The Metabolism of the Methyl Moiety of Aspartame

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Reviewed and Approved:

R. E. Ranney, Ph.D.
Director
Department of Drug Metabolism
& Radiochemistry

Contributors to this Report

Department of Drug Metabolism and Radiochemistry

Dr. R. E. Ranney, Director
Ms. P. A. McKeown, Administrative Secretary

Peer Review Panel

Department of Drug Metabolism and Radiochemistry

Dr. James A. Oppermann, Group Leader

Department of Biology

Dr. J. Potts, Associate Director

Department of Analytical Resources and Methodology

Dr. T. Hutsell, Group Leader

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I. Abstract

Aspartame (3-amino-N(α carboxyphenethyl)succinamic acid, methyl ester; the methyl ester of aspartylphenylalanine, SC-18862) is hydrolyzed in the gut to yield aspartic acid, phenylalanine and methanol. This review of the literature describes the metabolic paths followed by methanol in its conversion to CO_2 or its incorporation into body constituents. Methanol is oxidized to formaldehyde by alcohol dehydrogenase; formaldehyde is transformed to formate by a specific formaldehyde dehydrogenase; and formate is converted to CO_2 by a catalase/peroxidase system. About 70% of the ^{14}C from [Me-^{14}C]-aspartame follows this path to $^{14}CO_2$.

Formaldehyde and formate may enter into the biosynthesis of purines, pyrimidines and amino acids by entry into the one-carbon metabolic pool by formylation of tetrahydrofolic acid. About 25% of the ^{14}C from [Me-^{14}C]-aspartame enters into such transmethylation or formylation biosynthetic reactions.

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II. Introduction

Aspartame (3-amino-N-(α carboxyphenethyl)succinamic acid, methyl ester; the methyl ester of aspartylphenylalanine; SC-18862) is a sweetening agent that organoleptically has about 180 times the sweetness of sugar (1, 2). In considering the metabolism of this dipeptide, it is evident that extensive degradation of the compound may occur after it enters the digestive tract. The compound is stable in an acid medium and would be expected to be little changed by the gastric juice. However, in the small intestine chymotrypsin would hydrolyze the methyl group through its amino acid esterase activity (3), and the peptide hydrolases of the microvillar membrane would cleave the dipeptide to its constituent amino acids (4). If this were the case, then all moieties of the compound, methanol, phenylalanine and aspartic acid, should be handled by the body as natural constituents of the diet.

In a previous report, Oppermann et al. (5) have compared in monkeys the overall disposition of ^{14}C from [^{14}C]-methanol with the biotransformations of the methyl group of [Me-^{14}C]-aspartame. Ranney et al. (6) have described the comparative metabolism of these two compounds in various species. The present report describes from the literature the stepwise transformation of methanol to CO_2 as well as the alternate pathways the methyl group may follow through the one-carbon metabolic pool.

Recent reviews which have considered different parts of these metabolic pathways have been that of Stokstad and Koch (7) on the role of folic acid in the one-carbon metabolic pool, and that of Tephly et al. (8) which describes the biochemical toxicology of methanol.

III. The Absorption and Disposition of ^{14}C from [Me-^{14}C]-Aspartame

Oppermann et al. (5, 9) have compared in rats and monkeys the disposition of ^{14}C in plasma, urine, feces and expired air after intragastric administration of either [^{14}C]-methanol or [Me-^{14}C]-aspartame. Their data (Fig. 1) showed that after administration of equimolar amounts of either labeled compound to monkeys the conversion of administered ^{14}C to respiratory $^{14}CO_2$ occurred to the same extent with both compounds. This suggests that the methyl moiety was rapidly and completely cleaved from aspartame, and that this methyl group was oxidized in much the same manner as methanol. However, during the first hour after aspartame administration, there was [illegible] $^{14}CO_2$ expired, whereas ^{14}C output after methanol treatment was very rapid. This difference may be explained by ready absorption of methanol from the stomach while aspartame must have had to pass into the small intestine before hydrolysis and absorption occurred. Over an 8 hour period about 70% of the ^{14}C from either [^{14}C]-methanol or [^{14}C]-aspartame was excreted in the expired air.

In Fig. 2 the plasma levels of ^{14}C following [^{14}C]-methanol or [Me-^{14}C]-aspartame administration are shown. It can be seen that with both compounds the plasma levels of ^{14}C were low and that the dis-

appearance of radioactivity was slow. Furthermore, with both aspartame and methanol the rates of decline of radioactivity appeared to change with time. This may be due to the incorporation of the label into two or more metabolic pools, each of which had a different turnover time. Fig. 2 also illustrates the delay in the absorption of the label after its administration as part of aspartame.

The initial hydrolytic reaction which occurred when aspartame entered the small intestine was:

$$\text{aspartame} \xrightarrow[\text{esterases}]{\text{intestinal}} \text{methanol} + \text{aspartylphenylalanine} \qquad [1]$$

The major enzyme which participated in this reaction was chymotrypsin (EC 3.4.4.5), a very active esterase as well as a proteolytic enzyme (3).

IV. The Oxidation of Methanol to CO_2

The rapid appearance of $^{14}CO_2$ in the expired air after methanol administration to monkeys (Fig. 1) indicates that oxidation of the compound was initiated as soon as it entered the liver. This is presumably a "first pass effect" (10) which is the result of absorption and transport to the liver through the hepatic portal system.

The first event in the series of reactions that leads to CO_2 formation is the oxidation of methanol to formaldehyde. Two enzymatic systems may be responsible for this. In the rat, Tephly et al. (11) found this was mediated by a catalase:peroxidase system. Catalase (EC 1.11.1.6) provided the oxidizing hydrogen peroxide:

$$2\ H_2O + O_2 \xrightarrow{\text{catalase}} 2\ H_2O_2 \qquad [2]$$

and methanol acted as an electron donor for the peroxidase (EC 1.11.1.7) system:

$$CH_3OH + H_2O_2 \xrightarrow{\text{peroxidase}} CH_2O + 2\ H_2O \qquad [3]$$

There are species differences in this reaction, however, since Makar et al. (12) observed that in the monkey alcohol dehydrogenase (EC 1.1.1.1) was the enzyme responsible for the transformation of methanol to formaldehyde:

$$CH_3OH + 2\ NAD \xrightarrow{\text{alcohol dehydrogenase}} CH_2O + 2\ NADH \qquad [4]$$

This apparently was the dominant methanol metabolizing system in man also (13).

A specific dehydrogenase (EC 1.2.1.1, FDH) for formaldehyde was first described by Strittmatter and Ball (14). This system has been found to be glutathione (GSH) and NAD dependent:

$$CH_2O + GSH \longrightarrow HO\text{-}CH_2\text{-}SG$$

$$HO\text{-}CH_2\text{-}SG + FDH \longrightarrow [HO\text{-}CH_2\text{-}SG]FDH$$

$$[HO\text{-}CH_2\text{-}SG]FDH + NAD \longrightarrow [HCO\text{-}SG]FDH + NADH + H^+$$

$$[HCO\text{-}SG]FDH + H_2O \longrightarrow FDH + GSH + HCOOH \qquad [5]$$

This enzyme system has been found by Goodman and Tephly (13) to occur in both rat and human liver and it was observed to be specific in that it was unreactive toward acetaldehyde and benzaldehyde. The activity of FDH in human liver was several fold higher than in rat liver.

Minor alternate pathways for formaldehyde have been described by Abels and Lee (15) and by Hift and Mahler (16). These reactions were, respectively, alcohol dehydrogenase (EC 1.1.1.1, ADH):

$$CH_2O + NAD \xrightarrow{ADH} HCOOH + NADH$$

$$CH_2O + NADH \xrightarrow{ADH} CH_3OH + NAD$$

$$2\ CH_2O \xrightarrow{ADH} HCOOH + CH_3OH \qquad [6]$$

and an unclassified pyruvate:formaldehyde ligase:

$$CH_2O + CH_3\text{-}CO\text{-}COOH \longrightarrow CH_2OH\text{-}CH_2\text{-}CO\text{-}COOH \qquad [7]$$

Formic acid formed in the reactions described above may enter one of two major metabolic pathways. The major path is oxidation to CO_2. This is shown in Fig. 1, where about 70% of the ^{14}C from either [^{14}C]-methanol or [Me-^{14}C]-aspartame was converted to CO_2. The second pathway is to enter into the one-carbon metabolic pool via formylation of tetrahydrofolic acid. The latter reactions will be described in the next section of this report.

The oxidation of formate to CO_2 is carried out by a catalase:peroxidase system as is the oxidation of methanol in the rat [Eq. 2]. Chance (17) demonstrated that, in the rat, this system reacted with formate and with methanol at about the same rates. For formate oxidation the reactions were:

$$2\ H_2O + O_2 \xrightarrow{\text{catalase}} 2\ H_2O_2 \qquad [2]$$

$$HCOOH + H_2O_2 \xrightarrow{\text{peroxidase}} CO_2 + 2\ H_2O \qquad [8]$$

This in vitro identification of the route of formate oxidation was confirmed in rats in vivo by Nakada and Weinhouse (18).

V. Incorporation of Formate into Body Constituents

Although about 70% of the ^{14}C from [^{14}C]-methanol or [Me-^{14}C]-aspartame given to monkeys was oxidized to $^{14}CO_2$ about 25% was incorporated into body constituents while the remaining 5% was excreted in the urine and feces.

The entrance of formaldehyde and formate into amino acids, purine and pyrimidine body pools is via the one-carbon metabolic path for which folic acid is a precursor coenzyme (7). The structure of folic acid (pteroylglutamic acid, PtGlu) and the structures of the active formyl derivatives of folic acid are given in Fig. 3.

The major reactions which occur are: reduction of the pyrazine ring to yield tetrahydrofolic acid (H_4PtGlu); formylation at N^5 resulting in N^5-formyltetrahydrofolic acid; formylation at N^{10} to yield N^{10}-formyltetrahydrofolic acid; and the reaction with formiminoglycine or formiminoglutamate to yield N^5-formiminotetrahydrofolic acid. Other derivatives have also been described at methyl, formaldehyde and formate levels of oxidation.

Formate must be activated before it can enter into folate-dependent formylation reactions. An example of this is the formation of 10-CHO-H_4PtGlu from H_4PtGlu by the enzyme formyltetrahydrofolate synthetase (EC 6.3.4.3):

$$H_4PtGlu + HCOOH + ATP \longrightarrow 10\text{-}CHO\text{-}H_4PtGlu + ADP + P_i \quad [9]$$

This formylated folate derivative may be a methyl group donor for purine and pyrimidine biosynthesis.

Another reaction in which the methyl group from aspartame may enter the one-carbon pool is the reaction of formaldehyde with H_4PtGlu:

$$CH_2O + H_4PtGlu \longrightarrow 5,\ 10\text{-}CH_2 = H_4PtGlu \quad [10]$$

This is a non-enzymatic reaction. The product is reduced to form the $5\text{-}CH_3$ derivative which participates in methionine synthesis by 5, 10 methylene tetrahydrofolate reductase (EC 1.1.1.68):

$$5,\ 10\text{-}CH_2 = H_4PtGlu + FADH_2 \longrightarrow 5\text{-}CH_3 - H_4PtGlu + FAD \quad [11]$$

This compound acts as a methyl donor in an unclassified methyl transferase system that is cobalamin, FADH and S-adenosylmethionine dependent:

$$5\text{-}CH_3\text{-}H_4PtGlu + \text{homocysteine} \xrightarrow{FADH_2 + \text{cobalamin} + \text{S-adenosylmethionine}} \text{methionine} + H_4PtGlu \quad [12]$$

The methyl groups that are synthesized by folic acid-dependent enzyme systems are the labile methyl groups that serve in transmethylation reactions such as those involving choline and creatine. Fig. 4 summarizes reactions involving activated formate and formaldehyde.

A flow diagram which summarizes the various metabolic pathways followed by the methyl group of aspartame is shown in Fig. 5.

VI. The Nutritional Value of Methanol

Dietary methanol is derived in large part from fresh fruits and vegetables. It occurs as the free alcohol or esterified with fatty acids, or as the product resulting from the hydrolysis of methoxy groups of polysaccharides such as pectin (19-23). In fruit juices, the methanol content may range from 12 to 640 mg/liter with an average of 140 mg/liter (23). Fermented and distilled beverages may have an even greater methanol content than this, some neutral spirits having as much as 1.5 g/liter (23). Dietary methanol is metabolized in the body primarily to CO_2 (Part IV), although some may be excreted unchanged in the breath (24).

Concern that the use of aspartame as an artificial sweetening agent might lead to the ingestion of undesirable amounts of methanol seems groundless. Since this compound has an organoleptic sweetness of about 180 times that of sugar (2), much less aspartame will be needed to give an equivalent taste. For example, it has been estimated that an instant-mix soft drink adequately sweetened with aspartame would contain 0.4 g/liter. In terms of hydrolysis of the methyl group, this would be equivalent to 40 mg methanol/liter. This value is considerably less than the amount listed by Francot and Geoffroy (23) as the average content of fruit juices (140 mg/liter). The caloric value of methanol is 4.6 calorie/g.

VII. Endogenous Sources of Methanol

Methanol has been found to be a normal constituent of expired air and saliva and is detectable in the blood (24, 25). Because of the variability of methanol concentrations in the breath, Erickson and Kulkarni (24) concluded that dietary sources were only partial contributors to the total body pool of methanol.

Axelrod and Daly (26) confirmed this suggestion by identifying an unclassified methyltransferase enzyme in the pituitaries of rats, rabbits and man which formed methanol from S-adenosylmethionine:

$$\text{S-adenosylmethionine} + H_2O \longrightarrow \text{S-adenosylhomocysteine} + CH_3OH \quad [13]$$

The exact mechanism of the reaction was not identified and may have occurred by either the methylation of water or hydrolysis of the substrate S-adenosylmethionine. These investigators found the greatest activity of the enzyme in the posterior pituitary, and a survey of 10 other tissues showed negligible activity of the methanol-forming enzyme.

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IX. Figures

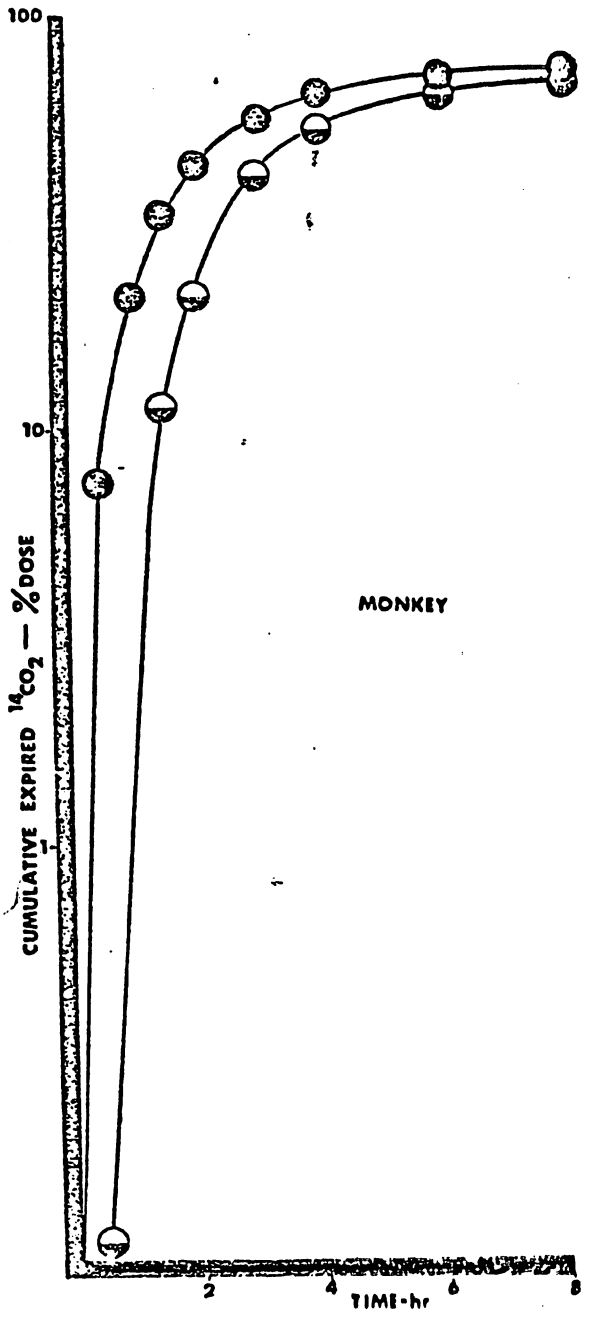

Fig. 1: Mean cumulative excretion of ^{14}C in expired air of monkeys given equimolar (0.068 mmoles/kg) oral doses of [^{14}C]-methanol (⊖——⊖) or [Me-^{14}C]-aspartame (⊖——⊖). Units: ordinate: cumulative percent of ^{14}C in expired air; abscissa: hours after administration of compounds (Fig. from ref. 9).

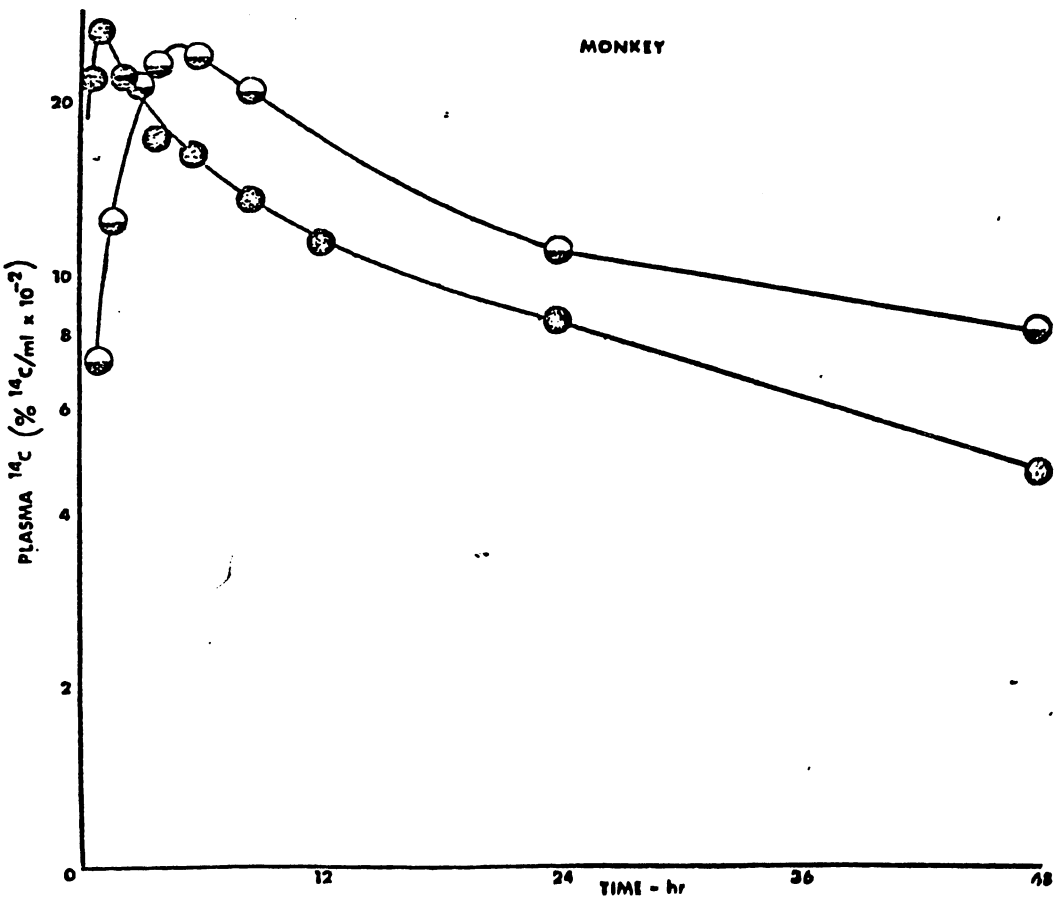

Fig. 2: Mean plasma ^{14}C of monkeys given equimolar (0.068 mmoles/kg) oral doses of [^{14}C]-methanol (●——○) or [Me-^{14}C]-aspartame (●——●). Units: ordinate: percent of ^{14}C/ml plasma; abscissa: hours after administration of compounds. (Fig. from ref. 9).

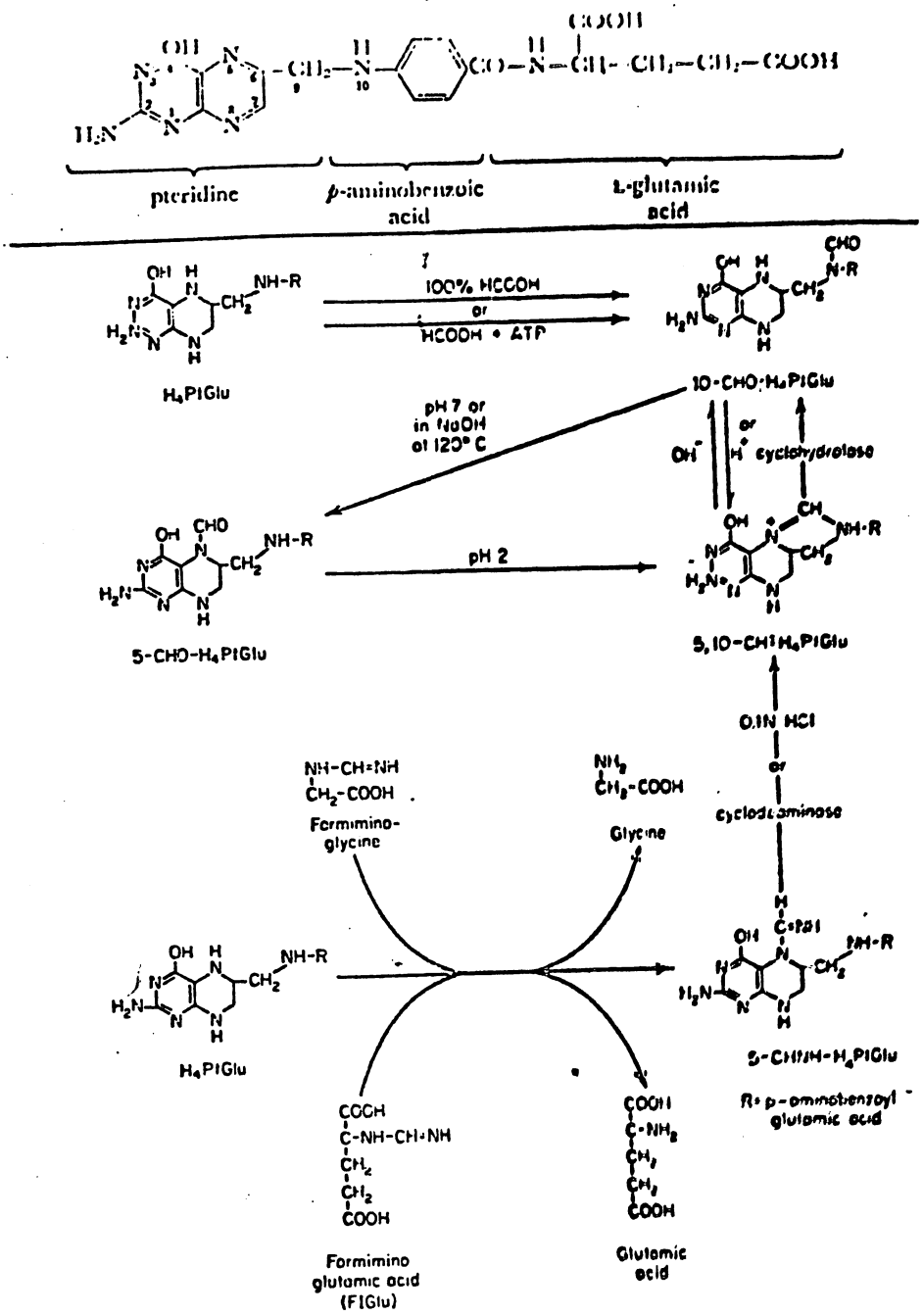

Fig 3: The structure of folic acid (above) and the reactions of formyl derivatives of folic acid (Fig. from ref. 7).

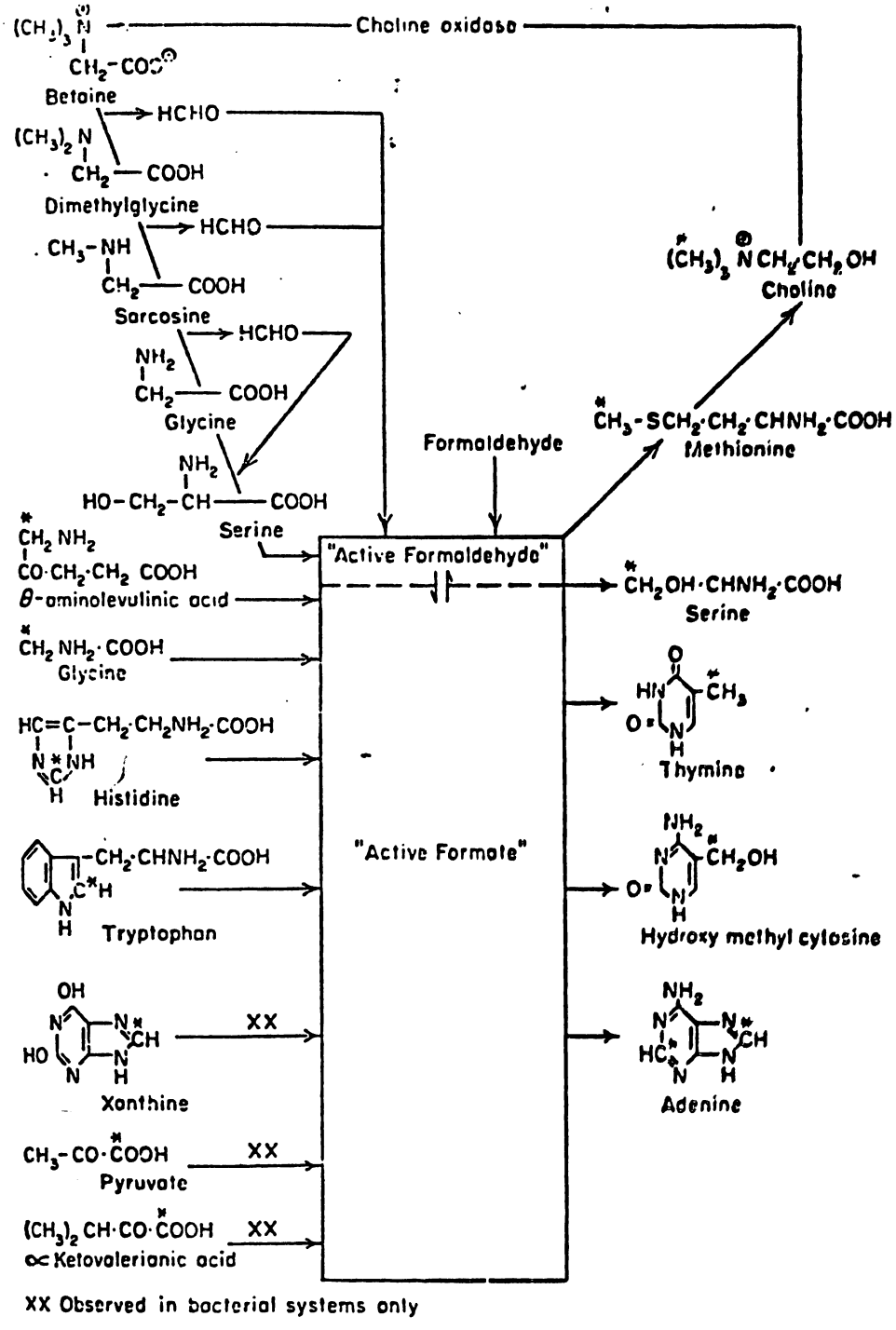

Fig. 4: Metabolic reactions involving activated formate and formaldehyde. Atoms identified by * indicate the source of the active formate carbon or the site of its addition in the biosynthesis of the compounds listed (Fig. from ref. 7).

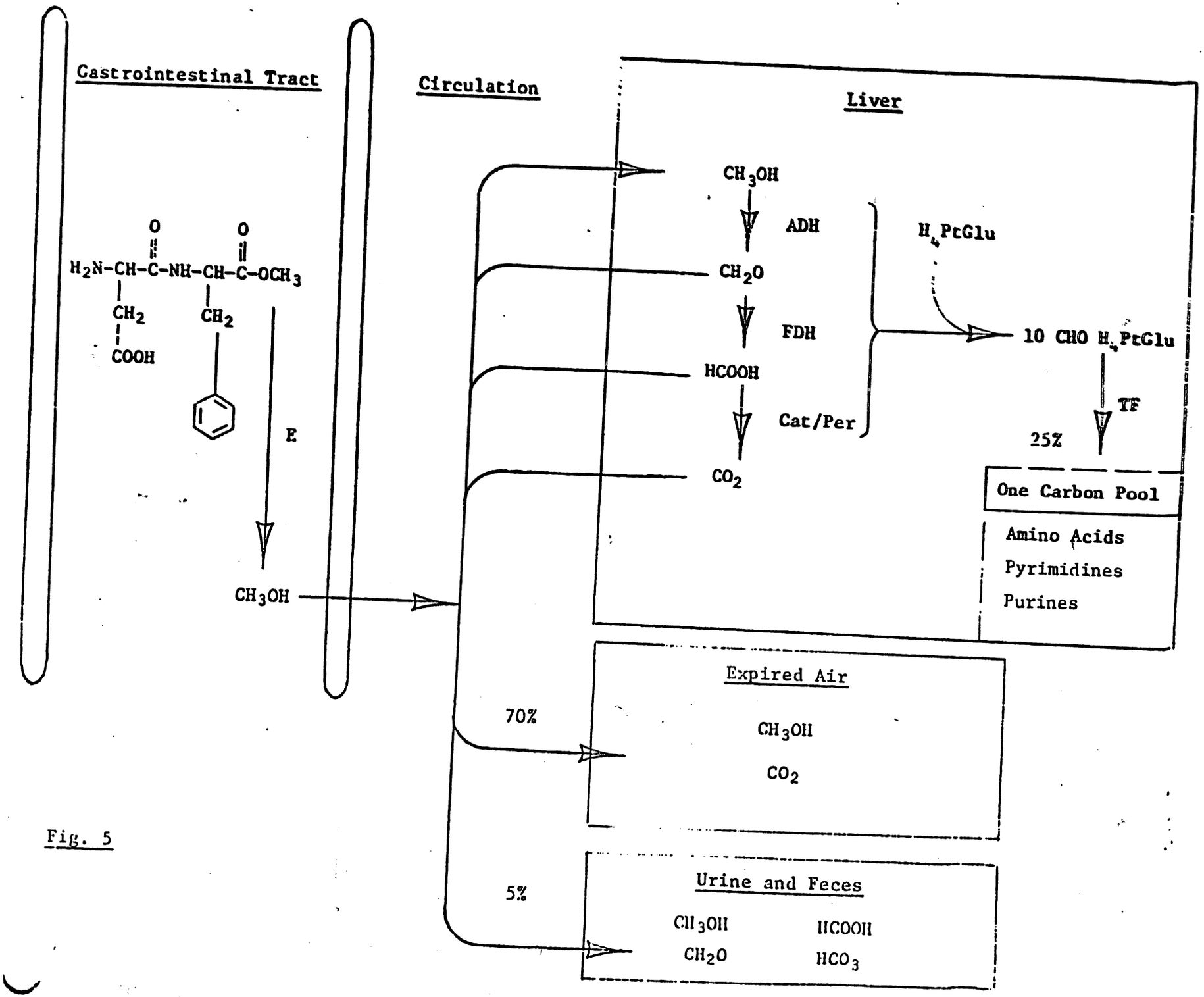
Gastrointestinal Tract
Circulation
Liver
CH_3OH
ADH
CH_2O
FDH
HCOOH
Cat/Per
CO_2
H_4PtGlu
10 CHO H_4PtGlu
TF
25%
One Carbon Pool
Amino Acids
Pyrimidines
Purines
E
CH_3OH
70%
Expired Air
CH_3OH
CO_2
5%
Urine and Feces
CH_3OH
HCOOH
CH_2O
HCO_3

Fig. 5

Fig. 5: The metabolism of the methyl moiety of aspartame. Symbols: E: intestinal esterases; ADH: alcohol dehydrogenase; FDH: Formaldehyde dehydrogenase; Cat/Per: catalase/perioxidase system; H_4PtGlu: tetrahydrofolate; TF: Transformylase. Percentage values indicate amounts of ^{14}C from [Me-^{14}C]-aspartame appearing in each fraction. It has been assumed that traces of methanol may contribute to the ^{14}C found in expired air and that traces of labeled methanol, formaldehyde, formate and bicarbonate were in urine and feces because these are normal constituents of these excreta.