amyl nitrite or propyl nitrite was added. After the solution had stood in a freezing mixture for one hour, the sodium hyponitrite was collected on a Büchner funnel. It was washed several times with absolute ethanol, then with absolute ether, and finally dried in a vacuum over sulfuric acid; yield, 0.69 g., or 13.9%.

The salt is not hygroscopic and will remain perfectly dry when exposed to the air in an open vessel for several days.

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RECEIVED FEBRUARY 21, 1927
PUBLISHED APRIL 7, 1927

CATALYTIC OXIDATION EFFECTS THAT RESEMBLE THE SPECIFIC DYNAMIC EFFECT[1]

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RECEIVED JULY 30, 1926 PUBLISHED APRIL 7, 1927

By a rational extension of earlier experiments on the catalysis of the oxidation of butyric acid and of glucose, results were obtained that show analogies to physiological data on specific dynamic action, as developed by Lusk and others.[2] The results obtained indicate that this effect is a true chemical catalysis and that its foundations are now capable of being investigated *in vitro*. Results on catalysis with hydroxy acids and with amino acids are given in this paper.

1. Oxidative Catalysis with Hydroxy Acids

Previous experiments showed that ammonium hydroxide catalyzes the oxidation of butyric acid with hydrogen peroxide, while sodium and potassium hydroxides do not.[3] This peculiarly specific action of ammonia suggested that perhaps substituted ammonias would also display this catalytic property. Experiments with glycine, which is one of the most abundant substituted ammonias present in living organisms, showed that they do. Additional experiments were then made with glycine, ammonium glycolate and potassium or sodium glycolate. All three compounds proved to be strongly catalytic for the oxidation of butyric acid. In fact the alkali glycolate appeared to be a little more strongly catalytic than either of the other two compounds. These results were surprising at first, since they indicate that when glycine is deaminized and ammonia

[1] These experiments were completed about five years ago while the author was a member of the Otho S. A. Sprague Memorial Institute, Chicago, Illinois, and are now published in this incomplete form because the writer may not have opportunity to pursue the subject again for some time.

[2] Lusk, "The Elements of the Science of Nutrition," W. B. Saunders, Philadelphia, 1919.

[3] Witzemann, *J. Biol. Chem.*, **49**, 123 (1921).

and glycolic acid are formed, both of these compounds can catalyze oxidation separately or if united as ammonium glycolate.

On searching the literature for similar cases the results of Euler and Bolin[4] on a chemical study of *Medicago Laccase* were found. These workers found that this oxidizing enzyme preparation is a mixture of the calcium salts of mono-, di- and tribasic organic acids, among which citric, malic and mesoxalic acids and probably much larger amounts of glycolic acid were identified. When manganese acetate and the above individual salts and mixtures of them were allowed to act upon hydroquinone, the results obtained resembled those obtained with the natural laccase.

Only a part of the experimentation suggested by the above results has been carried out. Enough has been done, however, to show that the catalysis of butyric acid oxidation is not limited to potassium or sodium glycolate. Test experiments with sodium and calcium citrate, lactate and malate showed that all of these compounds catalyze the oxidation of butyric acid in the same way that alkali glycolate does, but that the effect was considerably weaker with the calcium salt than with the sodium salt. Similar experiments on the influence of calcium and sodium salts of some hydroxy acids on the oxidation of glucose with hydrogen peroxide gave various results. These results will not be detailed in this paper.

In many tests, some of which have been published elsewhere[5] it was found that glucose never catalyzed its own oxidation. On the contrary it was observed that the salts of certain hydroxy organic acids (unpublished data) catalyzed their own oxidation. In the case of malic acid it was found that sodium malate catalyzes its own oxidation only, while calcium malate does not catalyze its own oxidation but does catalyze the oxidation of glucose. The results in hand are clear enough to indicate that other interesting results will be obtained when the subject can be fully worked out.

2. Oxidative Catalysis with Amino Compounds

In the preceding section the results on the catalytic influence of ammonium hydroxide and of glycine upon the oxidation of butyric acid were reviewed. The experiments with glycine suggested that perhaps more complicated amino acid compounds would also catalyze oxidation with hydrogen peroxide. Test experiments with gelatin showed that it catalyzed the oxidation of butyric acid, at least as well or better than any other compound tried, and that it also catalyzes the oxidation of glucose.

This result taken together with the results described in the preceding section suggests that possibly all of the proteins and many of their various

[4] Euler and Bolin, *Z. physiol. Chem.*, **57**, 97 (1908); **61**, 1, 72 (1909).

[5] Witzemann, *J. Biol. Chem.*, **45**, 1 (1920).

cleavage products share to a greater or less extent in catalyzing vital oxidation before undergoing oxidation themselves.

Specific Dynamic Action.—One of the most obvious conclusions from the above results is that they give a basis for a chemical interpretation of the specific dynamic effect.[6] It is not necessary to review the subject of specific dynamic action any further[7] than to say that two distinct and apparently opposed views have grown up which are represented by Grafe[8] and by Lusk,[9] respectively.

On the basis of the results given in Sections 1 and 2 it seems clear that if Lusk and Grafe had known of the existence of the catalytic properties of ammonium hydroxide, of glycine, of ammonium glycolate and of alkali glycolate they would not have assigned the specific dynamic effect of glycine to but one of the four compounds mentioned above. Their conclusions were carefully reasoned but there was no basis at that time for suspecting that all of these compounds, derivable from glycine, are capable of catalyzing oxidation.

The contents of this paper in so far as they relate to the specific dynamic effect must be regarded as an incomplete chemical supplement to the results and interpretation of this effect as developed by studies on metabolism.[2]

Experimental Part

The experimental results given here serve to illustrate the kind of results that were obtained. The reaction mixtures were analyzed by methods previously described,[3] that will not be given here again. The butyric acid solution used contained 0.25 g. in 5 cc.

Action of Glycine upon Butyric Acid Oxidation.—The experiments were set up thus: (1) 5 cc. of butyric acid solution (0.25 g.), 5 cc. of potassium hydroxide soln. (≡ 0.25 g. of butyric acid), 10 cc. of water, 0.25 g. of glycine and 100 cc. of 3% hydrogen peroxide; (2) 5 cc. of butyric acid solution, 5 cc. of potassium hydroxide solution (≡ 0.25 g. of butyric acid), 5 cc. of ammonium hydroxide solution (≡ 0.25 g. of butyric acid), 5 cc. of water and 100 cc. of 3% hydrogen peroxide.

After 48 hours at room temperature, the solutions were analyzed by the methods previously used.[3] The final distillate was redistilled from an excess of phosphoric acid in order to remove ammonia and any other volatile bases from the solution before determining the butyric acid by the distillation method.

[6] Compare in this connection the data of Ort and Bollman [THIS JOURNAL, **49**, 805 (1927)], obtained by a different method.

[7] Atkinson and Lusk, *ibid.*, **49**, 453 (1921). Ref. 2.

[8] Grafe, *Z. physik. Chem.*, **79**, 421 (1912); *Deut. Arch. klin. Med.*, **118**, 1 (1916). Warburg, *Ergebnisse Physiol.*, **14**, 253 (1914).

[9] Lusk, *Arch. Internal Med.*, **12**, 485 (1913); *J. Biol. Chem.*, **20**, 617; **49**, 543 (1921). Atkinson and Lusk, *ibid.*, **36**, 415 (1918). Ref. 2, p. 244.

No.		Acetone G.	Acetone %	Butyric acid recovered G.	Butyric acid recovered %
1	1st trial	0.0174	10.6	0.196	78.4
	2nd trial	.0292	17.8	.157	62.8
2	1st trial	.0374	22.8	.131	52.4
	2nd trial	.0486	29.6	.087	34.8

Action of Potassium and Ammonium Glycolate upon Butyric Acid Oxidation.—The experiments were set up thus: (3) 5 cc. of butyric acid solution, 5 cc. of ammonium hydroxide solution (≡ 0.25 g. of butyric acid), 10 cc. of water and 100 cc. of 3% hydrogen peroxide; (4) 5 cc. of butyric acid solution, 0.25 g. of glycolic acid, 11 cc. of potassium hydroxide solution to neutralize these acids and 100 cc. of 3% hydrogen peroxide; (5) 0.25 g. of glycolic acid (previously neutralized with ammonium hydroxide), 5 cc. of butyric acid solution, 5 cc. of potassium hydroxide solution and 100 cc. of 3% hydrogen peroxide.

After standing for three days at room temperature, these solutions were analyzed as usual.

No.	Acetone G.	Acetone %	Butyric acid recovered G.	Butyric acid recovered %
3	0.0242	14.7	0.159	63.6
4	.0351	21.3	.157	62.4
5	.0289	17.6	.173	69.2

In two other series in which these experiments were repeated, the amount of oxidation that occurred was somewhat less but the acetone yield was larger and the butyric acid recovered smaller in 4 than in 3 and 5.

Experiments with Lactic Acid.—The experiments were set up thus: (6) the same as 3; (7) the same as 4, except that 0.30 g. of lactic acid as the sodium salt was used; (8) the same as 5, except that 0.30 g. of lactic acid as the ammonium salt was used. The results for acetone formed were: (6) 0.021 g., or 13.1%; (7) 0.0147 g., or 8.9%; (8) 0.021 g., or 13.1%.

The unchanged butyric acid was not determined in this series.

Experiments with Other Organic Acids.—Preliminary tests with other organic acids showed that sodium citrate is a good oxidizing catalyst for butyric acid. Calcium lactate is not so good. Calcium acetate has even less value. Both calcium and sodium malate catalyze the oxidation of butyric acid but the sodium salt is more effective than the other.

Action of Gelatin upon the Oxidation of Butyric Acid.—Two portions of 0.5 g. of gelatin were treated for half an hour with 50 $M/128$ acetic acid (J. Loeb's method of preparing iso-electric gelatin). This was then filtered with suction, washed thrice with distilled water and dissolved in 50 cc. of water. Another portion of 0.5 g. of untreated gelatin was dissolved in 50 cc. of water. The experiments were set up as follows: (1) (acid to litmus) one portion of iso-electric gelatin, 0.25 g. of butyric acid

in 5 cc., and 50 cc. of 3% hydrogen peroxide; (2) (alkaline to litmus) one portion of iso-electric gelatin, 0.25 g. of butyric acid in 5 cc., 10 cc. of potassium hydroxide solution (5 cc. ≡ 0.25 g. of butyric acid), and 50 cc. of 3% hydrogen peroxide; (3) (neutral to litmus) 0.5 g. of gelatin, 0.25 g. of butyric acid in 5 cc., 5 cc. of potassium hydroxide solution (≡ 0.25 g. of butyric acid), and 50 cc. of 3% hydrogen peroxide.

These solutions were allowed to stand for two weeks at room temperature. They were then distilled after adding 50 cc. of water. The residue was treated with 10 cc. of phosphoric acid and 50 cc. of water and distilled again. Some manganese dioxide was added to the distillates containing hydrogen peroxide which were then redistilled. The results obtained follow.

	Acetone		Butyric acid recovered	
No.	G.	%	G.	%
1	0.0464	28.3	0.049	19.6
2	.00024	0.2	.190	76.0
3	.0512	31.2	.053	21.2

A blank experiment with 0.50 g. of gelatin, 0.25 g. of butyric acid and 10 cc. of phosphoric acid showed that unchanged butyric acid may be recovered completely and determined in the usual way in these experiments.

The same experiments were set up again except that only 0.25 g. of gelatin was used in each case and the potassium hydroxide was omitted in No. 3. The results obtained follow.

	Acetone		Butyric acid recovered	
No.	G	%	G	%
4	0.039	23.8	0.131	52.4
5	.0048	2.9	.241	96.4
6	.0575	35.1	.138	55.2

The results show that so-called iso-electric gelatin and ordinary gelatin are both good catalysts for the oxidation of butyric acid when an excess of alkali is not added.

Similar experiments using ordinary gelatin and glucose showed that gelatin catalyzes the oxidation of glucose about as well as it does that of butyric acid. The details of these experiments will not be given here.

Summary

In earlier experiments it was found that ammonium hydroxide catalyzes the oxidation of butyric acid with hydrogen peroxide, while potassium and sodium hydroxides do not. It has now been found that glycine, ammonium and alkali glycolates, ammonium and alkali lactates and gelatin in neutral or acid solution (but not in alkaline solution) also catalyze this oxidation of butyric acid to a marked extent. These results are believed to furnish the basis for a chemical interpretation of the well

established specific dynamic effect, associated especially with glycine and alanine, investigated so carefully by Lusk.

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ORTHO-CRESOLBENZEIN AND SOME OF ITS DERIVATIVES

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RECEIVED AUGUST 12, 1926 PUBLISHED APRIL 7, 1927

In connection with the chemical and spectrographic study of the phthaleins and sulfonephthaleins in this Laboratory, it was thought desirable to investigate *o*-cresolbenzein.

o-Cresolbenzein was first made in Doebner's Laboratory by Schroeter,[2] who found that it melted at 220–225° and gave results on analysis which agreed with the formula for dimethyldihydroxytriphenyl carbinol, similar to the dihydroxytriphenyl carbinol formula given to benzaurin (phenolbenzein) by Doebner. Schroeter concludes that like benzaurin, *o*-cresolbenzein is formed in two stages. It seemed highly probable that owing to the color of *o*-cresolbenzein the carbinol formula for it is not correct. Baeyer has shown that all carbinols are colorless and that color appears only when the carbinols lose water and the quinoid condition is established. It will be shown in this paper that the formula for the stable, colored form of *o*-cresolbenzein differs from the carbinol formula by one molecule of water, that is, that it is in the quinoid condition.

Experimental Part

***o*-Cresolbenzein (Quinoid Form).**—*o*-Cresol and benzotrichloride (2.1 moles:1 mole) were heated at 150° under reduced pressure until the evolution of hydrogen chloride ceased. The dark red material was dissolved in 5% sodium hydroxide solution, the benzein precipitated with hydrochloric acid, and the excess of *o*-cresol removed by steam distillation. The benzein, dissolved in sodium hydroxide solution, was treated with sulfur dioxide until a clear, brownish solution formed in the presence of considerable resinous material. (Phenyl-*o*-hydroxytolyl ketone was later recovered from these residues.) Acidification of the solution with hydrochloric acid decomposed the bisulfite compound, liberating the benzein. Repetition of this process produced a small amount of benzein which was boiled with water to remove traces of acid or sodium salts. Crystallization from absolute ethanol produced minute, red-orange crystal fragments which melted with decomposition at 260–262°.

Anal.[3] Subs., 0.1763, 0.1624: CO_2, 0.5379, 0.4968; H_2O, 0.0972, 0.0923. Calcd. for $C_{21}H_{18}O_2$: C, 83.41; H, 6.00. Found: C, 83.21, 83.43; H, 6.17, 6.36.

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[1] From a dissertation presented to the Faculty of the Graduate School, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, by S. Alice McNulty, holder of the Grasselli Fellowship in Chemistry at Cornell University, **1923–1924.**

[2] Schroeter, *Ann.*, **257**, 68 (1890).

[3] The values used for the atomic weights are those given in the 1925 International Table of Atomic Weights.