

COMPARATIVE STUDIES OF THE MODE OF OXIDATION  
OF PHENYL DERIVATIVES OF FATTY ACIDS  
BY THE ANIMAL ORGANISM AND BY  
HYDROGEN PEROXIDE.

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- I. The catabolism of  $\beta$ -phenylpropionic acid and  $\beta$ -phenyl- $\beta$ -oxypropionic acid.
- II. The oxidation of  $\beta$ -phenylpropionic acid,  $\beta$ -phenyl- $\beta$ -oxypropionic acid and acetophenone with hydrogen peroxide.
- III. The fate of phenylacetic acid and of  $\beta$ -phenylpropionylglycocoll in the body.

Experiments upon the fate of phenyl derivatives of fatty acids in the animal body are of special interest not only on account of the fact that these substances are produced in intestinal decompositions but also because the difficultly oxidizable aromatic nucleus affords an opportunity of detecting intermediate products of metabolism which in the case of the purely fatty acids would undergo further oxidation and so escape detection. If it were possible to trace the successive steps in the oxidation of the side chain of a phenylated fatty acid the information so gained would undoubtedly throw light upon the mode of oxidation of the purely fatty acids of related structure. The present paper contains the results of such an investigation.

*I. The fate of  $\beta$ -phenylpropionic acid in the body.*

$\beta$ -Phenylpropionic acid has long been known to undergo oxidation in the course of its passage through the animal body with production of benzoic acid which is excreted in the form of hippuric acid.<sup>1</sup>

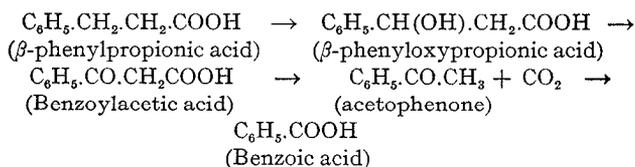


<sup>1</sup> E. and H. Salkowski: *Zeitschr. f. physiol. Chem.*, i, p. 169.

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The formation of benzoic acid from phenylpropionic acid is now commonly considered to be the result of the direct oxidation of the hydrogen attached to the  $\beta$ -carbon atom in accordance with the well-known ideas of Knoop.<sup>1</sup> Evidence in support of this view is found in the fact that phenylacetic acid does not undergo a similar oxidation in the body, this being generally assumed to indicate that phenylacetic acid is not one of the intermediate products of the catabolism of phenylpropionic acid.

So far as I am aware, no exact picture of the mechanism of the oxidation in the body of phenylpropionic acid to benzoic acid has hitherto been put forward. The results of the present investigation indicate that the reaction takes place at least in part in accordance with the following scheme:



The evidence for this belief is based on the fact that both  *$\beta$ -phenyl- $\beta$ -oxypropionic acid and acetophenone have been detected in the urine of dogs after subcutaneous injection of sodium  $\beta$ -phenylpropionate.* The  $\beta,\beta$ -phenyloxypropionic acid contains an asymmetric carbon atom and the acid found in the urine proved to be *levorotatory*. Benzoylacetic acid was not detected but its formation must be inferred from the observed excretion of acetophenone which is readily formed from benzoylacetic acid through loss of carbon dioxide.

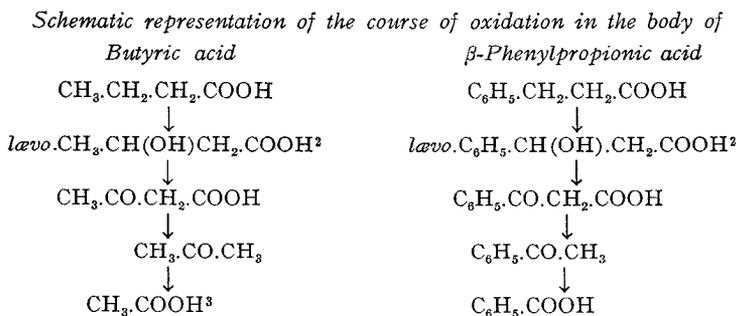
In harmony with the above hypothesis is the fact that each of the substances represented as products of intermediary catabolism of  $\beta$ -phenylpropionic acid ( $\beta,\beta$ -phenyloxypropionic acid, benzoylacetic acid and acetophenone) are themselves capable of further oxidation in the animal body with formation of benzoic acid.<sup>2</sup>

<sup>1</sup> F. Knoop: *Der Abbau aromatischer Fettsäuren im Tierkörper*, Freiburg (Baden) 1904, Ernst Kuttruff.

<sup>2</sup> Knoop: *Loc. cit.*; M. Nencki: *Journ. f. prakt. Chem.*, xviii, p. 288.

Additional evidence as to the correctness of the second step in the oxidation is furnished by the detection of acetophenone in addition to hippuric acid, in the urine of dogs which had received injections of inactive sodium  $\beta,\beta$ -phenyloxypropionate. Some unchanged phenyloxypropionic acid was excreted in the urine and showed a marked lævorotation. This result is analogous to that of McKenzie's, who investigated the fate of inactive  $\beta$ -oxybutyric acid in the dog.<sup>1</sup>

It will be seen from the following scheme that *there is the closest possible resemblance between the mode of oxidation in the body of phenylpropionic acid and that which is believed to represent the oxidation of butyric acid.* In each case a lævorotatory  $\beta$ -oxyacid is first formed, this is oxidized to a  $\beta$ -ketonic acid, which loses carbon dioxide passing into a ketone which then undergoes further oxidation with formation of lower acids.



These results afford the most convincing evidence of the occurrence of  $\beta$ -oxidation in the animal body. It is of interest to note that the excretion of  $\beta$ -phenyl- $\beta$ -oxypropionic acid and

<sup>1</sup> McKenzie: *Trans. Chem. Soc.*, 1902.

<sup>2</sup> The question whether the whole of the  $\beta$ -oxyacids pass through the stages of ketonic acid and ketone need not be considered here. It is probable that some acetic and benzoic acids are formed by direct oxidation without passing through the stages of acetone and acetophenone, respectively.

<sup>3</sup> Direct physiological evidence of the formation of acetic acid by the oxidation of acetone is not yet forthcoming. That acetone is to some extent oxidized appears to be demonstrated and there can be but little doubt that acetic acid is the first step in the process.

of acetophenone took place under conditions which were physiological except for the presence of the  $\beta$ -phenylpropionic acid in excess of the normal amount. None of the pathological conditions commonly accompanying the excretion of  $\beta$ -oxybutyric acid and acetone were present. It can hardly be doubted therefore that oxidation of the hydrogen attached to the  $\beta$ -carbon atom of saturated fatty acids constitutes the initial step in their normal catabolism.

*II. The oxidation of  $\beta$ -phenylpropionic acid and  $\beta,\beta$ -oxyphenylpropionic acid with hydrogen peroxide.*

In a number of previous communications<sup>1</sup> it has been shown that a surprisingly close analogy exists between the type of oxidation carried out under certain conditions with hydrogen peroxide and those occurring in the animal cell. It was therefore clearly of interest to try to imitate the reaction involved in the oxidation of  $\beta$ -phenylpropionic acid in the animal body, using peroxide of hydrogen as oxidizing agent. The investigation is not yet complete as the reaction is a complicated one, but the following facts have been determined.

1. Phenylpropionic acid when oxidized in the form of its ammonium salt with hydrogen peroxide yields a large amount of *acetophenone*, traces of benzaldehyde and benzoic acid and considerable amounts of aromatic oxy-acids chiefly composed of oxyphenylpropionic acids.

2.  $\beta$ -Phenyl- $\beta$ -oxypropionic acid oxidized under similar conditions yields *acetophenone*, traces of benzaldehyde and benzoic acid.

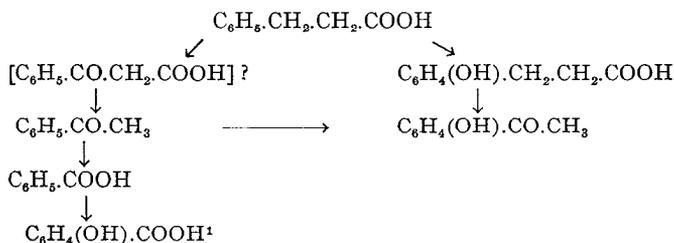
3. Acetophenone when oxidized with hydrogen peroxide in faintly ammoniacal solution yields *benzoic acid* and traces of oxybenzoic acids which result from the further oxidation of benzoic acid.<sup>2</sup> Part of the acetophenone undergoes oxidation in the nucleus, yielding oxyacetophenones.

It will be seen that the reaction is of a twofold nature in which oxidations may take place either in the side chain or in the nucleus or in both. The nuclear oxidation is similar in every

<sup>1</sup> This *Journal*, i, pp. 171 and 271, 1906; iv, pp. 63, 77, 91, 221, 227.

<sup>2</sup> *Ibid*, iii, p. 419, 1907.

way to that previously observed in the case of the oxidation of benzoic acid. The changes may be represented graphically as follows:



The results of the oxidations with hydrogen peroxide show that so far as concerns the side chains<sup>2</sup> the reactions have the closest resemblance to the similar oxidations in the animal body. The formation of acetophenone from phenylpropionic acid is perfectly similar to the formation of acetone from butyric acid<sup>3</sup> and can only be explained on the basis of the assumption of the initial oxidation taking place at the  $\beta$ -carbon atom.

There can be no doubt but that the reaction occurs, at least in part, in accordance with the scheme already put forward as representing the course of events in the tissue oxidation although at present it has not been possible to detect the  $\beta$ -oxy-acid among the hydrogen peroxide oxidation products.



It is clear that the formation of acetophenone from phenylacetic acid is perfectly analogous to the formation of ketones by the oxidation of the saturated fatty acids with peroxide of hydrogen, a reaction which has proved to be general for all straight chain fatty acids up to and including stearic acid.

<sup>1</sup> The direct formation of oxybenzoic acids by the oxidation of oxyacetophenones has not yet been demonstrated. The oxybenzoic acids are capable of further oxidation by hydrogen peroxide. (*This Journal*, iii, p. 431, 1907.)

<sup>2</sup> At present there is no evidence that phenylpropionic acid and acetophenone undergo nuclear oxidation in the animal body.

<sup>3</sup> *This Journal*, iv, p. 77.

*III. The fate of phenylacetic acid and of phenylpropionylglycocoll in the body.*

The fact that  $\beta$ -phenylpropionic acid undergoes oxidation in the animal body while phenylacetic acid is not oxidized but is paired with glycocoll and excreted as phenaceturic acid has generally been taken as evidence that oxidation of the hydrogen attached to the  $\alpha$ -carbon atom of phenylated fatty acids either does not occur or takes place with difficulty. This can hardly be regarded as a fair deduction since it may be urged that the fact of the phenylacetic acid being coupled with glycocoll to form phenaceturic acid protects the phenylacetic acid from oxidation for the stability of substances of this type is well recognized. In order to test the hypothesis of the protective action of the glycocoll grouping it was decided to prepare the analogously constituted glycocoll derivative of an acid known to be capable of animal oxidation. Accordingly  $\beta$ -phenylpropionylglycocoll,  $C_6H_5.CH_2.CH_2.CO.NH.CH_2.COOH$ , was prepared from phenylpropionyl chloride and glycocoll and was administered to dogs both by mouth and subcutaneously. It was found, however, that the glycocoll grouping did not protect the phenylpropionic acid from oxidation. No unchanged  $\beta$ -phenylpropionylglycocoll was recovered but much hippuric acid together with a smaller quantity of  $\beta$ -phenyl- $\beta$ -oxypropionic acid and acetophenone. The quantity of oxy-acid appeared to be larger than would be found in the urine after administration of corresponding amounts of phenylpropionic acid but apart from this no evidence of a protective action of the glycocoll grouping was obtained.

If the phenylpropionylglycocoll had been excreted unchanged, the conclusion would have been warranted that the non-oxidizability of phenylacetic acid might be due to its protection from oxidation through coupling with glycocoll. That this was not the case does not entitle one to draw the opposite conclusion. It may be, that phenylpropionylglycocoll is hydrolyzed in the body and that the phenylpropionic acid set free then undergoes oxidation in the usual way. The experiment must therefore be regarded as indecisive but in the meantime it is safer to refrain from drawing the conclusion that  $\alpha$ -oxidation does not occur, based on the fact that phenylacetic acid is not burned in the body. The facts so far only justify the conclusion that phenylacetylglycocoll

(phenaceturic acid) is incapable of oxidation in the animal body. It is true however that the resistance to oxidation in the organism shown by mandelic acid ( $C_6H_5.CHOH.COOH$ ) and phenylamidoacetic acid,  $C_6H_5.CHNH_2.COOH$ , the oxyphenylacetic acids and other derivatives of phenylacetic acid, which are for the greater part excreted unchanged and not in combination with glycocoll, makes it very probable that  $\alpha$  oxidation in the case of acids of this type occurs with great difficulty if at all.

#### EXPERIMENTAL PART.

*The fate of  $\beta$ -phenylpropionic acid in the body.* A number of similar experiments were made with uniformly concordant results in which four to eight grams of the acid in the form of the sodium salt in dilute aqueous solution were injected subcutaneously into a dog weighing about fifteen kilos. In every case the urine was collected for three days following the injection. In some cases after clarifying with charcoal, it was possible to observe in the unconcentrated urine a faint laevorotation, probably due to phenyl- $\beta$ -oxypropionic acid. With the exception of the substances resulting from the breakdown of the phenylpropionic acid, the presence of no abnormal urinary constituents was noted. In no case was any indication obtained of the excretion of unchanged phenylpropionic acid.

**DETECTION OF ACETOPHENONE.** The urines from two experiments in which 5.0 and 7.0 grams of  $\beta$ -phenylpropionic acid had been administered were neutralized with a little sodium carbonate solution and distilled until a few drops of the distillate gave no iodoform reaction. The distillate was then acidified with acetic acid and filtered to remove traces of fatty acids. A filtered solution of about 0.5 gram of paranitrophenylhydrazine in 40 per cent acetic acid was added to the filtrate. Precipitation of a hydrazone began at once and the separation was rendered more complete by immersing the liquid in a freezing mixture. The precipitates were then filtered off and washed with a little cold water. In one case 0.25 gram, in another 0.15 gram of crude hydrazone was obtained. It was purified by repeated crystallization from alcohol. At first the crystals melted at about  $172^\circ$  to  $175^\circ$  but after four additional recrystallizations, beautifully

formed needles melting sharply at 184° to 185° were obtained. The substance was identical in every way with acetophenone parani-trophenylhydrazone prepared for comparison from the pure ketone and corresponded exactly with the description given by Hyde.<sup>1</sup> The hydrazone dissolved with difficulty in dilute caustic soda with a rose color which became deep red on addition of alcohol. Part of the hydrazone was distilled with a little dilute sulphuric acid. The distillate smelt strongly of acetophenone, and gave intense positive reactions with the iodoform and the characteristic sodium nitroprusside tests.

DETECTION OF  $\beta$ -PHENYL- $\beta$ -OXYPROPIONIC ACID AND HIPPURIC ACID. After the acetophenone had been removed by distillation, the urines were concentrated, acidified with phosphoric acid, filtered and thoroughly extracted with ether in a continuous extractor. The ethereal extract was washed with a little water, evaporated and the residue boiled with water to remove volatile fatty acids. The solution was treated with a little charcoal and on filtering and cooling an abundant yield of hippuric acid was obtained. The hippuric acid usually amounted to one-half to two-thirds of the weight of the phenylpropionic acid administered. On recrystallizing twice from hot water the hippuric acid was obtained in the form of colorless crystals, melting-point 187°.

The filtrate from the first separation of hippuric acid was evaporated to a syrup and allowed to crystallize. The residue was then stirred with a little cold water and filtered from a small additional quantity of hippuric acid. The filtrate containing the  $\beta$ -phenyl- $\beta$ -oxypropionic acid was next examined in the polarimeter. In each of four experiments decided laevorotations varying from 1.20° to 2.05° were observed. On concentrating a crystalline residue was obtained, but it was not practicable to obtain crystals of the acid of correct melting point, owing to the presence of other substances. Accordingly the residue was boiled with dilute sulphuric acid so as to convert the oxy-acid into cinnamic acid, which could be more readily identified:



On extracting with ether a mixture of acids was obtained which was fractionally distilled from a very small flask. Some fatty

<sup>1</sup> *Berichte d. deutsch. chem. Gesellsch.*, xxxii, p 1814

acids came over first followed by benzoic acid derived from hippuric acid which had not been completely removed in the previous processes. When the thermometer reached  $265^{\circ}$ ,<sup>1</sup> the receiver was changed and a small fraction distilling between  $265^{\circ}$  and  $300^{\circ}$  was separately collected. This was crystallized twice from boiling water and yielded in one case slightly more than a tenth of a gram of pure cinnamic acid melting sharply at  $133^{\circ}$ . For further characterization the acid was converted into the dibromide by brominating in carbon disulphide solution. Platelets sparingly soluble in carbon disulphide, melting at  $195^{\circ}$  to  $196^{\circ}$ , identical with the product described by Glaser<sup>2</sup> were readily obtained. The formation of cinnamic acid from the  $\beta$ -phenyl- $\beta$ -oxypropionic acid is exactly analogous to the production of crotonic acid from  $\beta$ -oxybutyric acid and takes place even more readily than the latter reaction.

The presence of  $\beta$ -phenyl- $\beta$ -oxypropionic acid was further confirmed by the two following tests which are sensitive and serve for its detection in mixtures in which the amount of acid is too small to permit of the satisfactory isolation of the cinnamic acid.

(a) The crude acid, freed if necessary from phenylpropionic acid by steam distillation, is neutralized with ammonia and oxidized with hydrogen peroxide exactly as described on p. 428. If the oxy-acid be present acetophenone is formed and is readily identified by conversion into its nitrophenylhydrazone, by the iodoform reaction and by the nitro-prusside test. (b) The crude acid in concentrated solution is stirred in an open dish with a few drops of cold concentrated potassium permanganate solution. In the presence of  $\beta$ -phenyl- $\beta$ -oxypropionic acid a strong odor of benzaldehyde is at once recognizable.

**BENZOYLACETIC ACID.** Unsuccessful attempts to isolate this acid were made. In order to test qualitatively the urine was acidified with phosphoric acid and extracted with ether and the ethereal residue taken up in dilute alcohol and tested with ferric chloride which as Baeyer has shown, gives a violet color similar to the acetoacetic acid reaction.<sup>3</sup> The results were not sufficiently

<sup>1</sup> Benzoic acid boils at  $249^{\circ}$ .

<sup>2</sup> Liebig's *Ann. d. Chem.*, cxlvii, p. 91.

<sup>3</sup> *Ber. d. deutsch. chem. Gesellsch.*, xv, p. 2705.

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decisive to warrant the conclusion of the presence of this acid. It is not improbable, however, that a very small quantity of benzoylactic acid was present.

*The fate of  $\beta$ -phenyl- $\beta$ -oxypropionic acid in the body.*  $\beta$ -Phenyl- $\beta$ -oxypropionic acid was prepared by Fittig and Binder's<sup>1</sup> method by boiling  $\beta$ -bromhydrocinnamic acid (20 grams) with water (200 cc.) under a reflux condenser. The liquid was then cooled in ice and filtered from the precipitate of cinnamic acid containing a little adhering styrol. The filtrate was then repeatedly extracted with ether which on evaporation left 9 grams of the oxy-acid which readily crystallized. In each experiment three grams of the acid were neutralized with dilute caustic soda and injected subcutaneously into a dog weighing ten kilos. The urine was examined precisely as in the preceding case in which phenylpropionic acid had been administered. Hippuric acid amounting to about 1.0 gram was separated. Acetophenone was identified by means of its paranitrophenylhydrazone (0.09 gram) which melted at  $183^{\circ}$  to  $184^{\circ}$  after recrystallization from alcohol. Benzoylactic acid could not be detected. Unchanged  $\beta$ -phenyl- $\beta$ -oxypropionic acid was detected by the laevorotation ( $1.55^{\circ}$ ) of the aqueous solution of the ethereal extract and by the oxidation to acetophenone by hydrogen peroxide and to benzaldehyde by potassium permanganate.

*The oxidation of  $\beta$ -phenylpropionic acid with hydrogen peroxide.* The phenylpropionic acid (15.0 gram =  $\frac{1}{10}$  gram molecular) was dissolved in a slight excess of ammonia and then gently warmed under a reflux condenser with 400 cc. of neutral 3 per cent hydrogen peroxide.<sup>2</sup> After a time the solution was gently boiled for three hours and then distilled. The distillate contained much ammonia and was acidified with phosphoric

<sup>1</sup> Liebig's *Ann. d. Chem.*, cxcv, p. 138.

<sup>2</sup> It was of interest to ascertain if the reaction could be demonstrated to occur at low temperatures as was found to be the case in the oxidation of butyric acid. (This *Journal*, iv, p. 87). Accordingly an experiment was made in which the temperature was maintained at  $37^{\circ}$  for twenty-four hours. At the end of this time the excess of hydrogen peroxide was removed by agitation with freshly precipitated silver oxide. The liquid was then acidified with phosphoric acid and distilled. The distillate readily gave qualitative reactions for acetophenone with sodium nitroprusside and with the iodoform test, but the quantity of ketone was small.

acid and redistilled. The distillate contained oil drops which proved to be acetophenone with traces of oxy-acetophenone and benzaldehyde. The acetophenone was purified by warming the distillate with ammoniacal silver nitrate in the presence of caustic soda and then acidifying and redistilling. In this way about 1.0 gram of acetophenone was obtained.<sup>1</sup> It readily responded to all the tests for this substance including the iodoform and nitroprusside reactions and on treatment with paranitrophenylhydrazine in acetic acid solution gave an abundant yield of the hydrazone which crystallized from alcohol in needles melting sharply at 184° to 185°. The residue from which the acetophenone had been removed by distillation was acidified with phosphoric and repeatedly extracted with rhigolene to remove unchanged  $\beta$ -phenylpropionic acid. In this way more than two-thirds of the phenylpropionic acid was recovered. The liquid was then extracted with ether which on evaporation left 1.5 gram of residue which crystallized on standing. It was examined with a negative result for  $\beta$ -phenyl- $\beta$ -oxypropionic acid. The crystalline residue was fractionally crystallized, employing benzene and chloroform as solvents but it was impossible to obtain acids of constant melting point. The observed melting points varied from 80° to 120° and in every respect the substance behaved like a mixture of oxyphenylpropionic acids. The least soluble fraction was converted into the zinc salt which crystallized in small prisms and was sparingly soluble in cold water. The analysis agreed with zinc oxyphenylpropionate:

0.1065 gram salt dried at 100° gave 0.0219 gram Zn = 16.5 per cent Zn.  
 $C_{18}H_{18}O_6Zn$  requires 16.52 per cent Zn.

The most soluble fraction was converted into the calcium salt which was sparingly soluble in cold water:

0.1466 gram gave 0.0401 gram  $CaCO_3$  = 10.94 per cent Ca.  
 $C_{18}H_{18}O_6Ca$  requires 10.81 per cent Ca.

Both fractions gave a bluish-gray precipitate with ferric chloride and all the usual reaction for phenolic acids with Millon's reagent, diazo salts, etc. It is probable that the substance repre-

<sup>1</sup> Minute traces of oxy-acetophenones were present and were qualitatively detected with ferric chloride, Millon's reagent and the diazo-reaction.

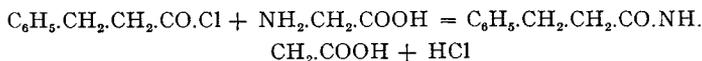
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sented a difficultly separable mixture of *ortho*, *meta* and *para*-oxyphenylpropionic acids.

*Oxidation of  $\beta$ -phenyl- $\beta$ -oxypropionic acid.* This acid was oxidized in exactly the same way as the  $\beta$ -phenylpropionic acid and the yield of acetophenone was approximately the same. As in the former case, the production of acetophenone can be demonstrated to occur even when the reaction is carried on at  $37^\circ$  but the change proceeds much more rapidly at higher temperatures. The acetophenone was separated by distillation and after traces of benzaldehyde had been removed by oxidizing with ammoniacal silver nitrate, it was redistilled from acid solution and identified by conversion into the paranitrophenylhydrazone which melted at  $184^\circ$  to  $185^\circ$ , after crystallization from alcohol. On acidifying the original solution from which the acetophenone had been distilled with phosphoric acid and extracting with ether a residue was obtained which contained a large quantity of unchanged  $\beta$ -phenyl- $\beta$ -oxypropionic acid and a little benzoic acid. The latter was separated by extraction with petroleum and crystallization from water. The amount of benzoic acid was not more than 1 per cent.

*Oxidation of acetophenone.* Acetophenone (5 cc.) was gently warmed under a reflux condenser with 3 per cent hydrogen peroxide (100 cc.) and a few drops of ammonia. Even at the boiling point the ketone is attacked with difficulty. After several hours the solution was made decidedly alkaline with ammonia and the unchanged ketone extracted with ether. It was found that the ketone so recovered gave a strong violet red coloration with ferric chloride similar to that given by ortho-acetylphenol ( $\text{CH}_3\text{CO.C}_6\text{H}_4\text{OH}$ ) so that it is probable that nuclear hydroxylation of the ketone had taken place to some extent as in the case of the other similar oxidations. After the unchanged ketone had been removed, the liquid was acidified with phosphoric acid and extracted with ether. The ethereal residue was small but quickly solidified and after two recrystallizations from water pure benzoic acid, melting-point  $121^\circ$  to  $122^\circ$ , was readily obtained. Minute traces of phenolic acids resulting from the further oxidation of benzoic acid could be detected in the mother liquor by means of ferric chloride and Millon's reagent, and the diazo reactions.

*Synthesis of phenylpropionylglycocoll and its fate in the organism.* Phenylpropionylglycocoll was prepared by the interaction of phenylpropionylchloride and glycocoll at low temperatures in the presence of excess of caustic soda.



The acid chloride was prepared in the usual way from phenylpropionic acid and phosphoric pentachloride and was fractionated *in vacuo*. Thirty grams of the acid gave 26 grams of chloride boiling at 119° to 121° under 18 mm. pressure. A number of different experiments were made to determine the best conditions for the interaction with glycocoll and the following was found most suitable.<sup>1</sup> Ten and eight-tenths grams of finely powdered glycocoll are added to 60 cc. of 20 per cent caustic soda solution contained in a flask which is immersed in a freezing mixture of ice and salt. Twenty-four grams of phenylpropionylchloride is then added in small portions at a time from a dropping funnel. The liquid is vigorously shaken and the temperature must be kept well below 0°. After all the chloride has been added and no oil drops are visible 7 cc. of concentrated sulphuric acid diluted with an equal volume of water are gradually added with shaking and cooling. The thick precipitate is filtered off, washed and dried and then washed again with dry alcohol-free ether, in order to remove any unchanged phenylpropionic acid. The yield of crude phenylpropionylglycocoll is practically the theoretical amount. It is purified by recrystallization from water containing a little alcohol. The substance crystallizes in long, thick needles melting at 114° to 115°. Phenylpropionylglycocoll is more readily soluble in water than hippuric acid and is almost insoluble in dry alcohol-free ether and petroleum ether, but very readily soluble in alcohol. It is readily hydrolyzed by acids with formation of phenylpropionic acid and glycocoll.

<sup>1</sup>I am indebted to Miss Mary Dows Herter for performing these experiments.

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On analysis:

0.1620 gram substance gave 0.3781 gm. CO<sub>2</sub> and 0.0914 gm. H<sub>2</sub>O.  
 0.2018 " " " 0.0135 gm. N (Kjeldahl).

	Calculated for C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N.	Found.
C.....	63.76 per cent.	63.65 per cent
H.....	6.28 " "	6.27 " "
N.....	6.77 " "	6.69 " "

In the first experiment 2.5 grams phenylpropionylglycocoll were mixed with chopped meat and fed to a dog weighing about ten kilos. The urine was collected and concentrated, acidified with phosphoric acid and extracted with ether to which some alcohol had been added. The ethereal extract was hydrolyzed by boiling under a reflux condenser with concentrated hydrochloric acid and then distilled in steam. The distillate was extracted with ether and gave much benzoic acid but no evidence was obtained of the presence of phenylpropionic acid. The glycocoll group therefore had not protected the phenylpropionic acid from oxidation.

In the next experiments the phenylpropionylglycocoll was administered subcutaneously as there was a possibility of the substance being hydrolyzed before absorption, if given by the mouth. Accordingly 3.0 grams of the substance were dissolved in very dilute alcohol and injected subcutaneously. The urine on distillation yielded a little acetophenone which was identified by the methods previously employed and the residue was then evaporated and extracted with alcoholic ether. The ethereal residue was hydrolyzed with sulphuric acid and then distilled in steam. The volatile acids were extracted with ether and then distilled from a very small flask. Fraction I, boiling-point up to 260° was practically pure benzoic acid. It was recrystallized from water and melted at 121° to 122°. Fraction II, 260° to 290°, was very small and contained a mixture of benzoic and cinnamic acids. Fraction III distilling over 290° weighed 0.25 gram and gave pure cinnamic acid, melting at 132° to 133°, after two recrystallizations from water. It was further identified by converting it into dibromhydrocinnamic acid, melting point 195° to 196° by brominating in carbon bisulphide solution. No indication was obtained of any unchanged β-phenylpropionic acid. β-phenyl-β-oxy-propionic acid was doubtless the mother substance of the

cinnamic acid for the former body has already been shown to occur among the oxidation products of phenylpropionic acid and the boiling with sulphuric acid for the purpose of hydrolysis would at once convert it into cinnamic acid.

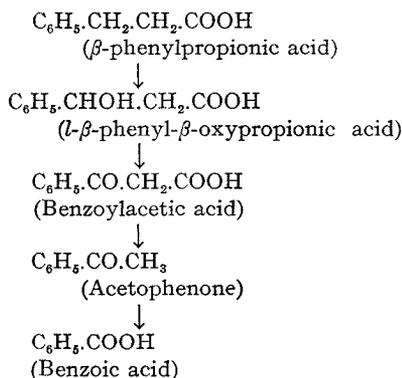
The investigation of the mode of oxidation of other phenyl derivatives of the fatty acids is being continued.

## SUMMARY.

The subcutaneous injection of  $\beta$ -phenylpropionic acid in the form of its sodium salt in aqueous solution in amounts equivalent to about 0.3 to 0.5 gram per kilo results in the excretion in the urine of  $\beta$ -phenyl- $\beta$ -oxypropionic acid ( $C_6H_5.CH(OH).CH_2.COOH$ ) and acetophenone in addition to benzoic acid (hippuric acid). The  $\beta$ -phenyl- $\beta$ -oxypropionic acid appears to be *lævorotatory* and was identified by its conversion into cinnamic acid, melting point  $133^\circ$ , and into dibromhydrocinnamic acid, melting point  $195^\circ$  to  $196^\circ$ , and also by its oxidation to acetophenone by means of hydrogen peroxide and to benzaldehyde with potassium permanganate. The acetophenone was identified by conversion into the paranitrophenylhydrazone, melting point  $183^\circ$  to  $184^\circ$ , and by other tests.

Similar injections of sodium- $\beta$ -phenyl- $\beta$ -oxypropionate resulted in the excretion of acetophenone, benzoic acid (hippuric acid) and a little unchanged *lævorotatory*  $\beta$ -phenyl- $\beta$ -oxypropionic acid.

The above results show that  $\beta$ -phenylpropionic acid undergoes oxidation in the body, at least in part, in accordance with the following scheme:



## 434 Oxidation of Phenyl Derivatives of Fatty Acids

In harmony with the above scheme is the fact that each substance represented as a product of intermediary metabolism is capable of oxidation to benzoic acid in the animal body.

Benzoylacetic acid could not be satisfactorily identified in the urine but its production must be inferred from the presence of acetophenone into which it readily passes with loss of carbon dioxide. The results afford clear proof that oxidation of hydrogen attached to the  $\beta$ -carbon atom of fatty acids takes place in the organism and show further that there is the closest analogy between the mode of oxidation in the organism of phenylpropionic acid and that which is believed to represent the similar oxidation of butyric acid. In each case a levorotatory  $\beta$ -oxy-acid is produced which then passes into a  $\beta$ -ketonic acid, which yields a ketone by loss of carbon dioxide. The ketone may be further oxidized to an acid.

In order to obtain chemical analogy for the type of oxidation believed to occur in the organism,  $\beta$ -phenylpropionic acid and  $\beta$ -phenyl- $\beta$ -oxypropionic acid were oxidized in the form of their ammonium salts with hydrogen peroxide. The reaction takes place to a slight extent at low temperature ( $37^{\circ}$ ) but more readily on warming. In each case acetophenone was produced in considerable amount. The formation of this substance from  $\beta$ -phenylpropionic acid can only be explained on the assumption of oxidation taking place at the  $\beta$ -carbon atom. Acetophenone on further oxidation with hydrogen peroxide yields benzoic acid. There is therefore a close analogy between the type of oxidation believed to occur in the organism and that capable of being effected by hydrogen peroxide. In addition to the products mentioned, oxidation with hydrogen peroxide results in the formation of phenolic derivatives through oxidation in the nucleus. Oxyphenylpropionic acids, and oxybenzoic acids are produced in this way. The course of oxidation of  $\beta$ -phenylpropionic acid with hydrogen peroxide is represented on p. 421.

In order to test the hypothesis that the resistance of phenylacetic acid to oxidation in the organism might be due to the fact that it undergoes condensation with glycocoll and is excreted as phenyl-acetylglycocoll and that the glycocoll might exercise a protective action, the homologous substance, phenylpropionylglycocoll, was synthesized and its fate in the organism investi-

gated. No clear proof of a protective action of the glycocholic acid could be obtained. When the substance was given either by mouth or subcutaneously the ordinary products of the catabolism of phenylpropionic acid were formed ( $\beta$ -phenyl- $\beta$ -oxypropionic acid, acetophenone and hippuric acid) but no unchanged phenylpropionic acid or its derivatives could be detected.