THE FATE OF BENZOYLACETIC ACID IN THE ANIMAL BODY.

By H. D. DAKIN.

(From the Herter Laboratory, 819 Madison Avenue, New York.)

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Studies upon the fate of the phenyl derivatives of fatty acids have thrown much light upon the possible transformation which the naturally occurring fatty acids may undergo in the course of their catabolism. Of these investigations of phenyl derivatives, that upon phenylpropionic acid has been particularly illuminating. By injecting cats with the sodium salt of this acid, it has been possible to determine the formation of the following intermediate substances in addition to the end product of oxidation, benzoic acid, which is excreted in the form of hippuric acid:

Phenyl-β-hydroxy-propionic acid: C₆H₅.COH.CH₂.COOH
Cinnamoyl-glycocoll: C₆H₅.CH:CH:CO.NH.CH₂.COOH
Benzoylacetic acid: C₆H₅.CO.CH₂.COOH
Acetophenone: C₆H₅.CO.CH₃
Hippuric acid: C₆H₅.CO.NH.CH₂.COOH

The relationships between these substances are somewhat complicated but they furnish a very striking demonstration of the essential accuracy of Knoop’s hypothesis of the oxidation of fatty acids at the β-position.

The relationship between acetophenone and benzoylacetic acid is evidently similar to that between acetone and aceto-acetic acid and all the evidence available tends to the belief that the ketones are derived from the ketonic acids by an irreversible reaction. In the case of the other substances the relationships are more complex. The unsaturated cinnamic acid readily passes

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1This Journal, iv, p. 419, 1908; v, p. 303, 1908; vi, p. 203, 1909.
over in part into phenyl-β-hydroxypropionic acid, while administration of salts of phenyl-β-hydroxypropionic may result in the excretion of cinnamic acid derivatives. The change here is evidently a reversible one:

$$\text{C}_6\text{H}_5\text{CH}::\text{CH}::\text{COOH} + \text{H}_2\text{O} \rightleftharpoons \text{C}_6\text{H}_5\text{CHOH}::\text{CH}::\text{COOH}$$

It has been shown that both cinnamic acid and phenyl-β-oxypropionic acid when administered as salts to cats may yield benzoylacetic acid (and hence acetophenone).

$$\text{C}_6\text{H}_5\text{CH}::\text{CH}::\text{COOH} \rightarrow \text{C}_6\text{H}_5\text{CHOH}::\text{CH}::\text{COOH}$$

It appeared not improbable that these reactions might also prove to be reversible, especially in view of the fact that Blum, Dakin, Wakeman, Friedmann and Maase have recently independently demonstrated the formation of l-β-hydroxybutyric acid by the asymmetric reduction in the body of aceto-acetic acid.

Experiments made a year ago with the object of determining this question showed that the reaction was in fact reversible. Sodium benzoylacetate when given intravenously or subcutaneously to cats in addition to yielding hippuric acid as observed by Knoop, in part was transformed into levora-rotatory phenyl-β-hydroxypropionic acid and cinnamoylglycocoll. In the meantime Friedmann arrived at the same conclusion although he did not succeed in isolating the β-hydroxy-acid. He obtained a levoro-rotatory acid soluble in ether which gave cinnamic acid on heating with hydrochloric acid. These results might equally well be due to the corresponding glycocoll derivative, C₆H₅CHOH.CH₂.CO.NH.CH₂.COOH, the synthesis of which has been described in this Journal. Since the writer had been successful

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1. This Journal, vi, p. 203, 1909.
3. This Journal, viii, p. 97, 1910.
7. This Journal, v, p. 303, 1908.
in actually isolating the pure lävo-rotatory phenyl-β-hydroxypropionic acid and found its properties to agree with those of the active acid resolved by McKenzie the results appeared worthy of record.

The details of the experiments are given in the following pages. The yield of phenyl-β-oxypropionic acid was relatively large; that of cinnamoylglycocoll was considerably less. Considerable amounts of unchanged benzoylacetic acid and acetophenone were always found in the urines.

In picturing the possible reactions concerned in the oxidation of phenylpropionic acid to benzoic acid in the animal body, it will therefore be necessary to take cognizance of the possible reversible interconversion of phenyl-β-hydroxybutyric acid and benzoylacetic acid. The following scheme is in harmony with the facts so far as at present ascertained.

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\begin{align*}
\text{C}_6\text{H}_5\cdot \text{CH}_2\cdot \text{CH}_2\cdot \text{COOH} & \quad \text{laevo-C}_6\text{H}_5\cdot \text{CHOH}\cdot \text{CH}_2\cdot \text{COOH} & \rightarrow & \text{C}_6\text{H}_5\cdot \text{CO}_2\cdot \text{C}_2\text{H}_5
\\
\text{C}_6\text{H}_5\cdot \text{CH}_2\cdot \text{COOH} & \quad \rightarrow & \text{C}_6\text{H}_5\cdot \text{COOH}
\\
\text{C}_6\text{H}_5\cdot \text{CH}:: \text{CH}_2\cdot \text{COOH} & \quad \rightarrow & \text{C}_6\text{H}_5\cdot \text{CH}:: \text{NH}_2\cdot \text{COOH}
\\
\text{C}_6\text{H}_5\cdot \text{CH}:: \text{CH}_2\cdot \text{COOH} & \quad \rightarrow & \text{C}_6\text{H}_5\cdot \text{CO}_2\cdot \text{CH}_3
\\
\end{align*}
\]

The experimental part of this paper also contains an account of the demonstration of the excretion of cinnamoylglycocoll when a solution of sodium cinnamate together with glycocoll was slowly injected into the femoral vein of cats. The amount of this substance recovered was, however, surprisingly small.

**EXPERIMENTAL PART.**

Benzoylacetic ethyl ester was prepared according to Claisen’s directions by the limited action of ammonia upon the sodium salt of benzoyl-aceto-acetic ester. The reaction proceeds smoothly and gives an excellent yield of the ester b.p.155-160° at 16mm. pressure. This method is decidedly more convenient than any of the older methods of preparation.
Benzoylacetic acid was prepared by the saponification of the ester as follows: 19.2 grams of the ester were shaken with 102 cc. of cold normal sodium hydrate solution. After standing for twenty-four hours, the solution was filtered from a little oil and acidified with sulphuric acid. An abundant precipitate of crystalline benzoylacetic acid was obtained and an additional quantity was recovered from the mother liquor by extraction with ether. The yield of acid was 14 grams.

Three experiments were made to determine the fate of benzoylacetic acid in the body. In two of these 2.0 and 2.5 grams of the acid were neutralized with caustic soda and injected subcutaneously into cats. In the third experiment the sodium salt corresponding to 3 grams of the acid was injected slowly in dilute aqueous solution into the femoral vein of a cat. From the rotations of the ether extract of the urines it was calculated that the amount of β-hydroxy-phenylpropionic acid excreted in the urine amounted to 0.8, 1.1, 0.5 gram respectively. The methods of urinary analysis were the same in each case so that a general description will suffice.

In each case a portion of the urine was acidified and shaken with ether. The ethereal extract was dissolved in a little alcohol and tested with ferric chloride. A positive reaction for unchanged benzoylacetic acid was invariably obtained. Another portion of the urine was distilled with phosphoric acid. The distillate gave the typical reactions for acetophenone strongly and on adding an acetic acid solution of p-nitro-phenylhydrazine, a sparingly soluble hydrazone was at once precipitated, which after recrystallization from dilute alcohol melted at 184-185°, corresponding to acetophenone p-nitro-phenylhydrazone.

The remaining urine was acidified with phosphoric acid and extracted in a continuous extractor with ether. The ethereal extract was distilled in steam to remove volatile fatty acids, clarified with charcoal and the concentrated solution examined in the polarimeter. A strong laevo-rotation was observed in each case corresponding to the amounts of l-phenyl-β-hydroxy-phenylpropionic acid previously recorded. The hydroxy-acid was separated as follows: The solution was concentrated to small volume and allowed to crystallize. The crystals consisting of a mixture of cinnamoylglycocoll and hippuric acid were filtered
off and reserved. The filtrate was twice shaken out with ether. The ethereal extract on evaporation left a chalky mass of the crude hydroxy-acid contaminated with some hippuric acid. The hydroxy-acid was separated by extraction with boiling chloroform but did not crystallize on evaporation of the solvent. On dissolving in water, however, and allowing the solution to stand a good yield of fine colorless needles of the pure phenyl-β-hydroxypropionic acid was readily obtained. The substance, after recrystallization from boiling toluene melted sharply at 115-116°. Its properties agreed in every respect with those of the laevo-acid recently obtained by McKenzie and Humphries by the resolution of inactive phenyl-β-hydroxypropionic acid.

Analysis: 0.0963 gm. gave 0.2280 gm. CO₂ and 0.0530 gm. H₂O

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<tr>
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<th>Found:</th>
<th>Calculated for C₅H₈O₃:</th>
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<tbody>
<tr>
<td>Carbon</td>
<td>64.6</td>
<td>65.1</td>
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<tr>
<td>Hydrogen</td>
<td>6.1</td>
<td>6.0</td>
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A small amount of the crystals served for the approximate determination of the optical rotation. The value obtained was very close to that determined by McKenzie and Humphries.

l=1; c=2.65; α=0.49°; [α]D = -18.5°.

On boiling the crystals for a few minutes with dilute hydrochloric acid and subsequently cooling, an abundant precipitate of cinnamic acid, m.p., 132-133°, was readily obtained.

The crude crystals consisting of a mixture of hippuric acid and cinnamoylglycocoll separated in an early stage of the analysis and previously referred to, amounted to about 1.5 grams from the three experiments. On recrystallizing repeatedly from hot water a small quantity of pure cinnamoylglycocoll m.p., 192-193°, crystallizing in the form of long shining needles was obtained. The substance dissolved in dilute sodium carbonate solution instantly reduced dilute potassium permanganate solution with production of benzaldehyde. The amount of pure substance was not sufficient for analysis.

Two experiments were made in which a solution containing 3.0 grams of cinnamic acid in the form of its sodium salt and 2.0 grams of glycocoll dissolved in 150 cc. of water was slowly injected into the femoral vein of large cats. The time of injection was between three and four hours and the mixture was evidently toxic. The urines secreted from both cats after the administration of the cinnamate was examined for cinnamoylglycocoll by the methods which have so frequently been made use of previously. In each case between 0.1 and 0.2 gram of cinnamoylglycocoll was separated from the urine. The separation of this substance from accompanying hippuric acid involves considerable loss but taking this into consideration the yield of cinnamoylglycocoll was surprisingly small.

SUMMARY.

Benzoylacetic acid (2-3 grams) administered to cats in the form of its sodium salt either intravenously or subcutaneously results in the excretion in the urine of cinnamoylglycocoll and of levorotatory phenyl-β-hydroxyphenylpropionic acid. The formation of the latter substance is completely analogous to the asymmetric reduction in the animal body of aceto-acetic acid to l-hydroxybutyric acid observed by Blum, Dakin and Wakeman and Friedman and Maase.

The l-β-hydroxyphenylpropionic acid was isolated from the urines in the pure state and had properties identical with those of the acid recently obtained by McKenzie and Humphries by the resolution of inactive phenyl-β-hydroxypropionic acid by means of alkaloids.

The experiments throw further light upon the mode of catabolism of phenylpropionic acid. These changes are represented diagramatically on page 125. β-Hydroxyphenylpropionic acid and benzoylacetic acid are mutually interconvertible in the animal body by means of reversible reactions involving oxidation and reduction respectively.

Experiments are described showing that an excretion of cinnamoylglycocoll may follow the intravenous injection of sodium cinnamate and glycocoll. The yield is poor, however.
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