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I hereby recommend that the thesis prepared under my supervision by Edgar S. Hill

entitled Spontaneous Oxidation of Dialuric Acid; the action of iron and cyanides on it, and its role in oxidizing amino acids.

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

Approved by:

A. P. Mathews.

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THE ACTION OF IRON AND CYANIDES ON THE SPONTANEOUS OXIDATION OF DIALURIC ACID

A dissertation submitted to the
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by

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INTRODUCTION

The catalytic action of iron in oxidations has been known for many years, and since iron is universally present in cells, physiologists and biochemists from very early times have almost universally ascribed to it an important role in oxidation. Professor J. U. Lloyd having suggested the study of iron in cell respiration, this investigation was begun under the direction and plan of Professor A. P. Mathews. Mathews and Walker (1) showed how greatly iron catalyzed the spontaneous oxidation of cysteine and more recently Warburg and Keilin have ascribed to it and its compound, cytochrome, or myohematin, the role of the respiratory enzyme. Warburg and Sakuma(2) believed that the whole of the oxidation of cysteine in air was due to iron and that cysteine entirely free from iron was incapable of autoxidation. Harrison(3), however, observing with even very pure cysteine some autoxidation, left the question open. Recently in this laboratory, Gerwe(4), in another investigation in this same series, has demonstrated that cysteine is spontaneously oxidizable, although at a very slow rate, and iron greatly catalyzes the oxidation. Gerwe also found that cyanide inhibited the catalytic effect of iron, but not the spontaneous oxidation of cysteine itself.

This work was begun with the view of studying the respiratory function of the cell nucleus. The nucleus has always been regarded as important in cell respiration since cells without nuclei respire at a very slow rate, even though they

blood corpuscles of mammals). Among the pyrimidine compounds of the nucleus, dialuric acid oxidizes at a very rapid rate, passing over into alloxan and alloxantin. This oxidation is being studied in this laboratory, and the relation of the oxidation to the reaction of the medium has already been published.

The present paper shows that (1) dialuric acid free from iron oxidizes spontaneously at a very rapid rate, (2) that this oxidation is at a maximum at pH 7.0-7.4 and falls rapidly on each side of the optimum to a minimum at pH 6.8 and 7.6, (3) that iron catalyzes the reaction on either side of the optimum, but not noticeably at the optimum, possible because oxygen could not enter the solution fast enough; and (4) that cyanides have no effect on the spontaneous oxidation, but inhibit only the catalytic action of iron.

In a previous paper, the author(5) has described the spontaneous oxidation of dialuric acid, a pyrimidine and an oxidation product of uric acid. This oxidation was found to be extremely sensitive to the reaction of the medium, going only with any considerable speed within the limits of neutrality. In this susceptibility and the position of the optimum, dialuric acid oxidation resembles that of cysteine and also closely parallels the condition in living matter where the oxidations are also extremely sensitive to such variations and in which there is the same optimum of oxidation at this alkalinity. It is the purpose of the present

investigation to discover: (1) if this reaction be accelerated by iron; (2) if dialuric acid be truly autoxidizable in the absence of iron(small amounts were present in the previous work); and (3) if the oxidation be interfered with by cyanides.

The effect of iron on the oxidation of another member of the pyrimidine family, thymine, has already been reported. Johnson and Baudisch(6) observed that thymine is readily oxidized by $FeSO_4$, $NaHCO_3$ and air, yeilding the products urea, acetol and pyruvic acid. Baudisch and Bass(7) studied the action of H_2O_2, and of H_2O_2 and $FeSO_4$ on thymine. They found the same products were formed as in the oxidation with $FeSO_4$ and air. Bass(8) demonstrated that under the influence of ultra-violet light oxygen was capable of attacking the thymine molecule in the presence of $FeSO_4$ so that subsequent hydrolysis gave urea and pyruvic acid.

I. The Action of Iron.

METHOD.

Dialuric acid was prepared according to the directions in the preceding paper(5) with various modifications in the final step of the process to insure freedom from iron. Crystallizations were carried out in fused quartz vessels until the product was free from iron. A two gm. sample of the final product, ignited in a quartz crucible and tested for iron, did not give the slightest trace of color when tested by the thiocyanate method described by Yoe(9). This method will detect 0.000,000,1 gm. of iron. The water, hydrochloric acid, and ammonia used throughout the experiments were distilled in quartz and samples of these solutions, in 500 cc. quantities, were evaporated in quartz dishes, and tested for iron. In no case was a trace of color obtained in the KCNS test.

The Barcroft respirometer was used, as before, for the measurement of the amount of oxygen absorbed by the oxidation of the dialuric acid. The experiments were made in the same manner. A 10 mg. sample of the dialuric acid was dissolved in iron-free distilled water, neutralized to the desired pH with iron-free ammonia and buffered with a buffer solution of the same pH. The buffer solutions used were combinations of varying amounts of 0.2 M KH_2PO_4 and 0.2 M NaOH, according to Clark(10), and gave values ranging from pH 6.0 to pH 8.0. The buffer solutions were essentially free from iron. 500 cc. quantities

were analyzed and found to contain 0.0005 mg. of iron. 50 cc. quantities gave no test, showing that they contained less than 1/10,000,000 gm. of iron, which is the sensitivity limit of the test. The 5 cc. of buffer solution used in each experiment could not have contained, therefore, more than 5/1,000,000,000 gm. of iron.

A series of experiments were carried out in which the oxidation of 10 mg. samples of iron-free dialuric acid was measured and a similar series in which 0.0001 mg., 0.001 mg., and 0.005 mg. of iron, respectively, were added to equal amounts of dialuric acid and the oxidation measured, These oxidations were measured at hydrogen ion concentrations varying from pH 6.0 to 8.0. The iron was added as $FeCl_3$, the various concentrations being prepared from a solution containing 0.1 gm. of standard iron wire dissolved in concentrated hydrochloric acid and diluted to a liter.

The respirometer was shaken throughout an experiment by a shaking machine, moving at a speed of 170±180 oscillations per minute, and the temperature in the oxidizing chambers maintained constant in a water bath at 25.0±.1 C.

The results obtained are tabulated in Tables I and II.

(Insert Tables I and II and Figure I here.)

Table I.

Effect of small amounts of iron.

pH-----	6.0	6.0	6.0	6.0	6.4	6.4	6.4	6.4
	Absorption, mm. difference of manometer level.							
Time shaken, min.	Dialuric acid alone	+ .0001 mg.	+ .001 mg.	+ .005 mg.	D. A. alone	+ .0001 mg.	+ .001 mg.	+ .005 mg.
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	51.	61.	76.	84.	24.	34.	46.	83.
10	64.	73.	88.	96.	33.	42.	60.	96.
15	69.	77.	95.	100.	35.	45.	66.	99.
20	69.	77.	98.	101.	36.	48.	70.	100.
25	70.	78.	98.	103.	36.	49.	71.	100.
30	70.	79.	100.	103.	35.	48.	71.	100.
35	70;	79.	101.	103.	36.	48.	72.	101.
40	70.	79.	101.	103.	35.	48.	72.	101.
45	70.	79.	101.	103.	35.	48.	72.	101.
50	70.	79.	101.	103.	35.	48.	72.	101.
55	70.	79.	101.	103.	35.	48.	72.	101.
60	70.	79.	101.	103.	35.	48.	72.	101.
Temperature, C--------	25.1	25.1	25.1	25.1	25.0	25.0	25.1	25.0
Total volume oxygen absorbed,--- cc.	.230	.259	.331	.338	.115	.157	.236	.331
% of total oxidation when absorption ceased-------	64.8	73.1	93.5	95.3	32.4	44.4	66.6	93.5

Table I.

Effect of small amounts of iron.

pH------	6.8	6.8	6.8	6.8	7.0	7.0	7.0	7.0
	Absorption, mm. of manometer level							
Time shaken, min.	Dialuric acid alone	+ .0001 mg. Fe	+ .001 mg. Fe	+ .005 mg. Fe	D. A. alone	+ .0001 mg. Fe	+ .001 mg. Fe	+ .005 mg. Fe
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	16.	28.	54.	85.	64.	74.	86.	85.
10	22.	34.	63.	95.	77.	87.	95.	94.
15	25.	37.	65.	100.	83.	91.	99.	99.
20	26.	39.	67.	101.	85.	95.	101.	102.
25	28.	39.	68.	102.	86.	96.	102.	103.
30	28.	40.	68.	104.	88.	96.	103.	103.
35	28.	39.	69.	105.	88.	98.	105.	105.
40	28.	40.	68.	105.	88.	98.	104.	105.
45	28.	40.	68.	105.	88.	98.	105.	105.
50	28.	40.	68.	105.	88.	98.	105.	105.
55	28.	40.	68.	105.	88.	98.	105.	105.
60	28.	40.	68.	105.	88.	98.	105.	105.
Temperature, C------	25.1	25.1	25.1	25.0	24.9	25.1	24.9	25.1
Total volume oxygen absorbed, cc.	.092	.131	.223	.344	.289	.321	.344	.344
% of total oxidation when absorption ceased	25.9	37.0	62.9	97.2	81.5	90.7	97.2	97.2

Table II.

Effect of small amounts of iron.

pH-----	7.4	7.4	7.4	7.4	7.4	7.6	7.6	7.6	7.6	8.0	8.0	8.0	8.0	8.0
						Absorption, mm. of manometer level								
Time shaken, min.	Dial-uric acid alone	+ .0001 mg. Fe	+ .001 mg. Fe	+ .005 mg. Fe	Dial-uric acid alone	+ .0001 mg. Fe	+ .001 mg. Fe	+ .005 mg. Fe	Dial-uric acid alone	+ .0001 mg. Fe	+ .001 mg. Fe	+ .005 mg. Fe		
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
5	68.	76.	86.	86.	16.	27.	50.	85.	40.	54.	71.	83.		
10	81.	87.	96.	97.	21.	34.	58.	95.	48.	62.	81.	94.		
15	86.	94.	100.	101.	22.	37.	62.	99.	52.	65.	87.	97.		
20	87.	97.	102.	103.	23.	38.	63.	104.	53.	66.	89.	101.		
25	89.	97.	103.	104.	24.	39.	65.	104.	55.	67.	89.	101.		
30	90.	99.	104.	104.	24.	40.	64.	105.	54.	67.	90.	102.		
35	90.	100.	106.	106.	24.	40.	65.	105.	55.	68.	90.	104.		
40	90.	100.	105.	106.	24.	40.	65.	105.	55.	68.	91.	104.		
45	90.	100.	105.	106.	24.	40.	65.	105.	55.	68.	91.	104.		
50	90.	100.	105.	106.	24.	40.	65.	105.	55.	68.	91.	104.		
55	90.	100.	105.	106.	24.	40.	65.	105.	55.	68.	91.	104.		
60	90.	100.	105.	106.	24.	40.	65.	105.	55.	68.	91.	104.		
Temper-ature, C----	25.1	25.0	25.0	25.0	25.1	24.9	24.9	25.0	25.1	25.0	25.0	25.0		
Total volume oxygen absorbed, cc.	.295	.328	.344	.348	.079	.131	.213	.344	.180	.223	.298	.341		
% of total oxidation when absorption ceased	83.2	92.6	97.2	98.1	22.2	37.0	60.1	97.2	50.9	62.9	84.2	96.2		

Discussion.

Fig. I shows comparatively the amounts of oxidation of samples of iron-free dialuric acid and of samples to which varying amounts of iron have been added. The concentration of hydrogen ions is plotted on the abscissa, the variation being confined to the limits of pH 6.0 to pH 8.0. The ordinates represent the mm. of negative pressure due to the absorption of oxygen after 60 minutes of shaking.

Tables I and II and Fig. I will show how great is the acceleration by small amounts of iron. It was found that the rates of oxidation of samples of iron-free dialuric acid were somewhat less than those recorded during the preceding work(5), attesting probably the more complete removal of iron, which was present in some of the buffers then used and was responsible for a part of the phenomenal oxidation in certain cases. The addition of 0.0001 mg. of iron produced a decided increase in oxidation rate at pH values other than 7.0-7.4; 0.001 mg. of iron, a much greater increase; while the addition of 0.005 mg. of iron was sufficient to produce almost complete oxidation, even at unfavorable pH values. In fact, the oxidations as accelerated by the addition of 0.005 mg. of iron may be considered to be complete, a slight divergence from the theoretical possible absorption being due possibly to some limiting factor in manipulation. The apparatus was not shaken at a maximum speed, and it is possible that oxygen could not enter the solution fast enough. At least, it can be seen in the oxidations at

pH 7.0 and 7.4 that maximum absorption is obtained, for here, the acceleration due to 0.005 mg. Fe is only very small and coincides with that produced by 0.001 mg. Fe.

Iron, in this oxidation undoubtedly acts the part of an oxygen carrier, as it is supposed to do in the cell. The mechanism of this acceleration possibly involves the formation of an intermediate compound of ferric iron and dialuric acid. Ferric iron may unite with the dialuric acid to oxidize it to alloxan; the compound immediately breaking up, the iron becoming free again as ferrous iron. The oxygen of the air oxidizes the iron back to the ferric state and the process is repeated.

II. The Action of Cyanides.

Closely related to the subject of acceleration by iron is the one of inhibition by cyanides. It has been shown by many investigators that small concentrations of KCN have the power to depress cellular oxidations. Mathews and Walker(11) showed that very small amounts of KCN were sufficient to check or prevent the spontaneous oxidation of cysteine to cystine. More recently, Gerwe(4) has shown that HCN inhibits only the accelerating action of iron, and not the spontaneous oxidation of cysteine. He thus confirmed the theory especially advocated by Warburg that cyanides inhibit respiration by combining with the iron. Warburg has maintained the view that if the oxidative process of any system is paralyzed by cyanide, then iron may be assumed to be the chief catalytic agent involved.

Many cases, however, have been reported where cyanide had no effect at all on oxidation. Thunberg(12) states that succino-dehydrogenase in muscle was not inhibited by cyanide in its methylene blue reducing power in the presence of succinic acid, whereas strong inhibition in oxygen uptake was observed. Hopkins and his co-workers also state that the Schardinger enzyme from milk and xanthin-oxidase are not at all sensitive toward cyanide. They concluded, therefore, that those oxidation systems had nothing to do with iron. Szent-Gyorgyi(13) has recently shown that the hexoxidase from cabbage leaves, probably related to the highly oxidizable hexuronic acid in the suprarenal cortex of animals, is not sensitive to cyanide.

Gerwe(4) has found that cyanides inhibit the acceleration added of the cysteine oxidation by iron, but that cysteine, entirely free from iron, is not affected in its rate of oxidation and is truly autoxidizable. This disproves Warburg's early supposition that cysteine had no true autoxidation. Cyanides stop the oxidation of cytochrome from yeast, described by Keilin(14) as an intracellular respiratory catalyst, but here again we are dealing with a compound containing iron. Cytochrome has as a nucleus an iron-pyrrol compound, similar to that in hemoglobin. Elvejhem(15) finds that the respiration of yeast is 80 per cent inhibited by KCN, but this is not startling as it has been already shown that iron and especially cytochrome is an essential element in the growth and metabolism of yeast.

Consequently, it is of importance to discover if the oxidation of dialuric acid be affected by cyanides. In this way its autoxidizability can be confirmed, because if this oxidation is a catalysis by iron, it should be inhibited by cyanides.

METHOD and RESULTS.

A series of experiments were carried out in which varying concentrations of KCN were added to oxidizing mixtures containing 10 mg. samples of dialuric acid free from iron. The concentrations used were M/20,000, M/10,000, M/5000, M/2000, M/1000, M/500, M/200, and M/50. These were the final concentrations in the solutions and the oxidations were measured at pH 7.0 and 7.4. In no case was there any inhibition of oxygen uptake (see TableIII) and these results show definitely that cyanides do not inhibit the oxidation of pure dialuric acid. These results support those of Richardson and Cannan(16), who found that the addition of HCN had no notable effect on the oxidation.

In order to test the effect of cyanide on the oxidation of dialuric acid to which iron had been added, M/200 KCN was added to reaction mixtures containing, in addition to the 10 mg. of dialuric acid, 0.0001 mg., 0.001 mg., and 0.005 mg. of iron, respectively, and the oxygen uptake measured. These mixtures, then, contained KCN in the final concentration of M/2000 and were buffered at pH 7.0. The oxygen uptake in these instances was just the same as when pure dialuric acid was used alone without KCN. (See Table IV).

Altogether, then, pure iron-free dialuric acid oxidizes at the same rate in the presence of varying amounts of KCN as in its absence. Cyanide inhibits the oxidation in the presence of iron only to the extent that the reaction has been catalyzed by iron.

Table III.

Effect of Cyanide on Oxidation of Dialuric Acid.

pH	Temperature, °C	Oxygen uptake of 10 mg. dialuric acid in mm./60 min.	HCN added to make final concentration	Uptake after addition of cyanide
7.0	25.0	88.0	M/10,000	89.0
7.0	25.0	89.0	M/5000	88.5
7.0	25.1	88.0	M/20,000	88.5
7.0	25.0	88.5	M/2000	89.0
7.4	25.1	90.5	M/1000	90.0
7.4	24.8	90.0	M/500	90.5
7.4	25.0	90.0	M/200	89.5
7.4	25.0	89.5	M/50	90.0

Table IV.

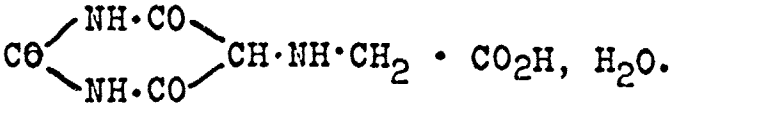
Effect of Cyanides on Iron Catalysis.

pH	Oxygen uptake of 10 mg. of dialuric acid	Fe added as $FeCl_3$	HCN added to make solution	Oxygen uptake
7.0	88.0	.0001 mg.	M/2000	89.0
7.0	88.5	.001 "	"	89.0
7.0	88.0	.005 "	"	88.5

III. The Oxidation of Amino-Acids by Dialuric Acid.

Strecker (17) was the first to examine the action of alloxan on amino-acids. He found that on warming a solution of alloxan with a solution of leucine, isovaleraldehyde and carbon dioxide were produced, and that alanine, when treated in the same way, gave acetaldehyde and carbon dioxide, while glycine gave no aldehyde, but did give carbon dioxide; in all three cases the liquid assumed the color of murexide. No quantities were given in Strecker's paper, and he identified his products by qualitative tests.

Pilotv and Finkh (18) in their paper on the constitution of murexide, describe the interaction of alloxan and glycine when concentrated solutions are mixed at 80°. In these circumstances, the color of murexide is produced, carbon dioxide is evolved, and on rapid cooling, a crystalline product is obtained which has the color of murexide. This product they describe as glycine purpurate. When, instead of cooling the mixture, it was heated until the color of the murexide had disappeared, an insoluble, amorphour substance was deposited, and the mother liquor yielded a yellow, crystalline solid, which they describe as uramiloacetic acid:



Piloty and Finkh made no reference to the production of formaldehyde.

The apparently abnormal behavior of glycine towards alloxan led Hurtley and Wootton (19) to repeat the earlier experiments. In the first place, they confirmed the production

of isovaleraldehyde from leucine and of acetaldehyde from alanine; they also tried the action of alloxan on α-amino-butyric acid, and were able to show that propaldehyde was produced. When molecular proportions of alloxan and glycine were heated in concentrated solution, no formaldehyde was produced, but they obtained the amorphous substance, and the uramiloacetic acid of Piloty and Finkh. When molecular proportions of alloxan and glycine in dilute solutions were distilled, formaldehyde was easily recognized in the distillate. Under these conditions, Piloty and Finkh's amorphous substance was not obtained. The liquid, which became purple soon after mixing, lost its purple color and yellow crystals identical with Piloty and Finkh's uramiloacetic acid were deposited on cooling. It is the opinion of Hurtley and Wootton that in the case of the concentrated solutions used by Piloty and Finkh, some of the uramiloacetic acid, or the uramil derived from it, had condensed with dialuric acid and formaldehyde to form the amorphous substance, and thereby concealing the presence of formaldehyde. The dialuric acid was produced by the reduction of a part of the alloxan during the oxidation of the amino acid.

Hurtley and Wootton also found that dimethylalloxan oxidizes an α-amino-acid to the next lower aldehyde and yields tetramethylmurexide. In addition to the amino acids mentioned before, they tried the action of alloxan on tyrosine, tryptophane, cystine, and on glucosamine; all of these give a strong murexide color.

Traube (20) found that alloxan likewise oxidizes anilinoacetic acid to benzaldehyde and carbon dioxide, the

solution becoming red. Besides alloxan, isatin, p-benzoquinone and toluquinone were shown to oxidize the amino acid to aldehyde. Fatty aromatic amines, for example, benzylamine, were in like manner oxidized to aldehydes by alloxan and isatin. Purely fatty amines,for example, isoamylamine, were not oxidized by alloxan.

Recognizing the ease with which dialuric acid is oxidized to alloxan, and with which it is obtained from alloxan on reduction, it was the purpose of this portion of the study to discover if amino acids could be oxidized by the use of dialuric acid itself. The reaction promises to be one of considerable biochemical interest.

METHOD

The amino - acids used were glycine, alanine, valine, glutamic acid and phenylalanine. The amount of amino-acid employed in an experiment was usually 0.5 gm. except for one 0.25 gm. sample of phenyl alanine and the amount of dialuric acid varied from 0.002 gm. to 0.1 gm. according to the experiment. (see Table V)

The reactions were measured by passing a stream of air, free from carbon dioxide and ammonia, through a mixture of the amino-acid and dialuric acid, in water solution, at room temperature. The amino-acid was dissolved in water (usually about 5c.c.) with the aid of 0.01 N HCl and 0.01 N NaOH according to the solubility of the acid and the solution neutralized to the desired pH with these two solutions. An equal volume of buffer solution (0.5 M K H_2PO_4 + 0.5 M NaOH) of the same pH was added and the solution placed in the reaction chamber, a large test-tube of about 60c.c. capacity, The dialuric acid was added to this solution in solid form and the mixture placed in the reaction train. This was arranged as follows: A stream of air was passed through two bottles containing concentrated K O H solution to remove carbon dioxide, then through a bottle of concentrated H_2SO_4 to remove ammonia and moisture. From this the air passed through the mixture of amino-acid and dialuric acid. It then passed through two large tubes, the first containing 25 c.c. of 0.01 H_2SO_4, the second, 50 c.c.of 0.01 N H_2SO_4, to absorb the ammonia given off; then, through a wash bottle of concentrated H_2SO_4 to remove moisture and then through two carbon dioxide absorption towers filled with "Ascarite" (sodium hydrate asbestos). The carbon dioxide towers were weighed

before and after each experiment. The H_2SO_4 solutions in the two ammonia absorption tubes were titrated at the end of an experiment with 0.01 N NaOH, using alizarin as an indicator, as stated above, the reaction chamber was at the temperature of the room. The air was passed through the apparatus at a rate of about 40 bubbles per minute.

The data and results are shown in Table V.

TABLE V

Oxidation of amino-acids by dialuric acid.

Amino acid	Wt. amino acid gm.	Wt. dialuric acid gm.	pH	Temperature, °C	Time hrs.	Amt. CO_2 absorbed gm.	Amt NH_3 absobbed gm.	% Oxidation.
glycine	0.5	0.1	7.0	24.5	2.0	.0046	.00172	1.50
glycine	0.5	0.1	7.0	23.8	1.66	.0042	.00161	1.40
glycine	0.5	0.005	7.4	25.0	5.83	.0073	.00281	2.45
glycine	0.5	0.002	7.0	24.0	5.0	.0011	.00039	.34
alanine	0.5	0.1	7.0	24.9	2.0	.0041	.00153	1.60
alanine	0.5	0.1	7.4	25.4	5.66	.0057	.00219	2.30
alanine	0.5	0.005	7.6	25.3	5.0	.0024	.00091	.96
valine	0.5	0.01	7.4	23.7	5.0	.0046	.00324	4.51
valine	0.5	0.01	7.0	25.1	6.0	.0042	.00295	4.11
glutamic acid	0.5	0.01	7.0	24.7	5.25	.0045	.00174	3.02
glutamic acid	0.5	0.01	7.4	25.0	5.08	.0066	.00255	4.42
glutamic acid	0.5	0.01	7.6	25.5	6.25	.0036	.00139	2.41
phenyl alanine	0.25	0.01	7.4	24.9	6.5	.0035	.00132	5.30
phenyl alanine	0.5	0.01	7.6	25.2	6.0	.0052	.00196	3.93

Results.

Table V shows comparatively the amounts of carbon dioxide and ammonia given off by different amino-acids at different pH values, different concentrations of dialuric acid, etc.

During the experiments, the solutions began to turn pink shortly after the dialuric acid had been mixed with the amino-acid, and this color increased slightly as the reaction progressed, but never reached any great intensity, due to the minute quantities of dialuric acid added. The color was strongest in those cases where the largest amount of dialuric acid was used, i. e., 0.1 gm. This color was due to the formation of murexide, and persisted throughout an experiment. After the completion of an experiment, the solution was allowed to stand for several hours, the color faded from the liquid, and a pinkish sediment was found in the bottom of the tube. This was assumed to be the salt of the amino-acid and purpuric acid as described by Piloty and Finkh. The sediment was not examined.

In the experiments where glycine was used, formaldehyde was found to be present in the solution after oxidation. Its presence was shown by the $FeCl_3$-milk-H_2SO_4 test. All of the solutions showed tests for aldehydes after oxidation. In the case of phenyl alanine, the solution smelled strongly of hyacinths, this odor being characteristic of phenyl acetaldehyde.

The course of the reaction appears to be as follows: the dialuric acid is oxidized to alloxan; alloxan then acts on the amino-acid, setting free ammonia and carbon dioxide, and making the aldehyde of the next lower acid. The alloxan is reduced in the process to dialuric acid and takes up oxygen again and so repeats the oxidation. Some of the alloxan unites with part of the dialuric acid to form alloxantin, which, with the ammonia, forms murexide. Proof that the solution contains alloxantin has been obtained by testing with barium hydrate.

SUMMARY.

1. The spontaneous oxidation of dialuric acid, occurring at unfavorable pH values, is greatly accelerated by the addition of small amounts of iron; but little or no acceleration by iron was obtained at pH 7.0-7.4. This may have been due to the fact that oxygen could not enter the solution fast enough at pH 7.0-7.4 to permit any acceleration.

2. The autoxidation of iron-free dialuric acid is not inhibited by cyanides.

3. The addition of KCN to dialuric acid of which the oxidation was catalyzed by iron reduced the oxygen consumption to that of dialuric acid alone.

4. In its acceleration by iron and the inhibition of iron catalysis by cyanide, the spontaneous oxidation of dialuric acid shows the same interesting parallelisms to the oxidations of cells, as is shown by cysteine and its compounds, such as glutathione.

5. Alloxan, formed by the oxidation of dialuric acid, acts upon amino acids setting free from them ammonia and carbon dioxide, and making the aldehyde of the next lower acid. The alloxan is reduced to dialuric acid in the process and takes up oxygen again and so repeats the oxidation.

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