney homogenates. This was not explained by instability of pyrrole carboxylate or of \( \Delta^1 \)-pyrroline-4-hydroxy-2-carboxylate in guinea pig kidney as tested by additions to homogenates.

**Observations with Human Kidney Preparations**

In contrast with the indecisive data on pyrrole carboxylate excretion by guinea pigs, there is clear evidence for pyrrole carboxylate excretion by man (15). In preliminary results with human kidney preparations, L-leucine oxidation could not be demonstrated either with a homogenate or with a fraction purified through ammonium sulfate precipitation to the stage of Fraction 4, Table III. However, apparent formation of pyrrole carboxylate from 0.4 M hydroxy-L-proline was catalyzed by the ammonium sulfate fraction, and the activity was inhibited over 90% by \( 8 \times 10^{-4} \) M benzole. The fractions tested contained \( \Delta^1 \)-amino acid oxidase, as detected by allohydroxy-\( D \)-proline oxidation, but the quantity of hydroxy-L-proline converted to pyrrole carboxylate (0.003%) exceeded the calculated contamination of hydroxy-L-proline with a \( \Delta^1 \)-epimer. The low level of reaction (less than 1% that of a rat kidney homogenate) is compatible with catalysis of pyrrole carboxylate formation from hydroxy-L-proline by \( \Delta^1 \)-amino acid oxidase (19) rather than by an L-amino acid oxidase of human kidney.

**Discussion**

Present data support the earlier conclusion, based on more limited and partly erroneous measurements (11), that in the rat exogenous hydroxy-L-proline is a far better source of urinary pyrrole carboxylate than is exogenous L-proline. In man (15), similarly, urinary pyrrole carboxylate was elevated either by an oral hydroxy-L-proline load or in congenital hydroxyprolinemia. In the rat, radioactive hydroxy-L-proline was a more efficient source of pyrrole carboxylate than was radioactive proline by 2 orders of magnitude, a difference which would appear to exceed the bias of diluting the labeled precursor into a larger body pool (Table V).

Further questions concern the site and mechanism of hydroxy-L-proline conversion to pyrrole carboxylate. Our findings, summarized in part earlier (11), and subsequent data provided by Iguchi et al. (12), implicate the L-amino acid oxidase of rat kidney. The observations detailed here conclusively extend earlier findings in that the homogeneous enzyme was shown to act on hydroxy-L-proline; additionally, in crude kidney homogenates the L-amino acid oxidase reaction accounted for most of the relevant oxidizing activity. This question was raised by our finding, reported separately (19), that mammalian kidney \( \Delta^1 \)-amino acid oxidase also catalyzes a slow oxidation of hydroxy-L-proline to the same pyrroline product. Perhaps surprisingly, in view of the many examples in which intestinal flora account for trace metabolic conversions in mammals (30), effective sterilization of the gut had no influence either on the excretion of pyrrole carboxylate following a hydroxy-L-proline load, or on its endogenous production.

In connection with the above experiments, two findings remain unexplained. Administration of unlabeled L-proline resulted in a small (not more than 3-fold), but seemingly definite, increase in urinary pyrrole carboxylate (Fig. 1). Possible explanations include stimulation of collagen turnover by a large proline load, as well as interference by high proline blood levels with the renal tubular reabsorption of pyrrole carboxylate, or of its precursor, \( \Delta^1 \)-pyrroline-4-hydroxy-2-carboxylate. We have investigated another possibility: that L-proline inhibits the main pathway oxidation of hydroxy-L-proline and hence makes the latter more accessible to the minor oxidative pathway via L-amino acid oxidase. Our findings were unexpected in that free hydroxy-L-proline of liver was decreased rather than increased, after a proline load. Experiments with kidney homogenates, however, did indicate marked inhibition of hydroxy-L-proline oxidase by high concentrations of L-proline, an observation not previously reported, to our knowledge. These findings, while of interest in themselves, did not provide an explanation for the stimulation of pyrrole carboxylate excretion by a proline load. A second observation that we cannot now explain is the 3- to 4-fold higher specific radioactivity of urinary pyrrole carboxylate than that expected from the specific radioactivity of the free hydroxy-L-proline of urine, whether the source of label was L-[\( U \)-\( 14 \)C]proline or hydroxy-L-[G-3H]proline (Table II). It should be noted that, in contrast, the expected specific activity ratio was obtained in the pyrrole carboxylate formed from hydroxy-L-proline by kidney homogenates. Since the same specific activity increment in pyrrole carboxylate, over that of the urinary hydroxyproline, resulted from administering either tritiated hydroxy-L-proline or carbon-labeled L-proline, both an isotope effect peculiar to tritium labeling, or a metabolic fate peculiar to proline seem doubtful as explanations. As an alternative we suggest possible time- or place-compartmentation of hydroxyproline oxidation (to the cyclic ketimine) relative to hydroxyproline excretion.

Another question concerns the site at which pyrrole carboxyl-
ate is formed. Several findings favor the conclusion that, as a product of administered hydroxy-L-proline, pyrrole carboxylate is formed in the urine from the labile ketimine oxidation product of hydroxy-L-proline, Δ¹-pyrroline-4-hydroxy-2-carboxylate. Thus, administered pyrrole carboxylate is converted almost entirely to urinary metabolic products, largely water-soluble, both in the present studies and in man (15). In contrast, when pyrrole carboxylate was formed in vivo from radioactive allolhydroxy-o-proline, no water-soluble radioactivity was detected in the urine other than residual allolhydroxy-o-proline itself, and no radioactive peaks in the ether-soluble fraction of urine were found other than free pyrrole carboxylate (Fig. 3E). Alkalination of rat urine, to stabilize the presumptive pyrroline precursor of endogenous origin, gave suggestive but not conclusive support to the postulation above: from these trials 50% of the pyrroline carboxylate could have been present in alkaline urine as the pyrrole precursor. These experiments would have appeared conclusive had none or all of the endogenously formed pyrrole carboxylate behaved as if derived from an alkali-stable, NaBH₄-reducible precursor. The result obtained is consistent with the hypothesis examined if the relatively slow rate of dehydration of Δ¹-pyrroline-4-hydroxy-2-carboxylate measured in simple solutions at pH 7 to 8 is accelerated by unknown components of urine. At present, our findings occur consistent with the thesis that much or all of the urinary pyrrole carboxylate, either of endogenous origin or after precursor administration, results from excretion of Δ¹-pyrroline-4-hydroxy-2-carboxylate and its conversion to pyrrole carboxylate at some stage after the formation of urine.

While our data are consistent with endogenous free hydroxy-L-proline as the major, if not the only, source of urinary pyrrole carboxylate, a number of alternative hypothetical pathways cannot be excluded. These include possible formation of pyrrole carboxylate from 3,4-dehydroproline (39) (itself hypothetically derivable from L-proline) from the in vivo degradation of sialic acids, from γ-hydroxyornithine (by transamination at the α-amino group (4)), or from 3-hydroxyproline. Certain of these possibilities appear unlikely from our data. Thus, radioactive L-proline, a likely precursor of 3,4-dehydroproline on a priori grounds, was not a significant precursor of pyrrole carboxylate beyond the rate compatible with conversion of L-proline to hydroxy-L-proline, nor did administration of unlabeled N-acetylenuraminate support the sialic acids as a physiological source (2). While γ-hydroxyornithine might give rise, by a transamination, to the pyrrole precursor of pyrrole carboxylate, there is no evidence for the former compound in animal tissues.

The role of L-amino acid oxidase in the formation of Δ¹-pyrroline-4-hydroxy-2-carboxylate from hydroxy-L-proline is well supported by our studies with rats. The low, or nil, pyrrole carboxylate excretion by guinea pigs is consistent with our inability (and that reported earlier (29)) to demonstrate unambiguous L-amino acid oxidase in guinea pig kidney. Adult humans excrete approximately 15-fold less pyrrole carboxylate, on a body weight basis, than do the immature rats studied in the present work; the estimated daily release of free hydroxy-L-proline from collagen in the two cases corresponds approximately to this ratio (15), and evidence like that cited for the rat also implicates hydrox-L-proline as a source of pyrrole carboxylate in human urine (15). Yet, activity for hydroxy-L-proline oxidation to the cyclic ketimine was barely demonstrable in human kidney homogenates, so that in man other pathways for this reaction should be considered.

Certain of the observations reported here bear on the metabolic fate of allolhydroxy-o-proline or Δ¹-pyrroline-4-hydroxy-2-carboxylate formed from it. In studies of kidney homogenates, we could demonstrate satisfactory balances, indicating that the pyrrole compound was stable under our incubation conditions and was the only product of allolhydroxy-o-proline. However, results after administering allolhydroxy-o-[G-3H]-proline in vivo pointed to extensive metabolism either of allolhydroxy-o-proline or its pyrrole product, since only 50% of the administered tritium was recovered. The possibility that the missing radioactivity had been diluted in body H₂O was considered under “Results.” A plausible product is 3-hydroxy-4-aminoobutyrate, earlier demonstrated to arise from Δ¹-pyrroline-4-hydroxy-2-carboxylate in in vitro experiments (4). As noted above, kidney homogenates did not catalyze formation of this product in our experiments, possibly because of the inclusion of catalase in incubation mixtures. A parenthetic point of interest was our failure to find radioactive hydroxy-L-proline in rat urine after administering allolhydroxy-o-[G-3H]-proline. This also argues against significant epimerization of hydroxyproline by gut bacteria in vivo (or reabsorption of the reaction product) and separately, implies that enzymatic reduction of Δ¹-pyrroline-4-hydroxy-2-carboxylate to hydroxy-L-proline is not a significant reaction in the rat; the corresponding conversion of Δ¹-pyrroline-2-carboxylate to L-proline was observed in several rat tissues (40).

Finally, the possible application of our findings merits some comment. The reaction we have studied would appear to represent a new pathway (although a minor one) for the metabolism of hydroxy-L-proline in mammals. Measurement of urinary pyrrole carboxylate therefore provides a possible index of collagen turnover which might respond differently to various alterations of collagen metabolism than do other indices such as urinary hydroxyproline (41). As yet we have no information under this heading, and ready accumulation of data would need a simpler assay for free pyrrole carboxylate than the one we have developed.

Some preliminary findings of ours are worth noting. Although most of the studies in this paper were based on immature male rats, a few observations with female rats of the same age and strain indicated no sex difference, by the relatively undiscriminating procedure of Method 1. Administration to rats of triiodothyronine (100 µg/100 g of body weight) led to no significant increase in urinary pyrrole carboxylate or in the apparent activity of kidney L-amino acid oxidase. Urinary pyrrole carboxylate in this case also was measured by the earlier method, which includes pyrrole carboxylate-unrelated components; however, any prominent increase in true pyrrole carboxylate excretion would have been detected. Our findings in this respect do not support the conclusion of Yaminishi et al. (14) that pyrrole carboxylate is increased in human hyperthyroidism.

REFERENCES

1. HEACOCK, A. M., AND ADAMS, E. (1973) Fed. Proc. 32, 613
2. HEACOCK, A. M. (1974) Ph.D. Thesis, University of Maryland
3. GOTTESCHALK, A. (1955) Biochem. J. 61, 298-307
4. RADHIKRISHNAN, A. N., AND MEISTER, A. (1957) J. Biol. Chem. 226, 559-571
5. YONEYA, T., AND ADAMS, E. (1961) J. Biol. Chem. 236, 3273-3279
6. LETELLIER, G., AND BOUTHILLIER, L. P. (1956) Can. J. Biochem. Physiol. 34, 1129-1139
7. GERBER, G. B., GERBER, G., AND ALTMAN, K. I. (1960) Nature 186, 767-768
8. ADAMS, E., AND GOLDSKODE, A. (1960) J. Biol. Chem. 235, 3492-3498
9. ADAMS, E. (1970) Int. Rev. Conn. Tissue Res. 5, 1-91
10. ADAMS, E. (1973) Mol. Cell. Biochem. 2, 109-119
11. HEACOCK, A. M., AND ADAMS, E. (1973) Biochem. Biophys. Res. Commun. 50, 392–397
12. IGUCHI, M., KIKUO, M., OHYAMA, H., AND MATSUMURA, Y. (1973) Biochim. Biophys. Acta 315, 285–292
13. GERBER, G. B., TRÄNELS, K. R., WOOD, D., AND ALTMAN, K. I. (1964) Clin. Chim. Acta 9, 185–187
14. YAMINISHI, Y., IGUCHI, M., OHYAMA, H., AND MATSUMURA, Y. (1972) J. Clin. Endocrinol. Metab. 35, 55–58
15. HEACOCK, A. M., AND ADAMS, E. (1974) J. Clin. Invest. 54, 810–818
16. GRYDER, R. M., AND ADAMS, E. (1969) J. Bacteriol. 97, 294–306
17. WILLIAMS, I. H., AND FRANK, L. H. (1974) Anal. Biochem. in press
18. KREBS, H. A. (1939) Enzymologia 7, 53–57
19. HEACOCK, A. M., AND ADAMS, E. (1974) Biochem. Biophys. Res. Commun. 57, 279–285
20. NEUMAN, R. E., AND LOGAN, M. A. (1950) J. Biol. Chem. 184, 200–306
21. PIEZ, K. A., IRREVERRE, F., AND WOLFF, H. L. (1966) J. Biol. Chem. 231, 687–697
22. STRECKER, H. J. (1957) J. Biol. Chem. 225, 823–834
23. NAKANO, M., SAGA, M., AND TSUTSUMI, Y. (1969) Biochim. Biophys. Acta 187, 19–30
24. NAKANO, M., AND Danowski, T. S. (1966) J. Biol. Chem. 241, 2075–2083
25. FRIEDEMANN, T. E., AND HAUGEN, G. E. (1943) J. Biol. Chem. 147, 415–442
26. WARBURG, O., AND CHRISTIAN, W. (1942) Biochem. Z. 310, 384–421
27. LETTELIER, G., AND BOUTHILLIER, L. P. (1957) Can. J. Biochem. Physiol. 35, 811–817
28. FINLAY, T. H., AND ADAMS, E. (1970) J. Biol. Chem. 245, 5248–5260
29. BLANCHARD, M., GREEN, D. E., NUKETO, V., AND RATNER, S. (1944) J. Biol. Chem. 156, 421–440
30. HARDMAN, K. (1973) Physiol. Rev. 53, 496–534
31. FINERMAN, G. A. M., DOWNING, S., AND ROSENBERG, L. E. (1967) Biochim. Biophys. Acta 135, 1008–1015
32. ROJKIND, M., AND DIAZ DE LEÓN, L. (1970) Biochim. Biophys. Acta 217, 512–522
33. KERSHENORICH, D., FIERRO, F. J., AND ROJKIND, M. (1970) J. Clin. Invest 49, 2246–2249
34. CHVAPIL, M., AND RYAN, J. N. (1973) Agents Actions 3, 38–44
35. WOLF, G., AND BERGER, C. R. A. (1958) J. Biol. Chem. 230, 231–240
36. WISS, O. (1949) Helv. Chim. Acta 32, 149–163
37. SUMMER, G. K. (1961) Science 134, 1527–1528
38. KAFFRAMER, J., AND MUHN, G. (1956) Hoppe-Seyler's Z. Physiol. Chem. 306, 78–83
39. WELLNER, D., AND SCANNONE, H. (1964) Biochemistry 3, 1746–1749
40. MEISTER, A., RADHAKRISHNAN, A. N., AND BUCKLEY, S. D. (1957) J. Biol. Chem. 229, 785–800
41. KIVIRIKKO, K. I. (1970) Int. Rev. Conn. Tissue Res. 5, 93–163
Formation and excretion of pyrrole-2-carboxylic acid. Whole animal and enzyme studies in the rat.
A M Heacock and E Adams

J. Biol. Chem. 1975, 250:2599-2608.

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