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THE REPLACEMENT OF CYSTINE BY SOME PHENOLS IN METABOLISM

Thesis for Degree of M. S.

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THESIS

THE REPLACEMENT OF CYSTINE BY SOME PHENOLS IN METABOLISM

Thesis

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Shwen-yuing Chen

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THE REPLACEMENT OF CYSTINE BY SOME PHENOLS

IN METABOLISM

Introduction

The combustion of the assimilable food material as glucose furnishes the energy necessary for the maintenance of the living organisms. But the proteins, carbohydrates, fats and their derivatives in the food are only oxidisable through the activation of oxygen (or hydrogen.) Generally the processes of physiological oxidations are held to be accomplished through two agencies. One is enzymic, which brings about direct oxidations. The other is brought about by H-accepters, as glutathion which affects oxidation through a reaction of dehydrogenation. The SH group in the cysteine compound of glutathion ($2CH_2SHCHNH_2COOH + 2COOHCH_2CH_2CHNH_2COOH - H_2O$) has the property of taking up and giving off hydrogen as represented by the following equation:

$$RS\text{-}SR + 2H \rightleftharpoons 2RSH$$

oxidised reduced

The reduced form of glutathion in being oxidised may produce the cystine complex and hydrogen peroxide as:

$$2RSH + O_2 \longrightarrow RS\text{-}SR + H_2O_2$$

This peroxide will thus make further oxidation possible, or the oxidised form reacting with water may liberate oxygen as:

$$RS\text{-}SR + H_2O \longrightarrow 2RSH + O$$

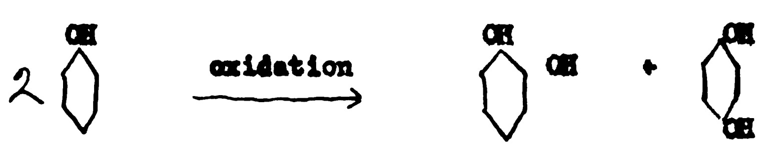
Abderhalden and Wertheimer (Arch. ges. Physiol. (Pflugers) 198, 415-20 1923) showed that the system cystine $\rightleftharpoons$ cysteine is a mechanism

which enables the cells to bring about the transformation of aldehyde into acid and alcohols. The addition of cystine favors the production of aldehyde while the addition of cysteine favors the formation of alcohol. In experimenting with pigeons they also found that the birds fed on a diet of polished rice exclusively showed a failure to utilise the oxygen of the blood normally. This was accompanied by a lowered body temperature and a depression of the gaseous metabolism. Since the tissue extracts of the rice-fed pigeons could not effect the reduction of cystine, it was interpreted that the direction of the well balanced reaction RS-SR $\rightleftharpoons$ RSH + SHR only goes from right to left resulting in the lack of mercaptor groups necessary for the oxidation. In cyanide poisoning the reaction goes from RS-SR $\longrightarrow$ RSH + HSR and utilization of the mercaptor groups for oxidation is impossible.

Goldschmidt (Arch. Ophthalmol., 1917, XCIII, 447) showed that there is diminution of cystine in adult life and that cataract is due to the lack of cysteine or inability of cystine to form cysteine.

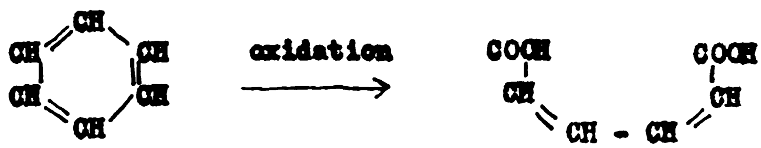
With regard to the effect of phenols in metabolism, Huston and Lightbody (Unpub. work) have found evidence that the dihydroxy-phenols may serve some physiological functions as shown by the addition of these phenols to some racket producing diets.

Tyrosine, one of the important amino acids in nutrition, in decomposition by colon bacteria yields phenol, which can be oxidized to form pyrocatechol or hydroquinone as:



The phenols are then excreted in the urine in combination with sulphuric acid or to certain extent with glucuronic acid.

Upon further oxidation in the course of metabolism the benzene ring is broken. The researches of Jaffe showed that on administration of benzene to dogs small amounts of muconic acid may be isolated from the urine. He represents this reaction as follows:

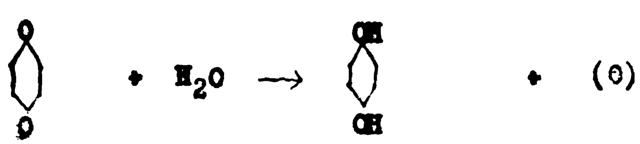


Muconic acid

Bijlsma and Versteigh (Arch. ges. Physiol. (Pflugers) 197, 415-23, 1922) have shown that hydroquinone administered to mice and guinea pigs in over dose causes marked motor disturbances of the heart, cramps, episthotonos and other effects.

The possible mechanism of the action of these dihydroxyphenols in metabolism may be suggested as following:

1) According to Weilands dehydrogenation idea (Ber. 1912, 45, 484.) hydroquinone is oxidised to quinone by oxygen free palladium. The oxidation consists of the removal of labile hydrogen which is taken up by palladium. Glucose is easily oxidised to carbon dioxide and water at low temperature by quinone as an acceptor for hydrogen in the absence of elementary oxygen. Bacot (Master thesis, Michigan State College, 1926) showed that quinone in water forms hydroquinone as a reversible reaction. The quinone attacks the water molecule with liberation of activated oxygen as:



2) Enzymic oxidation theory.

It has been known that in animals and plants there are oxidases or oxidising enzymes effecting oxidations, which ordinarily can not be

brought about by molecular oxygen alone such as the blueing of guaiacum, the conversion of hydroquinone to quinone, etc.

Chodat and Bach (Handbuch der Biochemischen arbeits methoden E. Abderhalden, Berlin, 1910, Vol. 3 pp. 42-47) consider an oxidase as composed of an oxygenase and peroxidase. The oxygenase being enzyme like unites with the oxygen of the air to form peroxides. The peroxidase acts upon the peroxide and renders one atom of its oxygen active for subsequent oxidation of an acceptor. The reaction is approximately as follows:

$$2AO + O_2 \longrightarrow 2AO_2 \text{ (Peroxide)}$$

$$AO_2 + \text{Peroxidase} = AO + O$$

According to Onslow (Biochem J. 14, 535) the oxidase is a three component system. One member is an organic substance and probably is an aromatic compound with two hydroxy groups in the o-position or a catechol substance. Upon oxidation the catechol will become orthoquinone. The other two are enzymes, oxygenase which catalyzes the production of peroxides (or the oxidation of the catechol) and a peroxidase which decomposes the peroxides with the formation of active oxygen. Thus the orthoquinone peroxide is acted upon by a peroxidase to yield active oxygen with the formation of catechol.

In experimenting with the tissue and tissue extracts of plants she found that those containing some substance with catechol grouping give a blue color with guaiacum tincture. She also found that catechol, adrenalin, and compounds with o-dihydroxy groupings tend to oxidise slowly when left in the air with the formation of peroxides, the oxygen of which can be activated by peroxidases.

The oxidation of guaiacum, paraphenylen-diamine, and phenolphthalin by hydrogen peroxide under the influence of the peroxidase of milk is great-

ly accelerated by phenol and cresol (Kastle and Porch Hygenic Laboratory Bull. No. 59). These peroxidase accelerators probably act in the capacity of auxiliary oxygen carriers and are themselves more or less completely oxidised in such processes.

Let P = Peroxidases,

A = Auxiliary oxygen carrier,

B = The peroxidase reagent,

Then

$$P + 2H_2O_2 \longrightarrow PO_2$$

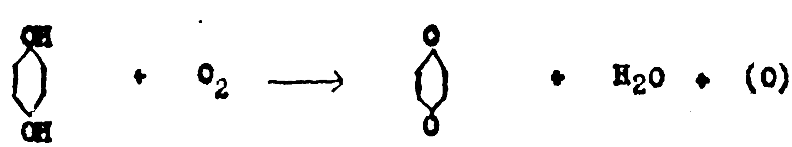
$$PO_2 + A \longrightarrow P + AO_2 \qquad \text{or}$$

$$2PO_2 + A \longrightarrow 2PO + AO_2$$

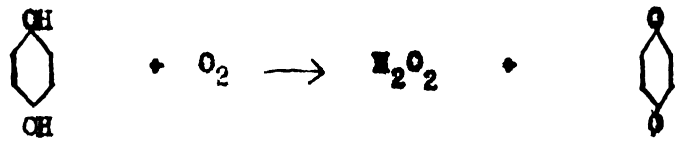
$$AO_2 + B \longrightarrow AO + BO$$

3) The peroxide theory of oxidation accounts for the phenomena of autoxidation and oxygen carrying upon the supposition that spontaneously oxidizable substances have the power of combining with partially dissociated molecules of oxygen to form peroxides. These peroxides may then react either with additional amounts of the autoxidizable substance itself, or with some other substance to form simpler oxides.

4) Rolf Mier (Arch. Exptl. Path. Pharm. 100, 137-48, 1923) found that in the presence of blood pigments acting as catalytic agents hydroquinone, amino-phenol and hydrazobenzene are quickly oxidised by atmospheric oxygen. As a reducing agent, with the catalytic effect of blood pigments, hydroquinone or pyrocatechol therefore may render the ordinary oxygen active in breaking up the molecular form to atomic oxygen as:



Quinone in water solution alone is sufficient to blue guaiacum. Bacot suggested this to be due to the formation of hydrogen peroxide from the reaction of hydroquinone and molecular oxygen as:



since quinone also forms an equilibrium mixture with hydroquinone.

5) On the other hand the work of Moreau and Dufraisse (J. of the Chemical Society Vol. 127 Jan., 1925) proved that hydroquinone or pyrocatechol is antioxygenic. They prevent autoxidation when added in very small quantities to autoxidizable substances. It is explained as follows: the antioxygen B acts by decomposing catalytically the peroxide AO_2 which results from the union of the autoxidizable substance with one molecule of free oxygen, with the formation of the peroxides AO and BO. These two peroxides being antagonistic to each other decompose to form A, B, and oxygen in their original state.

The catalysis may be positive, if the peroxide BO attacks the substance A in preference to the peroxide AO, or negative if the converse condition prevails. The equations are:

$$A + O_2 \longrightarrow AO_2$$

$$AO_2 + B \longrightarrow AO + BO$$

positive catalysis;

$$BO + A \longrightarrow B + AO$$

negative catalysis;

$$BO + AO \longrightarrow A + B + O_2$$

Experimentally hydroquinone plays the part of an antioxygen in the oxidation of benzaldehyde by interfering with the fixation of free oxygen. However recent work by Bacot in this laboratory showed that in high concentration of hydroquinone, the Moreau and Dufraisse effect does take place with glucose. In low concentration of hydroquinone such an effect is not noticeable.

Hydroquinone and pyrocatechol are antipyretic. This may be partly due to the depressed oxidation in the body, although they are well-known as vasodilators.

Nearly all the above results are based upon the work done in vitro as most of the striking biochemical oxidations of the living cell can be imitated more or less satisfactorily by experiments in test tubes. So far there is no evidence that suggests the oxidative processes of the living organisms differ in any fundamental way from chemical oxidations known to take place in inanimate nature. For example, A ketonic acid may undergo three types of change in the body according to the different conditions:

1) It may be oxidised to a lower fatty acid,

2) It may be reduced to a hydroxy acid,

3) Its ammonium salts may be reduced to an amino acid.

All are readily imitated in vitro;

1) by hydrogen peroxide,

2) and 3) by reducing agents as sodium amalgam.

In view of the above work, namely,

1) the relation of cystine to animal oxidation,

2) the evidence of a physiological function of dihydroxy phenols,

3) the relation of these phenols to plant oxidations, and to some types of oxidation in vivo, this work was undertaken. It was thought that the phenols might in part replace cystine in the oxidative functions.

Experimental

In order to determine if hydroquinone and pyrocatechol can be substituted for cystine which is essential for growth, four different diets were prepared:

Number one has the composition of:

Casein	9%
Salt mixture	4.5% (Osborne and Mendel, J. of Biochem., 37,557,1919)
Dextrin	70.65%
Cod liver oil	2%
Hydrogenated cotton seed oil	12.85%
Butter	1%

Diets number two, three, and four have the same composition as number one, except that in number two one per cent of the dextrin is replaced by laboratory prepared cystine, which is ten and forty-six hundredths per cent pure; in number three, one tenth per cent of the dextrin is replaced by hydroquinone; in number four, one tenth per cent of the dextrin is replaced by pyrocatechol.

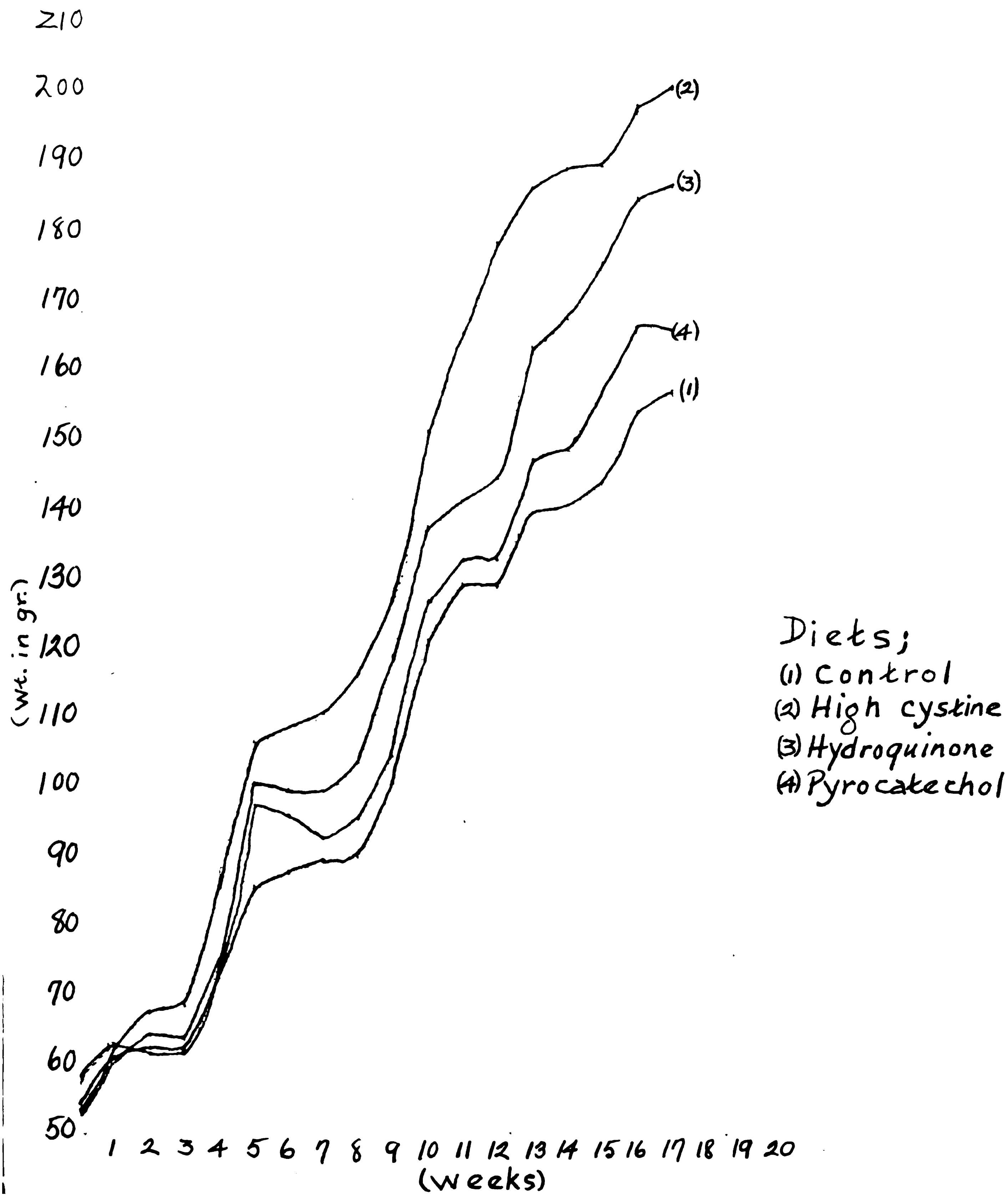
In addition to the above diets three grams of Fleischmanns dry yeast was given to each rat weekly, as a source of vitamine B. The Folin-Looney method for determination of cystine (J. of Biochem. Vol. 51, 1922, pp. 427) showed the dry yeast to contain one and seven hundred sixty eight thousandths per cent of a reducing substance. Considering this as cystine and the average food intake as fifty grams per week the cystine from the yeast amounted to one hundred and six thousandths per cent of the diet.

Six white rats at the age of four weeks were placed upon each diet. They were kept in individual cages and were weighed weekly.

The results are as follows:

Average Weight

Weeks	Diet 1 (low cystine content or control)	Diet 2 (high cystine content)	Diet 3 (hydroquinone)	Diet 4 (pyrocatechol)
1	55.25	53.61	53.43	58.5
2	60.97	62.23	59.5	62.5
3	62.38	67.77	64.15	61.78
4	62.07	68.27	62.95	61.82
5	72.083	85.41	74.12	73.77
6	85.33	106.12	100.31	96.93
7	87.75	108.37	99.25	95.43
8	89.36	110.45	99.07	92.4
9	89.4	115.95	103.15	94.95
10	100.13	128.3	118.6	104.15
11	121.4	150.95	136.93	125.95
12	128.6	166.3	140.68	132.7
13	128.9	178.25	144.02	132.5
14	139.33	185.86	163.6	146.5
15	140.27	188.1	167.75	148.15
16	143.77	189.3	175.03	156.71
17	153.77	197.97	184.65	166.4
18	156.28	200.5	185.98	165.31



210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
(wt. in gr.)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
(weeks)
(1)
(2)
(3)
(4)
Diets;
(1) Control
(2) High cystine
(3) Hydroquinone
(4) Pyrocatechol

Conclusion

Part of the cystine required for normal growth is replaceable by some phenols. Hydroquinone serves better than pyrocatechol.

If the growth of the control rats is considered as unity, then the growth of the rats on the other three diets may be expressed as, one and three tenths for high cystine diet, one and two tenths for hydroquinone diet, and one and six hundredths for pyrocatechol diet.

The mechanism of the action is probably that of the regulation of the oxidative processes.

With dihydroxy phenols the position of the hydroxy groups determine their value in metabolism. The para position is preferable to the otho.

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