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# The Effect of Catalytic Amounts of Epinephrine and of Adrenochrome on the Oxidation of Glycine

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## INTRODUCTION

A deficiency or an abnormal metabolism of glycine in schizophrenic patients is suggested by the following experimental findings. The first of these is the decreased ability of schizophrenic patients to detoxify benzoic acid by conjugation with glycine at a normal rate (1–6). However, if glycine be administered together with benzoic acid, the hippuric acid excretion is normal, indicating that the enzymes involved can function but that the available glycine is low (7–9). The second is the increased concentration of phosphoglycolic acid reported to occur in erythrocytes of schizophrenic patients (10, 11). This could be due to an increased rate of oxidative deamination of glycine to glyoxylic acid followed by reduction to glycolic acid and phosphorylation by adenosine triphosphate (ATP). A low availability of glycine could also be a contributing factor for the low blood glutathione levels found in schizophrenia (12, 13) since glycine is one of the amino acids comprising this peptide.

In schizophrenia there is also some evidence for an abnormal metabolism of epinephrine. Hoffer *et al.* (14) have noted the structural similarity between adrenochrome (an oxidation product of epinephrine), mescaline, and lysergic acid diethylamide, and claimed that adrenochrome when given to normal individuals produced mental disturbances. This observation does not appear to have been confirmed, but changes in the electroencephalogram following adrenochrome administration have been reported by Hoffer's group as well as by others (15, 16). Lea (17) has found a highly significant excess of deeply pigmented types among a population of schizophrenic individuals, compared with normal persons,

indicating an increase in melanin formation. This could be formed from epinephrine by way of adrenochrome.

Rinkel *et al.* (18) have also considered the possibility that the metabolism of epinephrine is involved in the production of schizophrenia.

Leach *et al.* (19) have reported an increased rate of oxidation of epinephrine by the serum of schizophrenic patients, and Akerfeldt (20) showed an increased concentration of ceruloplasmin and a decrease in ascorbic acid or other reducing material in serum in this disease. It was pointed out that neither of these tests is specific for schizophrenia however.

In view of these findings it was decided to determine whether epinephrine and/or its oxidation products had any effect *in vitro* on the metabolism of amino acids.

## Methods

The rat tissues were homogenized in water to give a 20% concentration of tissue; 0.5 ml. of homogenate was used in each Warburg vessel. The buffer was dimethylglycine, pH 8.2, in a final concentration of 0.25 *M* as used by Ratner (21) for measuring glycine oxidase activity. The final concentration of glycine was 0.25 *M*; that of some other amino acids was less than this because of difficult solubility. The pH of the amino acid solutions was adjusted to pH 8.2. Epinephrine (Upjohn U.S.P.), 1 mg./ml., was dissolved in water by adding HCl equivalent to the epinephrine. The pH was then adjusted to about 8.2 by adding NaOH. Adrenochrome was prepared by Dr. D. A. Shepherd of the Chemistry Department of The Upjohn Company. This was added to the main compartment immediately before placing the vessels in the bath. The total volume of the reaction mixture was 2 ml. The gas phase was either air or oxygen, the temperature 37°C. Oxygen consumption was measured by the manometric procedure, using Warburg vessels of about 15 ml. capacity, $CO_2$ being absorbed by KOH in the center well. $CO_2$ was determined as the difference in gas exchange in the presence and absence of KOH in the center well after liberating $CO_2$ retained by the buffer by the addition of acid to the medium.

Ammonia was determined by the method of Ma and Zuazaga (22), glycolic acid by the method of Dagley and Rodgers (23), glyoxylic acid by the method of Long (24). For the determination of formic acid the method of Grant (25) was first used, but the results obtained were very variable. The method of Weihe and Jacobs (26), however, although less sensitive, gave reproducible results. This method was modified as follows. The solution to be analyzed for formic acid (up to 1 ml.) was placed in the main compartment of a Warburg vessel together with 0.2 ml. *N* acetic acid. In the side bulb was placed 0.8 ml. mercuric acetate (10% solution in 0.5 *N* acetic acid). The vessel was equilibrated, closed, and read, and the mercuric acetate was tipped into the main compartment. The $CO_2$ evolved was measured and was found to be directly proportional to the formic acid present so that the

rate for the sample to be analyzed could be compared with the rate of a standard formic acid solution and the amount of formic acid in the sample estimated.

## Results

The effect of epinephrine and of adrenochrome on the oxidation of glycine in the presence of rat tissues is shown in Table I and in Figs. 1 and 2. Since the stimulating effects of epinephrine and adrenochrome were similar, it is possible that the same material was the active agent in both cases, as epinephrine is oxidized to adrenochrome and to further oxidation products spontaneously in air, as well as enzymically by cytochrome oxidase and certain other enzymes.

In the presence of tissues the glycine was oxidized predominantly to ammonia and glyoxylic acid (Table I), little or no formic acid being formed in most experiments. The reaction mixture gave a test for glycolic acid, but it was found that glyoxylic acid interfered with this determination giving 10% of the color with chromotropic acid when compared in equimolar amounts. The main course of the reaction can be written as follows:

$$\begin{array}{l}CH_2NH_2\\|\\COOH\end{array} + \tfrac{1}{2}O_2 \rightarrow \begin{array}{l}CHO\\|\\COOH\end{array} + NH_3$$

Added flavine adenine dinucleotide (FAD) had no effect on the course of the reaction (Table I).

Serotonin stimulated the conversion by brain of glycine to glyoxylic acid, although it was not as effective as adrenochrome (Table I).

When catalytic amounts of epinephrine or of adrenochrome were added to glycine in the absence of tissue, there was a marked increase in the oxidation of glycine (Figs. 3 and 4). After this observation was made it was found that Edlbacher and Kraus in 1928 (27) reported that in the presence of epinephrine or its oxidation products glycine was oxidized to $CO_2$, $NH_3$, and traces of formaldehyde. Under our experimental conditions we found $CO_2$, $NH_3$, formic acid, and some glyoxylic acid (Table II) as the products of the reaction. No formaldehyde or glycolic acid was found. The course of the reaction can be written

$$\begin{array}{l}CH_2NH_2\\|\\COOH\end{array} + O_2 \rightarrow NH_3 + CO_2 + HCOOH$$

Reducing the amount of catalyst reduced the rate of oxidation (Table

TABLE I

*Effect of Epinephrine and Adrenochrome on the Oxidation of Amino Acids by Rat Tissues*

| Tissue | Additions | Duration of experiment | $O_2$ consumed | $NH_3$ formed | C—O formed |
|---|---|---|---|---|---|
| | | *min.* | *μmoles* | *μmoles* | *μmoles* |
| Kidney | None | 220 | 2.9 | 5.2 | 0.9 |
| | Glycine | | 3.1 | 7.0 | 0.6 |
| | Adrenochrome | | 3.1 | 5.4 | 1.0 |
| | Glycine + adren.[a] | | 7.4 | 15.4 | 18.2 |
| Brain | None | 90 | 1.8 | 0.88 | 0.5 |
| | Glycine | | 2.3 | 6.9 | 0.5 |
| | Adrenochrome | | 1.2 | 3.0 | 0.5 |
| | Glycine + adren. | | 4.4 | 9.9 | 12.2 |
| Brain | None | 75 | 1.5 | | 0.2 |
| | Glutamic acid | | 3.9 | | 0.5 |
| | Adrenochrome | | 1.3 | | 0.5 |
| | Glutamic acid + adren. | | 5.1 | | 1.9 |
| Kidney | None | 75 | 2.4 | | 0.5 |
| | Glutamic acid | | 5.8 | | 1.2 |
| | Adrenochrome | | 2.0 | | 0.6 |
| | Glutamic acid + adren. | | 5.6 | | 3.0 |
| Brain | None | 175 | 1.8 | 4.3 | |
| | Glycine | | 2.2 | 5.2 | |
| | Adrenochrome | | 1.8 | 4.2 | |
| | Glycine + adren. | | 12.8 | 20.7 | |
| Kidney | None | 160 | 3.0 | 12.0 | |
| | Tryptophan | | 4.5 | 12.5 | |
| | Adrenochrome | | 2.8 | 13.9 | |
| | Tryptophan + adren. | | 5.9 | 18.9 | |
| Kidney | None | 100 | 2.5 | 5.2 | |
| | Glycine | | 3.0 | 7.5 | |
| | Adrenochrome | | 2.5 | 6.3 | |
| | Glycine + adren. 25 μg./ml. | | 4.9 | 12.4 | |
| | Glycine + adren. 50 μg./ml. | | 6.3 | 16.5 | |
| Kidney | None | 60 | 2.5 | 9.6 | |
| | L-Phenylalanine | | 4.3 | 11.6 | |
| | Adrenochrome | | 1.7 | 9.0 | |
| | L-Phenylalanine + adren. 5 μg./ml. | | 5.1 | 10.9 | |
| | " " 25 μg./ml. | | 5.5 | 13.1 | |
| | " " 50 μg./ml. | | 6.0 | 14.5 | |
| Brain | None | 105 | 1.7 | | 0.5 |
| | Adrenochrome 100 μg./ml. | | 1.3 | | 0.6 |
| | FAD 100 μg./ml. | | 1.7 | | 0.4 |
| | Glycine | | 2.2 | | 0.5 |

TABLE I—*Continued*

| Tissue | Additions | Duration of experiment | $O_2$ consumed | $NH_3$ formed | C—O formed |
|---|---|---|---|---|---|
| | | *Min.* | *μmoles* | *μmoles* | *μmoles* |
| Brain—(*cont.*) | Glycine + FAD 25 μg./ml. | | 1.0 | | 0.7 |
| | " " 50 μg./ml. | | 1.6 | | 0.7 |
| | " " 100 μg./ml. | | 1.3 | | 0.6 |
| | Glycine + adren. 50 μg./ml. | | 8.0 | | 27.3 |
| | Glycine + adren. 50 μg. + FAD 25 μg./ml. | | 7.7 | | 26.0 |
| | Glycine + adren. 50 μg. + FAD 50 μg./ml. | | 7.3 | | 21.8 |
| | Glycine + adren. 50 μg. + FAD 100 μg./ml. | | 7.8 | | 24.3 |
| | Glycine + adren. 100 μg. + FAD 25 μg./ml. | | 14.5 | | 36.7 |
| | Glycine + adren. 100 μg. + FAD 50 μg./ml. | | 12.5 | | 36.5 |
| | Glycine + adren. 100 μg. + FAD 100 μg./ml. | | 12.6 | | 40.5 |
| Brain | None | 240 | 2.3 | | 0.3 |
| | Glycine | | 2.7 | | 0.3 |
| | Serotonin | | 1.7 | | 0.5 |
| | Glycine + serotonin | | 2.5 | | 1.0 |
| Liver | None | 90 | 4.1 | | 0.6 |
| | Glycine | | 5.6 | | 0.6 |
| | Adrenochrome | | 3.6 | | 0.5 |
| | Glycine + adren. | | 5.9 | | 6.7 |

[a] Adren. = adrenochrome.

III). Reducing the amount of glycine also reduced the rate of oxidation. The glycine was not completely oxidized, but with lower amounts of glycine the percentage oxidized was higher (Table III). This may have been due to inadequacy of the buffer with the higher amounts of glycine or to the catalyst losing its activity after a certain period of time.

With a gas phase of pure oxygen, the rate of the catalytic oxidation of glycine by adrenochrome was less and the reaction stopped earlier than when the gas phase was air. With epinephrine as a catalyst, the initial rate was higher in $O_2$ than in air, and in some experiments in air there was a lag period before oxidation began at an appreciable rate. However, in oxygen the reaction stopped earlier and the total oxygen consumed

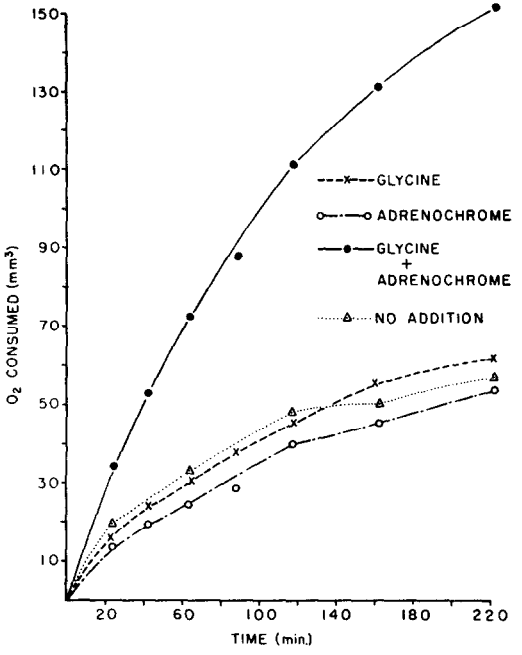



FIG. 1. Oxidation of glycine in the presence of rat kidney.

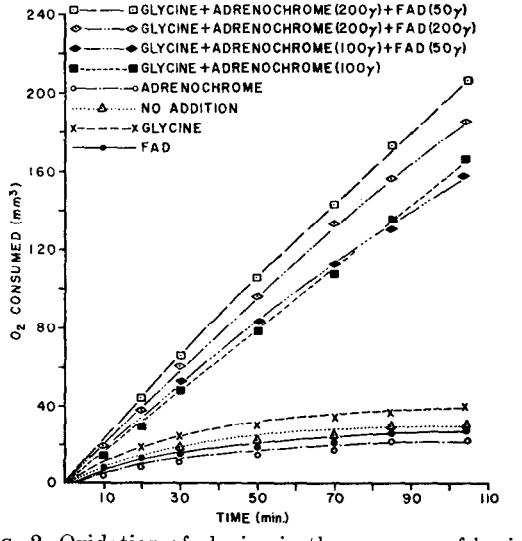



FIG. 2. Oxidation of glycine in the presence of brain.

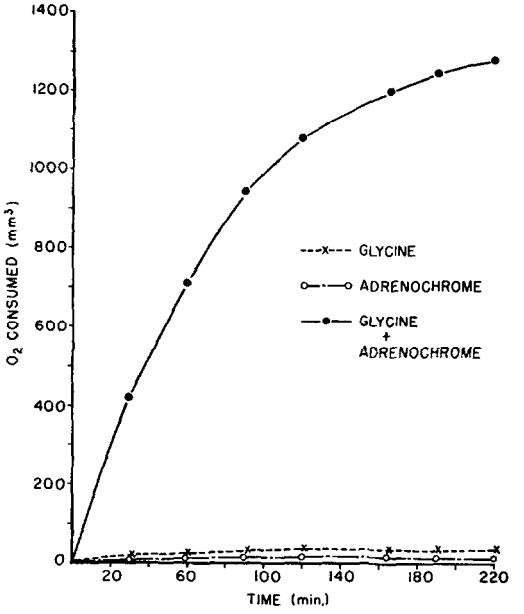



FIG. 3. Catalytic oxidation of glycine by adrenochrome.

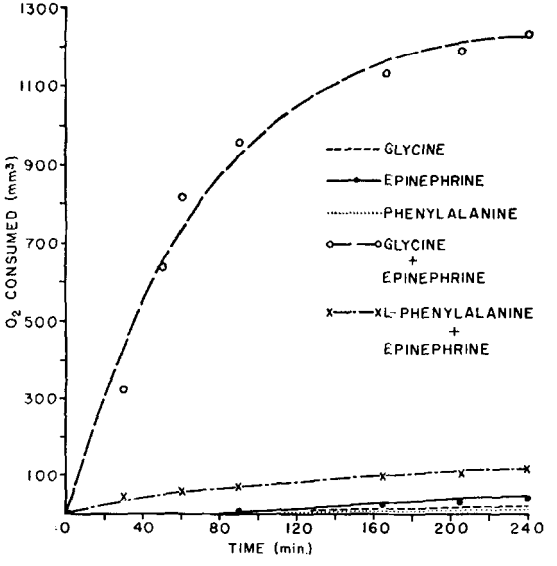



FIG. 4. Catalytic oxidation of glycine and of phenylalanine by epinephrine.

TABLE II

*Catalytic Oxidation of Glycine by Adrenochrome and the Effect of Catalase Thereon*

| Experiment | Enzyme added | $O_2$ absorbed | $CO_2$ evolved | Millimoles $NH_3$ formed | Formic acid formed | Glyoxylic acid formed |
|---|---|---|---|---|---|---|
| 1 | None | 20.0 | — | — | 17.0 | — |
| 2 | None | 28.0 | — | — | 20.0 | — |
| 3 | None | 19.5 | — | — | 20.3 | — |
| 4 | None | 14.0 | 14.0 | | | |
| 5 | None | 17.5 | 18.3 | — | — | — |
| 6 | None | 56.0 | — | 59.0 | — | — |
| 7 | None | 54.0 | — | 59.0 | — | — |
| 8 | None | 65.0 | — | — | 52.0 | 16.0 |
| 9 | None | 55.5 | — | — | — | 19.5 |
| | Catalase | 19.9 | — | — | — | 58.5 |
| 10 | None | 64.5 | — | — | — | 18.2 |
| | Catalase | 37.0 | — | — | — | 103.0 |

TABLE III

*Effect of Variation in Glycine and Adrenochrome Concentration on the $O_2$ Consumption*

| Glycine | Adrenochrome | Duration of experiment | $O_2$ consumed | Per cent of glycine oxidized |
|---|---|---|---|---|
| *mmoles* | *μg.* | *min.* | *mmoles* | |
| 0.05 | 100 | 240 | 0.02 | 40 |
| 0.05 | 100 | 408 | 0.02 | 40 |
| 0.25 | 100 | 220 | 0.06 | 23 |
| 0.05 | 50 | 240 | 0.01 | 20 |

was much less than when the gas phase was air. This may be due to an irreversible oxidation of the catalyst under the higher oxygen tension.

Under anaerobic conditions with equimolar amounts of adrenochrome and glycine there was no appreciable formation of $NH_3$.

Neither dimethylglycine, glycolic acid, nor formic acid was oxidized catalytically by adrenochrome. Glyoxylic acid was oxidized very slowly compared with glycine.

Glycylglycine was oxidized slowly. For one mole $O_2$ consumed, one mole $NH_3$ and two carbonyl equivalents (determined as dinitrophenyl hydrazone) were formed. Other amino acids were oxidized slowly in the presence of adrenochrome with the liberation of $NH_3$ (Table IV).

Serotonin (5-hydroxytryptamine) had no effect on the rate of oxidation of glycine. Hydrogen peroxide oxidized glycine rapidly, $NH_3$ and formic acid being detected among the reaction products.

TABLE IV

*Effect of Adrenochrome on the Oxidation of Amino Acids*

The final concentration of the amino acids was 0.25 *M* except for leucine, tryptophan, and tyrosine in which cases the final concentration was 0.125 *M*.

| Amino acid | Cu. mm. $O_2$ at 200 min. Adrenochrome alone | Amino acid alone | Adrenochrome + amino acid |
|---|---|---|---|
| L-Alanine | 19 | 43 | 72 |
| DL-Alanine | 19 | 42 | 75 |
| L-Arginine | 0 | 142 | 166 |
| L-Asparagine | 0 | 39 | 99 |
| L-Aspartate | 19 | 42 | 99 |
| L-Glutamate | 12 | 58 | 97 |
| D-Glutamate | 12 | 58 | 102 |
| Glycine | 10 | 30 | 1260 |
| Glycylglycine | 5 | 5 | 125 |
| L-Leucine | 20 | 15 | 59 |
| L-Lysine | 20 | 67 | 100 |
| L-Phenylalanine | 10 | 14 | 73 |
| L-Tryptophan | 15 | 36 | 76 |
| L-Tyrosine | 15 | 26 | 42 |

When catalase was added to glycine and catalytic amounts of adrenochrome, the rate of oxygen consumption was decreased and the oxidation went only as far as glyoxylic acid (Table II).

With boiled rat tissue, glycine, and adrenochrome, the oxygen consumption and the products formed were the same as without tissue, the reaction going mainly to formic acid.

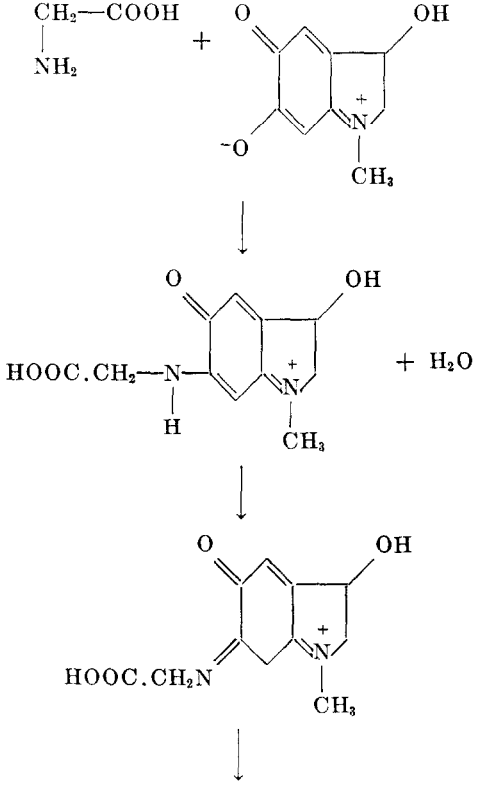
## Discussion

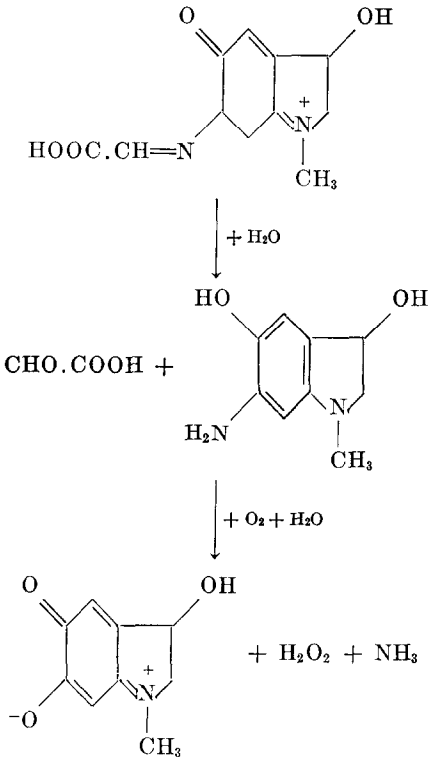
In the experiments described above it may be seen that when catalytic amounts of epinephrine or adrenochrome were added to glycine in the presence of tissue there was a marked increase in the rate of oxidation, and the oxygen consumed was accounted for largely by the formation of glyoxylic acid and ammonia. These are the products of the enzymic oxidation of glycine by glycine oxidase (21).

When adrenochrome or epinephrine, in catalytic amounts, was added to glycine without tissue there was also a marked increase in oxygen consumption. This had been reported previously by Edlbacher and Kraus in 1928 (27) who reported the products of oxidation as being $CO_2$, $NH_3$, and traces of formaldehyde. Kisch in 1930–32 reported that an oxidation product of epinephrine, an adrenaline quinone,

deaminated glycylglycine, the ethyl ester of glycine, serine, and leucine (28–30). Other substances which catalyzed oxidative deamination at pH 9–11 were hydroquinone, *p*-quinone, pyrogallol, phloroglucinol, resorcinol, and homogentisic acid (31). With resorcinol as a catalyst, 50 % of the glycine was deaminated at pH's between 9 and 12 in about 40 hr.

Under our experimental conditions the products of the catalytic oxidation of glycine by epinephrine or by adrenochrome were formic acid, ammonia, and $CO_2$ in a ratio of 1:1:1, with occasionally small amounts of glyoxylic acid. The oxidation of glyoxylic acid was also catalyzed by adrenochrome, but the magnitude of the effect was much less than with glycine. In a discussion of these experiments with Dr. Melvin Calvin, of the University of California, Berkeley, California, he suggested the following mechanism for the reaction between adrenochrome and glycine.






$$H_2O_2 + CHO.COOH \rightarrow HCOOH + CO_2 + H_2O$$

This idea was tested by adding catalase to a glycine solution in the presence of adrenochrome, in order to destroy the $H_2O_2$. If this be the mechanism of the reaction, one could expect glyoxylic acid to accumulate in the presence of catalase. This was indeed found to be the case (Table II). In the presence of catalase the $O_2$ uptake was reduced and the glyoxylic acid was markedly increased and accounted for all the $O_2$ consumed.

The oxidation of glycine by adrenochrome in the presence of tissue is thus essentially a catalytic (non-enzymic) oxidation of glycine which is stopped at the glyoxylic acid stage because the $H_2O_2$ is destroyed by the tissue catalase. In the absence of catalase the $H_2O_2$ reacts with the glyoxylic acid to form formic acid and $CO_2$.

The rate of oxidation of glycine catalyzed by epinephrine or adrenochrome in the presence of tissue is considerably less than half that found in the absence of tissue. If the tissue contributed only catalase, one would

expect the oxygen consumption without tissue to be double that with tissue for an experiment of the same duration. Two possible explanations for this reduced catalytic activity with presence of tissue are: (*a*) The tissue contains amine oxidase which causes destruction of epinephrine by oxidative deamination, thus preventing the formation of adrenochrome. (*b*) It is possible that adrenochrome is reduced or that epinephrine is prevented from oxidation to some extent by substances in the tissue such as glutathione.

There is some evidence that in schizophrenia, epinephrine or its precursors is oxidized to adrenochrome and other oxidation products (17, 19). If this be true and if the reactions described above which occur *in vitro* occur also *in vivo*, there might be a deficiency of glycine and thus a decreased ability to detoxify benzoic acid by the formation of hippuric acid as reported in schizophrenia (1–9). These reactions could also account for the increased formation of phosphoglycolic acid reported in the erythrocytes of blood from schizophrenic patients (10, 11) since glyoxylic acid is readily converted to glycolic acid (32).

These findings might also account for the decrease in blood glutathione reported in schizophrenia. Since glutathione can protect epinephrine against oxidation, a lack of this material could result in increased oxidation of epinephrine thus leading to a vicious cycle in which a glycine lack led to a deficit of glutathione. This in turn could produce conditions in which epinephrine would be oxidized and the oxidation products of epinephrine could stimulate oxidative deamination of amino acids.

A defective glycine metabolism has been reported in Cushing's syndrome (33), in rheumatoid arthritis (34), as well as in scorbutic guinea pigs (35).

If the marked stimulating effect of adrenochrome on glycine oxidation, described above, occurred also *in vivo*, grave pathological effects could ensue. A deficiency of this amino acid could cause a decreased synthesis of proteins and thereby of enzymes or perhaps the formation of abnormal proteins and enzymes deficient in the amino acid in question.

## Summary

Adrenochrome or epinephrine, without tissue, catalyzed the oxidation of glycine to formic acid, $CO_2$, and $NH_3$. Glyoxylic acid and $H_2O_2$ appeared to be intermediates. In the presence of rat tissue homogenates or purified catalase the reaction stopped at the glyoxylic acid stage as the $H_2O_2$ was destroyed. The possible implication of this reaction in schizophrenia is discussed.

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