CARBON ATOM 2 OF L-HISTIDINE-2-C¹⁴, A SOURCE OF THE CARBON OF LABILE METHYL GROUPS IN LIVER*

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Data presented in this report point to carbon atom 2 of L-histidine as an important source of the carbon of labile methyl groups. The results obtained in direct studies of the isolated perfused normal and cirrhotic rat liver are qualitatively and quantitatively similar to those obtained with intact normal rats and rats kept on a choline-deficient diet (1) for a short time (10 days) and a long time (1 year), the latter rats developing cirrhosis after 3 to 4 months.

While this work was in progress, several brief reports appeared focusing attention on the metabolic rolé of carbon atom 2 of L-histidine. Soucy and Bouthillier (2) fed L-histidine-2-C¹⁴ to rats and found 25 to 33 per cent of the radioactivity of the liver protein hydrolysates to be in the serine recovered from these hydrolysates. Of the radioactivity found in similar hydrolysates of the combined remaining tissues, 9 to 14 per cent was recovered in serine. Reid and Landefeld (3) presented data indicating the use of carbon atom 2 of L-histidine for the synthesis of purines and the methyl groups of creatine and choline. In an earlier study in which L-histidine labeled with N¹⁵ in the γ-nitrogen of the imidazole ring was employed, Tesar and Rittenberg (4) were unable to find any evidence for the rolé of histidine as a precursor of creatine or purines.

The work reported in this paper was started under the stimulus of a preliminary report by Coon and Levy (5), later followed by a more complete presentation (6), in which formate was found to be an effective precursor of histidine in yeast. Edlbacher (7) demonstrated a rat liver enzyme which opened the imidazole ring of histidine to give a product which yielded formate, probably from carbon atom 2, upon acid hydrolysis. Mackenzie (8), in discussing his finding formaldehyde and formic acid on oxidation of C¹⁴-methyl-labeled sarcosine by liver homogenates and slices,

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came to the conclusion that all biologically labile methyl groups were sources of formaldehyde and formate in the rat. Du Vigneaud, Ressler, and Rachele (9), du Vigneaud, Verly, and Wilson (10), and Sakami and Welch (11) found formate to be a precursor of methyl groups. Taken together, these findings indicated the interesting possibility that both the synthesis and the breakdown of L-histidine might involve formaldehyde or formate, some of which could be traced to newly formed methyl groups.

The work reported here, showing significant incorporation of the radioactivity of carbon atom 2 of L-histidine into the methyl groups of choline and creatine, in general confirms the data of Reid and Landefeld (3). In addition, data are presented showing an enhancement of this methyl group synthesis from histidine in the presence of choline deficiencies and a small contribution of carbon atom 2 of histidine to fatty acid synthesis.

EXPERIMENTAL

Synthesis of L-Histidine-2-C14 Hydrochloride—The L-histidine-2-C14 hydrochloride used in this work was synthesized essentially by the method described by Tesar and Rittenberg (4), based on previous methods of Kossel and Edlbacher (12) and Ashley and Harington (13). The C14-sodium thiocyanate used in this synthesis was obtained by the procedure of Castiglioni (14) from sulfur and C14-sodium cyanide, which was prepared by the method of Claus et al. (15). Certain modifications in these procedures introduced in this laboratory will be described elsewhere. The purity of the product was confirmed by nitrogen analysis, optical rotation, and homogeneity on paper chromatography by the ninhydrin reaction and radioautography.

Intact Animal—Adult, white, Wistar strain rats, 200 to 400 gm. in weight, fasted for approximately 18 hours previous to the experiments, were fed L-histidine-2-C14 by stomach tube with or without supplements as indicated in the individual experiments. After 6 hours the rats were anesthetized with ether and as much blood as possible removed by heart puncture. The animals were then killed by cutting the diaphragm, and the liver and other organs removed and immediately frozen, as was also the remaining carcass. The livers were lyophilized before lipide extraction.

Liver Perfusion—The technique followed in these experiments was developed by Miller, Bly, Watson, and Bale (16). In this procedure the appropriate experimental dose was added to the perfusing homologous blood 5 or 10 minutes after the start of the perfusion. 6 hours after the dose was added, the liver was removed and frozen, later lyophilized, and the perfusing blood centrifuged for separation of the plasma. The plasma was also lyophilized before lipide extraction.

Supplements Used—In addition to receiving 17 mg. of L-histidine-2-C14
hydrochloride (radioactivity equivalent to $17.4 \times 10^7$ disintegrations per minute per mM) in the experimental dose, each rat was given one of the following three supplements: (1) glucose or starch only = 2.1 gm. of glucose or starch, (2) glucose or starch plus amino acids for intact animals = 1.5 gm. of glucose or starch plus 600 mg. of essential amino acids (equivalent to 346 mg. of Merck Vuj-N mixture in L isomers) and 425 mg. of non-essential amino acids, (3) glucose plus amino acids for liver perfusions = 500 mg. of glucose plus 160 mg. of essential amino acids (Merck Vuj-N mixture) and 167 mg. of non-essential amino acids. The composition of the essential and non-essential amino acid mixtures is given by Miller et al. (16). Starch was used instead of glucose in the later experiments because the ingestion of a large dose of glucose led to excessive accumulation of fluid in the gastrointestinal tract and produced hemoconcentration.

Diet of György and Goldblatt (1)—To make a kilo of diet the following were used: 640 gm. of sugar, 100 gm. of casein, 200 gm. of lard, 40 gm. of inorganic salt mixture (Nutritional Biochemicals No. 2, U. S. P. vitamin A test diet), 20 gm. of cod liver oil, 2 gm. of L-cystine, 70 mg. of niacin, 7 mg. of thiamine hydrochloride, 3.5 mg. of calcium pantothenate, 1.4 mg. of pyridoxine hydrochloride, 3.5 mg. of riboflavin.

Isolation of Choline from Liver and Blood Plasma—The lyophilized specimens were subjected to successive extractions of at least 8 hours each with the following solvents: ether-ethyl alcohol, 1:3, methyl alcohol-chloroform, 1:1. The combined extracts were distilled to dryness in vacuo in a nitrogen atmosphere. From this total lipid extract, the phospholipide fraction was isolated according to the procedure described by Popják and Beeckmans (17). After the second reprecipitation of the phospholipides, the material was redissolved in petroleum ether (100 ml.) and washed with 1 liter of 0.2 per cent inert L-histidine in water to remove any possible contamination by L-histidine-2-C\textsuperscript{14}. Although this does not rule out the possibility of contamination with some other radioactive material, it is unlikely that any other known significantly radioactive substance would be carried through the procedure as outlined here and persist in the final isolated products and their degradative fragments. The petroleum ether solution was then evaporated in a stream of carbon dioxide, hydrolyzed with barium hydroxide, and the choline isolated as the reineckate according to the procedure of Brante (18). The radioactive choline reineckate was weighed, inert carrier choline reineckate was added, and the proper correction made in calculating the millimolar activities of the subsequent products. The phospholipide fatty acids were also isolated from the hydrolysis mixture. The choline reineckate was converted to the chloro-

1 We are indebted to Merck and Company, Inc., Rahway, New Jersey, for a generous gift of amino acids.
platinum and then degraded to trimethylamine as described by Burke, Nystrom, and Johnson (19), with a minor modification. It was found quite suitable in the degradation procedure to precipitate the trimethylamine directly as the chloroplatinate by drawing the liberated amine into an alcoholic solution of chloroplatinic acid. The choline chloroplatinates were analyzed for platinum and samples of the choline chloroplatinates and the corresponding trimethylamine chloroplatinates assayed for radioactivity. The residual degradation mixture was also assayed, in most cases, for radioactivity trapped as carbon dioxide in the alkaline medium, by acidifying the alkaline mixture and evolving the carbon dioxide into the ionization chamber of the assay apparatus.

The per cent dose incorporated as liver choline was calculated on the basis of the weight of phospholipide actually isolated, a distribution of lecithin to cephalin in rat liver phospholipide of 60:40,2 and the observed millimolar activity of the isolated choline. The values obtained are not strictly quantitative since they depend on the weight of phospholipide actually isolated and not on an estimated total weight of phospholipide present in each liver. However, this calculation provides a minimum figure for comparison of the per cent dose incorporated as choline under the various experimental conditions. A similar calculation was carried out for the fatty acids isolated from the liver phospholipides after hydrolysis. Here again the calculation was based on the weight of phospholipide actually isolated, the average figures of 68 per cent for the fatty acid content of phospholipide and 280 for the molecular weight of the fatty acids, and the observed C14 activities of the isolated fatty acid mixture.

Isolation of Choline and Creatine from Carcasses -The frozen eviscerated rat carcasses were chopped and ground and then treated according to the procedure of du Vigneaud et al. (20), with several modifications as noted below, for the isolation of choline and creatine (as creatinine). In preparing the potassium creatinine picrate, the last two recrystallizations were made from a 0.2 per cent solution of inert L-histidine instead of distilled water. The hydrolysis of creatinine was carried out in a bomb tube (at 110° for 48 hours) instead of under a reflux. After separation of the BaCO3, the filtrate plus washings was distilled almost to dryness in vacuo after addition of a few ml. of 20 per cent NaOH. This procedure removed the ammonia formed during the hydrolysis and eliminated the necessity for fractional crystallizations to obtain pure methylamine chloroplatinate from the degradation of sarcosine. As in the case of the trimethylamine above, the methylamine was precipitated directly as the chloroplatinate by drawing the liberated amine through an alcoholic solution of chloroplatinic acid. Platinum analyses of the methylamine chloroplatinates

2 MacLachlan, P. L., unpublished data.
proved the feasibility of these modifications. Samples of the choline chloroplatinates, trimethylamine chloroplatinates, potassium creatinine picrates, methylamine chloroplatinates, and barium carbonates were assayed for radioactivity. Most of the residual degradation mixtures were also assayed for activity trapped as carbon dioxide in the alkaline medium.

Isolation of Carcass Fatty Acids - In the course of the procedure of du Vigneaud et al. (20) for the isolation of carcass choline and creatine, the carcass fatty acids were also isolated and assayed for radioactivity.

Radioactivity Assays—Assays of C\textsuperscript{14} activity were carried out with the apparatus designed and described by Bale.\textsuperscript{3} This apparatus makes use of the wet oxidation mixture of Van Slyke and Folch (21) and C\textsuperscript{14} activity of the resulting carbon dioxide is measured in an ionization chamber coupled with a dynamic vane electrometer and an Esterline-Angus automatic recorder. The carbon dioxide of the barium carbonate samples and of the degradation mixtures was liberated by acidification with hydrochloric acid.

In general, the accuracy of the C\textsuperscript{14} assays reported is at least within ±4 per cent. If the radioactivity of a sample fell to twice the background level, or lower, with a consequent decrease in accuracy, the values corresponding to such a sample are placed in parentheses in Tables II to V.

Results

Although the number of animals used under any particular set of conditions in the experiments reported here was small and the data would obviously not bear statistical scrutiny, all the results obtained so far uniformly point to the validity of the comparisons and conclusions presented in this paper.

A comparison of the radioactivities of the choline chloroplatinates derived from the livers of intact rats and perfused rat livers (Tables I and II respectively) and their corresponding trimethylamine chloroplatinates shows that approximately 80 to 90 per cent of the activity of the choline chloroplatinate is found in the trimethyl group. Cirrhotic Rat HR-18 provided the only exception, all the activity being found in the methyl groups. Furthermore, the isolated rat livers perfused with whole blood convert as much radioactivity from L-histidine-2-C\textsuperscript{14} into methyl groups as do the intact animals.

If normal rats and the isolated perfused liver can use carbon atom 2 of histidine for the synthesis of methyl groups, the stress of choline deficiency should enhance this process. This prediction received support from the data of Tables I, II, and III. In Rat HR-17, on the choline-deficient diet for only 10 days and with starch as the only supplement in

\textsuperscript{3} Bale, W. F., data to be published.
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the experimental dose, a greatly enhanced utilization of carbon 2 of histidine for methyl group synthesis was produced (Table I). The small enhancement in methyl group synthesis shown by Rat HR-18 is probably related to the presence of other sources of methyl group carbon in the amino acid supplement given to this rat in the experimental dose. Methionine and serine are to be considered as sources of carbon for labile methyl groups.

**TABLE I**

Incorporation of Radioactivity of Carbon Atom 2 of L-Histidine-2-C\(^{14}\) into Liver Choline of Intact Rats

<table>
<thead>
<tr>
<th>Rat preparation</th>
<th>Supplements*</th>
<th>Choline chloroplatinate</th>
<th>Trimethylamine chloroplatinate, activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent dose incorporated</td>
<td>Per cent P08</td>
</tr>
<tr>
<td>Normal, male (HR-1)</td>
<td>None</td>
<td>31.43</td>
<td>31.9</td>
</tr>
<tr>
<td>female (HR-11)</td>
<td>Glucose</td>
<td>162.54</td>
<td>31.8</td>
</tr>
<tr>
<td>&quot; (HR-12)</td>
<td>&quot; + amino acids</td>
<td>15.11</td>
<td>31.5</td>
</tr>
<tr>
<td>Choline-deficient diet, male (HR-17)</td>
<td>Starch</td>
<td>214.22</td>
<td>31.8</td>
</tr>
<tr>
<td>Choline-deficient diet 10 days, male (HR-18)</td>
<td>&quot; + amino acids</td>
<td>46.42</td>
<td>32.2</td>
</tr>
<tr>
<td>Cirrhotic, male (HR-9)</td>
<td>Glucose</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>&quot; (HR-10)</td>
<td>&quot; + amino acids</td>
<td>21.15</td>
<td>31.3</td>
</tr>
</tbody>
</table>

* All rats received 17 mg. of L-histidine-2-C\(^{14}\) hydrochloride, equivalent to 17.4 \(\times\) 10\(^7\) disintegrations per minute per mM plus supplements as indicated.
† C\(^{14}\) activity in disintegrations per minute per mM \(\times\) 10\(^{-4}\).
‡ Calculated as a minimum value (see "Experimental").
§ Theoretical platinum content, 31.7 per cent.
\|| Cirrhosis induced by feeding rats for approximately 1 year on the diet of György and Goldblatt (1), low in protein and vitamin E, and devoid of choline.

The comparatively low incorporation seen in Rats HR-9 and HR-10, both definitely cirrhotic and not far from the terminal stage, may in part be ascribed to the marked hemoconcentration observed in both rats at the time of sacrifice. In the case of the perfused rat liver, in which the circulation of the blood was maintained much more uniformly from experiment to experiment, the cirrhotic livers showed a large increase in methyl group synthesis from histidine over that in the normal perfused liver.

The increase in methyl group synthesis shown by Rat HR-11, a normal female, with only glucose as a supplement, contrasts with the low synthetic activity shown by Rat HR-12, also a normal female rat dosed with glucose
and amino acids as a supplement. This difference is referable to at least two factors, sex and amino acid supplements. We have observed that female rats are very slow to develop experimental cirrhosis when kept on the György and Goldblatt diet (1), while male rats generally develop cirrhosis in 3 or 4 months. A similar observation was made by Griffith (22) in a study of the effects of choline deficiency on young rats. In other

**Table II**

*Incorporation of Radioactivity of Carbon Atom 2 of L-Histidine-2-C\(^{14}\) into Choline of Perfused Rat Liver and of Perfusing Blood Plasma Phospholipides*

<table>
<thead>
<tr>
<th>Rat liver preparation</th>
<th>Supplements*</th>
<th>Choline chloroplatinate</th>
<th>Trimethylamine chloroplatinate activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Blood plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Per cent dose in</td>
<td>Per cent activity†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>incorporated§</td>
<td></td>
</tr>
<tr>
<td>Normal, male (RLP-61)</td>
<td>Amino acids</td>
<td>25 0.09 32.0 (10)</td>
<td>31.8 20</td>
</tr>
<tr>
<td>Normal, male (RLP-66)</td>
<td>Glucose +</td>
<td>14 0.32 31.6 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Cirrhotic, male (RLP-60)</td>
<td>amino acids</td>
<td>77 0.59 31.5 25</td>
<td>31.8 (14)</td>
</tr>
</tbody>
</table>

* Perfusing blood received 17 mg. of L-histidine-2-C\(^{14}\) hydrochloride, equivalent to \(17.4 \times 10^9\) disintegrations per minute per mm plus supplements as indicated.

† \(C^{14}\) activity in disintegrations per minute per mm \(\times 10^{-4}\). \(C^{14}\) activities in parentheses in this and the following tables correspond to measured net values less than twice background \(C^{14}\) activity.

§ Calculated as a minimum value (see "Experimental").

\$ Theoretical platinum content, 31.7 per cent.

‖ Cirrhosis induced by feeding rats for approximately one year on diet of György and Goldblatt (1), low in protein and vitamin E, and devoid of choline.

words, Rat HR-11, a normal female, presented with histidine as a restricted source of carbon for methyl group synthesis, utilized this source more efficiently than a normal male rat in a similar situation and also to a greater extent than a corresponding female rat presented a greater choice of sources of carbon for methyl group synthesis in the form of an amino acid supplement including methionine and serine.

The level of radioactivity in the choline of blood plasma phospholipide from perfused normal livers (Table II) follows the same pattern as the level of radioactivity found in the liver choline. This is in keeping with
TABLE III

Incorporation of Radioactivity of Carbon Atom 2 of \( l \)-Histidine-2-\( C^{14} \) into Rat Carcass

<table>
<thead>
<tr>
<th>Rat preparation</th>
<th>Supplements*</th>
<th>Choline chloroplatinate</th>
<th>Trimethylamine chloroplatinate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Activity†</td>
<td>Per cent Pt (theoretical 31.7)</td>
</tr>
<tr>
<td>Normal, male (HR-1)</td>
<td>None</td>
<td>1.32</td>
<td>31.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>Glucose + amino acids</td>
<td>0.53</td>
<td>31.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>Starch</td>
<td>2.84</td>
<td>31.2</td>
</tr>
<tr>
<td>Choline-deficient, male (HR-17)</td>
<td></td>
<td>0.58</td>
<td>31.8</td>
</tr>
<tr>
<td>Choline-deficient, male (HR-18)</td>
<td></td>
<td>0.62</td>
<td>31.8</td>
</tr>
<tr>
<td>Cirrhotic, male (HR-10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All rats received 17 mg. of \( l \)-histidine-2-\( C^{14} \) hydrochloride, equivalent to \( 17.4 \times 10^6 \) disintegrations per minute per \( \text{mm} \) plus supplements as indicated.
† \( C^{14} \) activity in disintegrations per minute per \( \text{mm} \times 10^{-4} \).

TABLE IV

Incorporation of Radioactivity of Carbon Atom 2 of \( l \)-Histidine-2-\( C^{14} \) into Rat Carcass

<table>
<thead>
<tr>
<th>Rat preparation</th>
<th>Supplements*</th>
<th>Potassium creatinine picrate</th>
<th>Methylamine chloroplatinate</th>
<th>Barium carbonate, activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Activity†</td>
<td>Per cent dose incorporated</td>
<td>Per cent creatinine</td>
</tr>
<tr>
<td>Normal, male (HR-1)</td>
<td>None</td>
<td>0.35 (0.06)</td>
<td>0.078</td>
<td>19.7</td>
</tr>
<tr>
<td>Normal, female (HR-12)</td>
<td>Glucose + amino acids</td>
<td>0.0010</td>
<td>18.4</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Choline-deficient, male (HR-17)</td>
<td>Starch</td>
<td>0.62</td>
<td>0.169</td>
<td>18.8</td>
</tr>
<tr>
<td>Choline-deficient, male (HR-18)</td>
<td>&quot; + amino acids</td>
<td>0.14</td>
<td>0.034</td>
<td>18.3</td>
</tr>
<tr>
<td>Cirrhotic, male (HR-10)</td>
<td>Glucose + amino acids</td>
<td>0.22</td>
<td>0.020</td>
<td>19.0</td>
</tr>
</tbody>
</table>

* All rats received 17 mg. of \( l \)-histidine-2-\( C^{14} \) hydrochloride, equivalent to \( 17.4 \times 10^6 \) disintegrations per minute per \( \text{mm} \) plus supplements as indicated.
† \( C^{14} \) activity in disintegrations per minute per \( \text{mm} \times 10^{-4} \).
‡ Analyzed for creatinine colorimetrically by the Jaffe reaction. Theoretical creatinine content (1 mole of creatinine per mole) = 18.6 per cent.
§ Theoretical platinum content (2 methyl carbons per mole) = 41.4 per cent.
the notion that the liver is a major, if not the sole, source of plasma phospholipide synthesis (23, 24). However, the fraction of blood choline ac-

Table V

<table>
<thead>
<tr>
<th>Rat preparation</th>
<th>Supplements*</th>
<th>Fatty acids From liver phospholipides</th>
<th>Fatty acids From carcass</th>
<th>Activity† Per cent dose incorporated†</th>
<th>Activity† Per cent dose incorporated†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, male (HR-1)</td>
<td>None</td>
<td>0.14</td>
<td>0.012</td>
<td>(0.04)</td>
<td>0.21</td>
</tr>
<tr>
<td>&quot; female (HR-11)</td>
<td>Glucose</td>
<td>1.15</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; (HR-12)</td>
<td>&quot; + amino acids</td>
<td>3.91</td>
<td>0.173</td>
<td>0.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Choline-deficient, male (HR-17)</td>
<td>Starch</td>
<td>0.67</td>
<td>0.041</td>
<td>(0.02)</td>
<td>0.15</td>
</tr>
<tr>
<td>&quot; &quot; (HR-18)</td>
<td>&quot; + amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhotic, male (HR-10)</td>
<td>Glucose + amino acids</td>
<td>1.24</td>
<td>0.056</td>
<td>0.22</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Perfused liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, male (RLP-61)</td>
<td>Amino acids</td>
<td>1.59</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhotic, male (RLP-60)</td>
<td>Glucose + amino acids</td>
<td>0.34</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; (RLP-62)</td>
<td>&quot; &quot;</td>
<td>0.78</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All rats and the perfusing blood received 17 mg. of L-histidine-2-$^{14}$C hydrochloride, equivalent to $17.4 \times 10^7$ disintegrations per minute per mm plus supplements as indicated.
† $^{14}$C activity in disintegrations per minute per mm $\times 10^{-4}$.
‡ Calculated as a minimum value (see "Experimental").

tivity in the methyl groups is definitely lower than that in the liver choline methyl groups.

The effect of the amino acid supplement on the utilization of carbon atom 2 of histidine for methyl group synthesis is revealed in the activities found in carcass choline (Table III). Rat HR-17, on a choline-deficient
diet for 10 days with starch as the only supplement in the experimental
dose, shows a 2-fold increase in activity over Rat HR-1, a normal animal
on a normal diet. Rats HR-12, HR-18, and HR-10, a normal rat, a rat
on the choline-deficient diet for 10 days, and a definitely cirrhotic rat,
respectively, all received the amino acid supplement and all show decreased
activity in the choline isolated. Rats HR-12 and HR-10 also exhibit a
definite decrease in the usual fraction of total choline $^3$H activity found in
the methyl groups. By the same token, this indicates greater incorpora-
tion of histidine-2-$^3$H in the ethanolamine moiety of choline, probably
via serine (25).

The same effect of the amino acid supplement on methyl group synthesis
from carbon 2 of histidine is found in the $^3$H data of Table IV, dealing
with creatine in the rat carcass. Rat HR-17 again shows a definite in-
crease in activity over Rat HR-1, and Rats HR-12, HR-18, and HR-10,
receiving the amino acid supplement, not only show a decrease in activity
incorporated but also a decrease in the fraction of the total creatine ac-
tivity found in the methyl group. The very low activity in the BaCO$_3$
samples from the hydrolysis of creatinine samples indicates that very little
activity of the creatine arose from carbon dioxide fixation in the guanidine
moiety.

From the data in Table V, it appears that carbon atom 2 of histidine
makes a small contribution to fatty acid synthesis in the rat, probably
via formate. Brady et al. (26) have reported no incorporation of radio-
activity into fatty acids from $^3$H-formate and formaldehyde by rat liver
slices. Their negative result, however, is not necessarily incompatible
with our result because of the relatively low concentration and activity
of the substrates used by Brady and the fact that conversions performed by
liver slices are quantitatively lower than comparable conversions in intact
rats or isolated perfused rat livers. Since the experiments reported here
were designed to remove radioactive contamination only in the form of
histidine-2-$^3$H, little can be said at present of the significance of these
findings. However, the incorporation of radioactivity from carbon 2 of
histidine into fatty acids is not very surprising in view of the facts that
about 15 per cent of the radioactivity incorporated into liver choline is
found in the ethanolamine moiety and that serine can go to pyruvate and
thence to fatty acids. Work is now in progress to rule out radioactive
contamination of any kind and to determine the distribution of activity
in the fatty acids.

**DISCUSSION**

The results presented here, partly in confirmation of those reported
by Reid and Landefeld (3), show conclusively that carbon atom 2 of L-
histidine is a normally available source of the carbon of methyl groups in the synthesis of choline and creatine and contributes also to the ethanolamine moiety of choline and to fatty acid synthesis.

At present there are no direct data to demonstrate the actual quantitative importance of carbon atom 2 of histidine as a source of the carbon of labile methyl groups. However, taken in conjunction with our data, results reported in the recent and older literature warrant the speculation that histidine is a very important source of methyl groups under stress of a choline deficiency. Griffith (22), using a diet containing 15 per cent casein and various amounts of added choline, found that young rats required approximately 1 mg. of choline chloride daily to prevent hemorrhagic degeneration of the kidneys. 2 to 3 times as much choline are required for the prevention of fatty livers, but Griffith also found that there was a marked decrease in the choline requirement of rats over 30 days of age. On the basis of the per cent dose incorporated into the methyl groups of choline from 17 mg. of L-histidine-2-C¹⁴ hydrochloride, Rat HR-17 synthesized approximately 0.05 mg. of choline in the 6 hours of the experiment.

Further work by Griffith and Mulford (27) can now be interpreted to indicate the importance of the synthesis of methyl groups from histidine. On a diet containing 24 per cent of a mixture of amino acids (28) devoid of glycine, serine, and methionine, and with a histidine hydrochloride content of 2.7 per cent, only one of eight rats developed any hemorrhagic lesions. These rats consumed an average of 2.1 gm. of food per day, thus receiving approximately 14 mg. of histidine hydrochloride daily, an amount almost identical with the dose used in the experiments described in this paper.

Rose, Burr, and Sallach (29), in a study of the growth of rats on a diet devoid of glycine, serine, and cystine, and low in choline, came to the conclusion that the ability of the rats to gain at slightly subnormal rates while feeding on the above diet demonstrated a source other than interconversion of related amino acids for at least one of the compounds in question. Carbon atom 2 of histidine, with its demonstrated conversion to the labile methyl groups of choline, especially under the stress of choline deficiency, can now be proposed as one source which might explain the continued growth of rats on the deficient diet of Rose et al. (29).

Du Vigneaud et al. (20), in discussing the results of their study of the synthesis of labile methyl groups in germ-free and normal rats fed deuterium oxide, speculate as to the manner in which the new deuterium-labeled methyl group is created. They propose the possibility that the new methyl group is formed during the synthesis of methionine from homocysteine, eventually reaching choline through transmethylation, but do not indicate a possible source for the labile methyl carbon. Under the conditions of
the experiments performed by du Vigneaud et al. (20), methyl group synthesis from carbon 2 of histidine-2-C\textsuperscript{14} in the presence of deuterium oxide should yield methyl groups labeled with both deuterium and C\textsuperscript{14} and thus afford an explanation for their findings (20).

In a reinvestigation of the lipotropic action of various amino acids, Eckstein (30) concluded that no amino acid other than methionine showed any such action. In view of the relatively low daily intake of histidine by the rats on the various diets used in his study and the large variations observed between different groups on the same diets, it is difficult to correlate his results with our proposal that histidine should exhibit lipotropic activity.

On the basis of the wide-spread distribution of histidine in dietary proteins which often have a higher histidine than methionine content, it would seem that histidine must now be considered as a source of labile methyl groups of major importance in the economy of the rat. However, the ultimate evaluation of the true potency of carbon atom 2 of L-histidine as a source of carbon of labile methyl groups must await further diet experiments with histidine as the sole possible source of the carbon of these methyl groups.

**SUMMARY**

1. The demonstration of the conversion of carbon atom 2 of L-histidine-2-C\textsuperscript{14} to the carbon of labile methyl groups of liver, blood, and body choline and body creatine as a normal process in the intact rat has been confirmed and extended.

2. A choline deficiency of short duration in intact rats has been shown to enhance the synthesis of methyl groups from carbon atom 2 of histidine. The same has been shown to hold true for the isolated perfused cirrhotic liver (from rats on a choline-deficient diet for 1 year).

3. The responses of the isolated perfused livers of normal and experimental cirrhotic (choline-deficient) rats are qualitatively and quantitatively similar to those of intact rats in the conversion of carbon atom 2 of histidine to the carbon of labile methyl groups.

4. A supplement of essential and non-essential amino acids fed with the experimental dose containing the L-histidine-2-C\textsuperscript{14} produces a definite decrease in methyl group synthesis from histidine, probably by providing other sources of carbon for these methyl groups.

5. A small contribution of carbon atom 2 of histidine to fatty acid synthesis has also been demonstrated.

6. The amino acid histidine must now be counted as an important dietary source of the carbon of labile methyl groups in the rat.

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