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I hereby recommend that the thesis prepared under my supervision by Charles David Stevens

entitled THE SOURCE OF THE FORMIC ACID PRODUCED ON ACID HYDROLYSIS OF NUCLEIC ACIDS

be accepted as fulfilling this part of the requirements for the degree of DOCTOR OF PHILOSOPHY

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THE SOURCE OF THE FORMIC ACID PRODUCED ON ACID HYDROLYSIS OF NUCLEIC ACIDS

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requirements for the degree of

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Charles David Stevens

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THE SOURCE OF THE FORMIC ACID PRODUCED ON ACID HYDROLYSIS OF NUCLEIC ACIDS.

by

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I. Introduction

1. Discovery of Nucleic Acids

In 1868 Friedrich Miescher at the laboratory of Felix Hoppe-Seyler in Tübingen undertook a chemical examination of pus cells. Surgical bandages from a neighboring clinic were extracted with dilute sodium sulfate and the heavy pus cells were readily separated from adherent serum and salt solution by careful decantation. By treatment with dilute HCl and also by treatment with artificial gastric juice, the outer portions of the cells were dissolved leaving the nuclei as an insoluble grey powder. This powder when dissolved in dilute sodium carbonate solution gave, on addition of acetic acid, a flocculent precipitate containing phosphorus and giving protein color tests. This precipitated material Miescher called "nuclein" (50, 39, 22).

Hoppe-Seyler delayed publication of Miescher's work until he and his students had confirmed it. Then in 1871 there appeared in Hoppe-Seyler's Medicinisch-chemische Untersuchungen five papers on the nucleins of pus cells (50, 17), egg yolk (51), red blood cells of birds and reptiles (60) and casein (48). In a further note which Hoppe-Seyler did not accept for publication, Miescher expressed the thought that characteristic differences among these nucleins might be found, inasmuch as they varied markedly in phosphorus content. This note appeared only after Miescher's death, in his book (54).

Shortly after completing his work with pus cells Miescher left Hoppe-Seyler to take charge of a department at Basel. There he became interested in the Rhine salmon. He discovered on examining its sperm that the spermatozoa heads, which are cell nuclei, are made up almost exclusively of a single chemical substance, the salt of an organic base rich in nitrogen and an organic acid containing phosphorus (52). The base he called "protamine" and the acid, which Altmann called "nucleic acid", he still called "nuclein", although it was protein-free and much purer than his earlier preparations. His analyses indicate that it was as pure as most preparations made today.

2. Elementary Analyses of Nucleic Acids

At this point one may consider the elementary analyses of thymonucleic acid. In Table I are listed a number of these, together with several theoretical calculations. Considerable variation is to be observed among most of the figures, and particularly those for nitrogen and phosphorus which in general are low compared with theory for a tetranucleotide containing a desoxypentose.

(Insert Table I.)

3. Discovery of Purines in Nucleic Acids

On warming a specimen of his nuclein with nitric acid Miescher found (52a) that a yellow spot was formed which turned bright red when moistened with alkali. Realizing the significance of this he asked his colleague, Piccard, (1874) to examine salmon sperm for xanthine bodies (purines). Piccard soon extracted material by use of boiling hydrochloric acid which gave the well-known gelatinous purine precipitate with ammoniacal silver nitrate. He reported (59) the only xanthine bodies present in the nuclein from salmon sperm were guanine and sarcine (hypoxanthine), the latter probably being adenine which was at that time always mistaken for hypoxanthine.

It remained for Kossel (25) in 1885 to discover

Table I.

Elementary Analyses of Thymonucleic Acid.

	C	H	N	P
1874 Miescher (52)	36.11	5.15	13.09	9.59
1896 Miescher (53)	37.8	4.5	15.8	9.7
1897 Mathews (49)			15.34	9.59
1901 Levene (33)	36.50	4.69	16.70	8.73
1901 Levene (33)	36.67	5.10	17.18	8.65
1901 Levene (33)	36.40	5.24	17.30	9.03
1901 Levene (33)	34.76	5.16	16.77	9.15
1901 Levene (33)	36.65	4.57	17.89	8.93
1905 Levene (36)	37.8	4.8	16.5	
1906 Inouye (20)	37.5	4.4	16.0	9.7
1908 Levene and Mandel (44)			17.0	10.0
1911-12 Peters (58)			13.94	8.51
1911-12 Peters (58)			15.28	8.82
1912 Steudel (67)	31.93	4.97	13.32	7.60
1922 Levene (38)	36.72	4.58	14.59	9.05
1922 Levene (38)	36.21	4.30	14.54	8.94
1922 Levene (38)	36.51	4.17	14.07	8.74
1922 Levene (38)	36.34	4.40	14.37	9.10
1922 Levene (38)	35.69	4.05	15.25	9.05
Hexose tetranucleotide (38) $C_{43}H_{59}N_{15}P_4O_{33}$	36.30	4.19	14.79	8.73
Desoxypentose tetranucleotide (39) $C_{39}H_{51}N_{15}P_4O_{25}$	37.35	3.99	16.76	9.89

adenine in pancreas, which he then isolated from spleen and from yeast. He proceeded to isolate adenine from yeast nuclein and reported this the same year (26).

4. Discovery of Pyrimidines in Nucleic Acids

The pyrimidines were later established as components of nucleic acids. Thymine was discovered and named by Kossel and Neumann (29) in 1893, who also reported the discovery of cytosine the next year (30). Ascoli, a student in Kossel's laboratory, found uracil in yeast nucleic acid in 1900 (3).

5. Carbohydrates of Nucleic Acids

Richard Altmann (1) in 1889 introduced the term "nucleic acid" and also a convenient and general method for its preparation free from protein. Kossel had begun work on the nucleins in 1879 in Hoppe-Seyler's laboratory (24) and shortly after publication of Altmann's work, had the chance to make a detailed study of the nucleic acid Altmann had prepared from yeast. With the exceptions already noted, the work on protein-free nucleic acids began with that which Kossel communicated to the Physiological Society of Berlin in 1891.

In that paper of January 30, 1891 (27) Kossel reported that hydrolysis of yeast nucleic acid with dilute mineral acids had yielded phosphoric acid, adenine,

guanine, and a substance with carbohydrate properties, including the odor of caramel and quick reduction of alkaline copper solutions.

Two years later Kossel and Horbaczewski (28) reported further on yeast nucleic acid from which they obtained a pentose, its phenylhydrazone and phenylosazone. The work of many investigators has since identified this pentose as d-ribose, then unknown.

Kossel and Neumann (30) later(1894) realized that the yeast nucleic acid previously used might have been contaminated with yeast gum, but pointed to the fact that their work on thymus nucleic acid presented in this paper probably confirmed the work on yeast nucleic acid. In this paper they first reported the presence of a carbohydrate in thymus nucleic acid, a name which they gave to the acid two years later (31). They found that on treating thymus nucleic acid for two hours at 150° C. with 30% (by volume) sulfuric acid, acids were obtained which could be extracted with ether and remained as a brown, thick fluid on evaporation of the ether. This fluid was subjected to distillation and about one-fourth of it distilled over at 100 - 200° C. The temperature then rose rapidly to 245° C. and most of the material distilled at 245 - 255° C. A thick, red-brown residue remained in the flask and solidified on cooling.

The first fraction gave all the reactions for formic acid, which they did not then discuss further. The second fraction was identified as levulinic acid by its solubilities, iodoform reaction, sodium nitroprusside reaction, phenyl hydrazone and silver salt, the last two being examined as to crystal forms and elementary analysis.

Noll (56), working in Kossel's laboratory in 1898 also reported the presence of levulinic acid in the hydrolysate of nucleic acid from sturgeon sperm (12 grams of nucleic acid treated two hours at 150° C. with 100 ml. of 30% sulfuric acid).

Neumann (55) reported in a footnote that levulinic acid was isolated by him after treating thymus nucleic acid with sulfuric acid.

Araki (2), working in Kossel's laboratory in 1903 also reported the presence of levulinic acid in the hydrolysate of nucleic acid from intestinal mucosa (2.8 grams of nucleic acid treated three hours on a water bath with 30 ml. of 30% sulfuric acid).

In 1902-03 Levene (34) reported that he could find only a possible trace of levulinic acid on hydrolysis of nucleic acid he prepared from spleen.

Inouye (19) prepared nucleic acid from beef spleen and on hydrolysis with 20% sulfuric acid obtained levulinic acid. In the same paper he likewise reported

levulinic acid could be obtained from the nucleic acid prepared from bull testicles which according to Levene (35) should contain no levulinic acid-forming radicles. Inouye also duplicated these results with the nucleic acid from the sperm of Muraenoesox cinereus Forsk.

Since then many other isolations of levulinic acid from thymonucleic acid and its derivatives have been reported (41, 66, 68, 43, 45, 46), and the presence of formic acid in the distillate has been repeatedly confirmed.

The simultaneous formation of these acids from nucleic acid was interpreted by Kossel and others as indicating the probable presence in the nucleic acid of a hexose of an unstable nature. This conclusion was based on the fact that hexoses when heated with acid yielded levulinic and formic acids.

In 1906 and 1907 Steudel (63, 64, 65) reported the isolation of an isomer of saccharic acid obtained by oxidation of nucleic acid with nitric acid. He has apparently not repeated this, and Levene (39) failed to confirm this finding.

In 1908 Levene (37) observed that hexoses, as well as pentoses and thymonucleic acid give a green color with orcin in the presence of traces of cupric ion.

In 1912 Levene and Jacobs (40) reported a substance

isolated from thymonucleic acid which analyzed for a guanine hexoside and gave after hydrolysis an osazone which resembled glucosazone. No repetition of this has been reported.

All other workers failed in attempts to isolate a sugar from thymonucleic acid.

Feulgen in 1914 (9) and 1917 (10) emphasized the fact, realized by investigators in the field, that due to its great instability and quick melanine formation the sugar of thymonucleic acid must differ in its stability from the class of common sugars. He first suggested that the carbohydrate was not an ordinary hexose and showed that on mild hydrolysis thymonucleic acid after losing its purines contained one or more free aldehyde groups and would restore the color of a fuchsine solution decolorized with sulfurous acid. He suggested that the carbohydrate might be of the nature of glucal; but pointed out that it was probably not glucal itself.

In 1920 Fischer et al. (13) found that purified glucal does not possess the properties of simple aldehydes and gives no color to decolorized fuchsine. Hence the question was as to whether Feulgen's test was due to a secondary decomposition product or actually to the sugar present in thymonucleic acid. Steudel and Peiser (68) (1924) took the former view and thought that both

of Feulgen's tests were due to traces of furfural, and that the sugar present was glucose, the queer behavior of which could be explained by its firm union with phosphoric acid.

In 1929 Levene and London (42) discovered a desoxypentose as a component of guanine nucleoside from thymonucleic acid, and later Levene and collaborators (43, 45, 46) identified the sugar as d-ribodesose (2-desoxy-d-ribose). They showed that desoxyribose passed readily into levulinic acid, thus accounting for the levulinic acid appearing on acid hydrolysis of thymonucleic acid, but leaving unexplained the formic acid simultaneously formed and the appearance of which, together with levulinic, was supposed to be characteristic of a hexose.

Willibald Klein in 1934 (23) also isolated desoxyribose from thymonucleic acid, confirming the earlier isolation.

Professor Mathews suggested that I determine the amount of formic acid produced and its origin, as the origin of the formic acid found in the decomposition products of thymonucleic acid was not known. In the case of the nucleic acid of the tubercle bacillus, Elmer Brown and Treat B. Johnson (4) had concluded in 1923:

> "It has been shown that the sugar functioning in tuberculinic acid is a hexose. This was proven by identification of levulinic and formic acids as products of its hydrolysis."

The same sort of proof has been given for thymonucleic acid by Kossel and Neumann in 1894. No quantitative determinations of the amount of formic acid produced on decomposition of any of the nucleic acids have yet been published.

The following investigation showed that acid hydrolysis of thymonucleic acid prepared from calves' thymus yielded an amount of formic acid which was far less than would be obtained by acid hydrolysis of any hexose examined under similar conditions, and corresponded approximately to the amount derived from the adenine present in nucleic acid. The conclusion is therefore, that the formic acid does not originate from the carbohydrate but from adenine chiefly.

II. Experiments

1. Materials used in formic acid production

The materials were dried in vacuo over phosphorus pentoxide with a few exceptions as noted.

Sulfuric acid, H_2SO_4, Grasselli C.P. quality.

Sodium formate, HCOONa, Mallinckrodt C.P. quality.

Tartaric acid, $C_4H_6O_4$, Merck C.P. quality. Its melting point was found at 166-7° C.

Levulinic acid, m.p. 33°, $C_5H_6O_3$, Eastman. This material was received in a partially liquid state. On

standing in vacuo over magnesium perchlorate at room temperature for several days it completely liquified. The same was true when sulfuric acid was used instead of the perchlorate. Hence it was used in the liquid state.

d-Glucose, $C_6H_{12}O_6$, anhydrous, Mallinckrodt C.P. quality. Melting point found was 144-5° C.

d-Galactose, $C_6H_{12}O_6$, anhydrous. This was made in this laboratory by Dr. McNary from Coleman and Bell Company C.P. lactose. Melting point found was 164-5° C.

d-Levulose, $C_6H_{12}O_6$, anhydrous, Coleman and Bell C.P. quality. Melting point found was 95-6° C.

l-Arabinose, $C_5H_{10}O_5$, Coleman and Bell C.P. quality. Melting point found was 151-2° C.

l-Xylose, $C_5H_{10}O_5$, Kahlbaum. Melting point found was 146-7° C.

Adenine sulfate, $(C_5H_5N_5)_2.H_2SO_4.2H_2O$, Eastman. Dried in vacuo over sulfuric acid this was found to contain 34.6 and 33.5% nitrogen in two analyses. Theory is 34.63% nitrogen.

Guanine hydrochloride, $C_5H_5N_5O.HCl.H_2O$, Eastman. This was found to contain 33.6 and 33.7% nitrogen in two analyses. Theory is 34.07% nitrogen.

Uric acid, $C_5H_4N_4O_3$, Coleman and Bell C.P. quality. This was found to contain 32.8 and 33.7% nitrogen in two analyses. Theory is 33.32% nitrogen.

Uracil, $C_4H_4N_2O_2$. This was synthesized by Dr. Kuizenga in this laboratory by the method of Davidson and Baudisch (8). It was found to contain 25.5 and 25.1% nitrogen in two analyses. Theory is 25.00% nitrogen.

Thymine, $C_5H_6N_2O_2$. This was kindly given us by Dr. Heyroth of the Basic Science Laboratory where it was synthesized by the Johnson and Harkins (21) modification of the method of Wheeler and Merriam (71). There was too little for an analysis by us.

Cytosine, $C_4H_5N_3O.H_2O$. This was also kindly given us by Dr. Heyroth. It was prepared from uracil by the method of Hilbert and Johnson (16). There was too little for an analysis by us.

Yeast nucleic acid, $C_{38}H_{49}N_{15}P_4O_{29}$, Pfanstiehl. This was kindly given us by Dr. Presnell of the Physiology Department. The analysis given on the label of the container was 13 - 16.5% nitrogen and 7 - 10.5% phosphorus. It was found to contain 13.9 and 14.8% nitrogen in two analyses, and 7.44 and 7.48% phosphorus in two analyses. Theory is 16.12% nitrogen and 9.53% phosphorus. The N/P ratio found was 1.9 . Theory is 1.69 .

Sodium salt of thymonucleic acid, $C_{39}H_{47}N_{15}P_4O_{25}Na_4$. This was prepared from calf thymus by the Levene and Bass (39) modification of the method of Levene (38). The thymus glands were obtained during slaughtering, packed

in ice, brought to the laboratory, immediately ground, weighed and poured into boiling salt solution. Just before the precipitation of nucleic acid with ethanol, the solution was made alkaline to litmus. About 100 grams of the sodium salt (which gave no biuret test) were obtained from 2 kilos of glands. This material was purified according to Steudel and Peiser (69) by re-precipitating three times, washed with ethanol and with ether and dried in vacuo over sulfuric acid.

About 45 grams of nearly white sodium nucleate were obtained which gave no biuret test on standing twenty-four hours, or even then after addition of ethanol and solid KOH (T.B. Osborne's modification (5)). It also failed to give Millon's test either on slow heating or standing one week at room temperature. It was found to contain 12.9 and 13.4% nitrogen in two analyses, and 7.55 and 7.76% phosphorus in two analyses. Theory is 15.66% nitrogen and 9.25% phosphorus. The N/P ratio found was 1.7 . Theory is 1.69 . The figures for nitrogen and phosphorus agree with those given by Steudel (67); but are lower than those of many other authors. The reason for this variation is unknown.

2. Nitrogen and phosphorus analyses.

Nitrogen determinations were carried out by micro-Kjeldahl and semi-micro Kjeldahl analyses. The former

were run by the procedure of Dr. Lieb (47). The latter were run using selenium oxychloride as catalyst (32a). The results are shown in Table II.

(Insert Table II.)

Phosphorus determinations were carried out by the Fiske-Subbarow method (14). Amino-naphthol sulfonic acid was used as reducing agent in the color formation. The results are shown in Table III.

(Insert Table III.)

3. Hydrolyses and formic acid determinations.

The method of hydrolysis employed was similar to that used for the production of levulinic acid by Araki (2) in Kossel's laboratory at Heidelberg where he treated nucleic acid with 30% (by volume) sulfuric acid on a water bath for three hours. In the present work the materials were subjected, with a few exceptions as noted, to 30% (by volume) sulfuric acid through which steam was continually passed, the periods of treatment varying in length from 3 to 12 hours on a single day, but often carried on for several days.

The apparatus used was that for the determination of formic acid by the official method of analysis of the Association of Official Agricultural Chemists (57) into

Table II.

Nitrogen Analyses

Materials analyzed	Weight of material (mgs.)	% Nitrogen found	% Nitrogen theory
Adenine sulfate	51.4	34.6	34.63
Adenine sulfate	44.3	33.5	
Guanine hydrochloride	42.9	33.6	34.07
Guanine hydrochloride	51.1	33.7	
Uric acid	44.8	32.8	33.32
Uric acid	54.4	33.7	
Uracil	3.485	25.47	25.00
Uracil	5.245	25.12	
Yeast nucleic acid	128.8	13.9	16.12
Yeast nucleic acid	109.0	14.8	
Sodium thymonucleate	10.892	12.94	15.66
Sodium thymonucleate	6.089	13.36	

Table III.

Phosphorus analyses

Materials analyzed	Weight of material (mgs.)	% Phosphorus found	% Phosphorus theory
Yeast nucleic acid	1.20	7.44	9.53
Yeast nucleic acid	1.20	7.48	
Sodium thymonucleate	1.34	7.76	9.25
Sodium thymonucleate	1.37	7.55	

which an additional spray trap (Hopkins distilling head) was introduced between the flask containing barium carbonate and the condenser to avoid losses during rapid and prolonged distillations.

In this method the formic acid produced by the action of strong mineral acid on the substance studied was continuously steam distilled into a constantly boiling suspension of $BaCO_3$. Volatile acids, such as formic, were caught here. The filtrate from the $BaCO_3$ was then treated with reagents, containing $HgCl_2$, HCl, NaCl and Na acetate, and the reduced HgCl filtered off and weighed. Any volatile acid capable of reducing $HgCl_2$ under these conditions was estimated as formic acid.

The procedure for formic acid determinations followed that of the Official Agricultural Chemists (57), with five exceptions. (A) The samples of material (with exceptions as noted) were placed in 30% sulfuric acid instead of in 2% tartaric acid solutions. (B) The volume of solution in which the samples of material were placed was kept at about 20 ml. instead of 50 ml. to obtain more rapid removal of formic acid from the site of its production. (C) The rubber stopper of the flask containing the sample was wrapped with lead foil to protect it from acid. (D) Blank analyses on reagents were not carried out with 150 ml. of barium chloride solution

but with the filtrate from 2 grams of barium carbonate boiled with 100 ml. of water to more closely duplicate the actual method of determination. (E) The filtrates from barium carbonate were refiltered after standing over night to ensure complete removal of solids.

Results

To test the method of formic acid determination controls were run with sodium formate in (A) 2% tartaric acid solution, (B) 30% sulfuric acid solution, and (C) 65% sulfuric acid solution. Blank analyses were carried out with (A) and (B) alone. The results are shown in Table IV. They showed that with 2% tartaric or 30% sulfuric acid heated for 3 hours about 85 - 95% of the formic acid added could be recovered. Some of it is probably converted by the stronger acid to CO and H_2O, since with 65% sulfuric only 22% was recovered as formic acid.

(Insert Table IV.)

To test the interference of any levulinic acid formed in the hydrolysis with the formic acid determination (62), controls were run with levulinic acid in (A) 2% tartaric acid solution, (B) 5% tartaric acid solution, and (C) 30% sulfuric acid solution. 13 - 20 cgs. of levulinic acid were added to the tartaric or

Table IV.

Formic Acid Recovery from Sodium Formate

Material treated	Wt. of material (grams)	Wt. of HgCl (mgs.)	Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH found	% of HCOOH theory	Hours of treatment	
HCOONa	0.0151	102.4	101.0	9.85	65.2	67.67	3	
HCOONa	0.0226	145.2	143.8	14.02	62.0	67.67	3	
HCOONa	0.0217	140.3	138.9	13.54	62.4	67.67	3	3
HCOONa	0.0227	130.4	130.0	12.68	55.9	67.67	3	3
HCOONa	0.0225	139.4	139.0	13.55	60.2	67.67	3	3
HCOONa	0.0212	34.0	32.6	3.18	15.0	67.67	3	6
(Blank analysis) (no formate added)		0.6	0.2				24	
(Blank analysis) (no formate added)		1.4	1.0				24	
(Blank analysis) (no formate added)		2.2	1.8				24	3
(Blank analysis) (no formate added)		1.1	0.7				24	3

Table IV.

id Recovery from Sodium Formate

. of OOH gs.)	% of HCOOH found	% of HCOOH theory	Hours of treatment	Acid used	Vol. of distillate (milliliters) total	Vol. of distillate (milliliters) per hr.	Remarks
.85	65.2	67.67	3	2% tartaric	900	300	Recovered 96.3%
.02	62.0	67.67	3	2% tartaric	900	300	Recovered 91.6%
.54	62.4	67.67	3	30% sulfuric	1200	400	Recovered 92.2%
.68	55.9	67.67	3	30% sulfuric	1200	400	Recovered 82.6%
.55	60.2	67.67	3	30% sulfuric	1200	400	Recovered 88.9%
.18	15.0	67.67	3	65% sulfuric	1200	400	Recovered 22.1%
			24	2% tartaric	8400	350	
			24	2% tartaric	8400	350	
			24	30% sulfuric	8400	350	
			24	30% sulfuric	8400	350	

sulfuric acid and treated by the usual procedure for 24 hours. The calomel precipitate formed under these circumstances in the final stages of the determination was yellow in color, as was always noted when levulinic acid was present, a confirmation of other work (62). The results are shown in Table V. They show that possibly small amounts of some volatile acid are formed from levulinic acid under these circumstances, or possibly small amounts of levulinic acid are volatile or are carried into the barium carbonate suspension.

(Insert Table V.)

To see if sulfites (which reduce the mercuric chloride used in formic acid determination by this procedure) might interfere with the formic acid determinations, sodium sulfite was heated 12 hours in 20 ml. of 30% sulfuric acid while distilling 4200 ml. of water through the apparatus in the usual manner. The filtrate from barium carbonate was made up to 200 ml. in a volumetric flask and divided into two 100 ml. portions.

To one portion was added 50 ml. of water and the mercuric chloride and other reagents as used for formic acid determination. This was heated 2 hours in a steam bath and the HgCl produced weighed according to the usual procedure for formic acid determination. It was found that 33.0 mg. of anhydrous sodium sulfite gave no

Table V.

The Interference of Levulinic Acid with Formic Acid De

Material treated	Wt. of material (grams)	Wt. of HgCl (mgs.)	Wt. of HgCl - blank (mgs.)	Equivalent wt. of HCOOH (mgs.)	Equivalent % of HCOOH	Hours o treatme
Levulinic acid	0.14	25.7	25.3	2.47	1.8	24
Levulinic acid	0.14	22.1	21.7	2.12	1.5	24
Levulinic acid	0.13	3.5	2.1	0.20	0.15	24
Levulinic acid	0.20	33.7	32.3	3.15	1.6	24
Levulinic acid	0.13	24.6	23.2	2.26	1.7	24
Levulinic acid	0.20	31.0	29.6	2.89	1.5	24
Levulinic acid	0.14	19.2	18.8	1.83	1.3	24
Levulinic acid	0.14	28.1	27.7	2.70	1.9	24

Table V.

Levulinic Acid with Formic Acid Determination.

Equivalent wt. of HCOOH (mgs.)	Equivalent % of HCOOH	Hours of treatment	Acid used	Vol. of distillate (milliliters) total	per hr.
2.47	1.8	24	2% tartaric	8400	350
2.12	1.5	24	2% tartaric	8400	350
0.20	0.15	24	5% tartaric	4400	185
3.15	1.6	24	5% tartaric	4300	180
2.26	1.7	24	30% sulfuric	3900	160
2.89	1.5	24	30% sulfuric	3800	160
1.83	1.3	24	30% sulfuric	8400	350
2.70	1.9	24	30% sulfuric	8400	350

HgCl beyond the amount of the blank analysis, and, in the duplicate analysis, 32.8 mg. of sodium sulfite gave only 0.1 mg. HgCl beyond the blank.

To the other 100 ml. portion of filtrate was added 1 ml. of 10% barium chloride and two drops of 10% HCl. It was then acid to methyl orange. Bromine was added and the solution heated on a steam bath. No precipitate formed. On addition of 1 to 2 mg. of sodium sulfite, which dissolved, and then addition of bromine, the solution became turbid. The duplicate analysis gave similar results, indicating the absence of sulfites in the 100 ml. portion of filtrate.

To the 20 ml. of 30% sulfuric acid in which the sulfite had originally been placed was added a slight excess of hot barium hydroxide solution. The mixture was filtered. About half the filtrate was refiltered, acidified to methyl orange with 10% HCl (less than 0.5 ml. was required) and 1 ml. of 10% barium chloride added. On addition of bromine and warming a slight turbidity appeared, indicating that a small quantity of sulfite may have remained in the sulfuric acid. The duplicate analysis gave similar results.

To further test the possibility of sulfites interfering with the formic acid determination, glucose was heated 6 hours in 20 ml. of 30% sulfuric acid while

distilling 2400 ml. of water through the apparatus in the usual manner. The filtrate from barium carbonate was made up to 200 ml. in a volumetric flask and divided into two 100 ml. portions. In one of these portions formic acid was determined as usual, and the result multiplied by two. It was found that 100.9 mg. of glucose gave 90.2 mg. of HgCl, equivalent to 8.79 mg. of formic acid, a yield of 8.71%. The duplicate analysis was lost. The other 100 ml. portion of filtrate was acidified to methyl orange with 10% HCl after addition of 1 ml. of 10% barium chloride. Bromine was then added and the solution heated on a steam bath. No precipitate formed, indicating the absence of sulfites. On addition of 1 to 2 mg. of sodium sulfite, which dissolved, and then addition of bromine, the solution became turbid.

To further test the possibility of sulfites interfering with the formic acid determination, sodium sulfite was placed in a suspension of 2 grams of barium carbonate in 100 ml. of water and the mixture boiled at a rate which evaporated 200 ml. of water per hour. The volume of the mixture was kept near 100 ml. by frequent additions of water. After boiling the mixture was cooled, filtered, 1 ml. of 10% barium chloride added, the cloudy mixture refiltered, acidified to methyl orange with 10% HCl and bromine added. No precipitate formed, indicating the

absence of sulfites. On addition of sodium sulfite a precipitate formed immediately and the mixture was decolorized. This procedure was carried out with 35.3 mg. of sodium sulfite boiled 1/2 hour, 29.8 mg. boiled 2 hours, 30.4 and 31.5 mg. boiled 2 1/2 hours. Results were similar in all four experiments.

This series of experiments shows that sulfites do not interfere with formic acid determination by this method. This finding extends the observation of Zerban (73) that sulfites do not interfere with formic acid determination by the official method of the A. O. A. C.

To measure the production of formic acid from known sugars, when hydrolysed under similar conditions, glucose, galactose, levulose, arabinose, and xylose were heated for a similar time in 30% sulfuric acid solutions. The amounts of formic acid obtained are shown in Table VI.

It will be seen that only minimal amounts were obtained from the pentoses but 6 - 18% from the hexoses, levulose giving most and glucose least but still about 14% in 24 hours heating.

(Insert Table VI.)

To measure the production of formic acid from pyrimidines and purines, these substances were heated with 30% sulfuric acid for similar periods and the formic acid produced determined: uracil, thymine, cytosine,

Table VI.

Formic Acid Production from Sugars

Material treated	Wt. of material (grams)	Wt. of HgCl (mgs.)	Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatment	Acid
Glucose	0.1024	64.2	62.8	6.12	5.98	3	30% su
		119.3	116.5	11.36	11.09	6	30% su
Glucose	0.1008	115.5	114.1	11.11	11.02	12	30% su
		152.4	149.6	13.59	13.48	24	30% su
Glucose	0.2003	205.2	203.8	19.87	9.92	12	30% su
		292.3	289.5	28.23	14.09	24	30% su
Galactose	0.1024	93.1	91.7	8.94	8.73	3	30% su
		160.2	157.4	15.35	14.99	6	30% su
Levulose	0.0997	188.2	187.8	18.31	18.4	12	30% su
Levulose	0.1016	191.7	191.3	18.65	18.36	12	30% su
Arabinose	0.4819	15.6	15.2	1.48	0.307	12	30% su
Arabinose	0.5624	34.0	33.6	3.28	0.583	12	30% su
Xylose	1.0164	31.7	30.3	2.95	0.290	3	30% su
		59.4	56.6	5.52	0.543	12	30% su
		61.8	57.6	5.62	0.553	24	30% su
Xylose	0.6659	27.5	26.1	2.54	0.381	3	30% su
		44.1	41.3	4.03	0.605	12	30% su
		46.5	42.3	4.13	0.620	24	30% su

Table VI.

Formic Acid Production from Sugars

Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatment	Acid used	Vol. of distillate (milliliters) total	per hr.
62.8	6.12	5.98	3	30% sulfuric	1200	400
116.5	11.36	11.09	6	30% sulfuric	2400	400
114.1	11.11	11.02	12	30% sulfuric	1800	150
149.6	13.59	13.48	24	30% sulfuric	3800	160
203.8	19.87	9.92	12	30% sulfuric	1500	125
289.5	28.23	14.09	24	30% sulfuric	3600	150
91.7	8.94	8.73	3	30% sulfuric	1500	500
157.4	15.35	14.99	6	30% sulfuric	2600	435
187.8	18.31	18.4	12	30% sulfuric	4800	400
191.3	18.65	18.36	12	30% sulfuric	4800	400
15.2	1.48	0.307	12	30% sulfuric	2400	200
33.6	3.28	0.583	12	30% sulfuric	2400	200
30.3	2.95	0.290	3	30% sulfuric	600	200
56.6	5.52	0.543	12	30% sulfuric	2800	235
57.6	5.62	0.553	24	30% sulfuric	5300	220
26.1	2.54	0.381	3	30% sulfuric	600	200
41.3	4.03	0.605	12	30% sulfuric	2800	235
42.3	4.13	0.620	24	30% sulfuric	5300	220

uric acid, adenine sulfate, and guanine hydrochloride. The results are shown in Tables VII and VIII. The pyrimidines when heated with 30% sulfuric gave minimal amounts or no formic acid. The one result with cytosine when heated for 48 and 60 hours was anomalous. A repetition gave no reduction. Of the purines adenine was especially able to form formic acid.

(Insert Tables VII and VIII.)

To measure the production of formic acid from nucleic acids, analyses were made with the sodium salt of thymonucleic acid and with yeast nucleic acid in 30% sulfuric acid solutions, and with the sodium salt of thymonucleic acid in 65% sulfuric acid solution and in 40% phosphoric acid solution. The results are shown in Table IX.

(Insert Table IX.)

To test the production of formic acid from a purine-free hydrolysate of thymonucleic acid, an analysis was carried out by treatment of sodium nucleate as usual for 1.5 hours in 30% sulfuric acid. The flask containing the hydrolysate was cooled in an ice bath in situ and ammonium hydroxide added to alkalinity. Sufficient ammoniacal silver nitrate was added to precipitate the purines which were filtered off. The filtrate was again brought to a concentration of 30% sulfuric acid

Table VII.

Experiments with Thymine, Cytosine, Uracil and Uric Aci

Material treated	Wt. of material (grams)	Wt. of HgCl (mgs.)	Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatment	Acid
Thymine	0.0357	3.6	3.2	0.31	0.88	48	30% su
Thymine	0.0355	2.3	1.9	0.19	0.52	48	30% su
Cytosine	0.0182	6.7	6.3	0.61	3.4	24	30% su
		11.5	10.7	1.04	5.71	60	30% su
Cytosine	0.0182	0.4	0.0	0.00	0.00	48	30% su
Uracil	0.4048	1.8	0.4	0.04	0.01	12	30% su
		2.8	0.0	0.00	0.00	24	30% su
Uracil	0.5482	5.6	4.2	0.41	0.07	12	30% su
		11.7	8.9	0.87	0.16	24	30% su
Uric acid	0.5011	2.3	0.9	0.09	0.02	24	30% su
Uric acid	0.8766	4.5	3.1	0.30	0.03	24	30% su

Table VII.

iments with Thymine, Cytosine, Uracil and Uric Acid.

Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatment	Acid used	Vol. of distillate (milliliters) total	per hr.
3.2	0.31	0.88	48	30% sulfuric	9600	200
1.9	0.19	0.52	48	30% sulfuric	9600	200
6.3	0.61	3.4	24	30% sulfuric	4800	200
10.7	1.04	5.71	60	30% sulfuric	12000	200
0.0	0.00	0.00	48	30% sulfuric	9600	200
0.4	0.04	0.01	12	30% sulfuric	3100	260
0.0	0.00	0.00	24	30% sulfuric	6100	255
4.2	0.41	0.07	12	30% sulfuric	3100	260
8.9	0.87	0.16	24	30% sulfuric	6100	255
0.9	0.09	0.02	24	30% sulfuric	4300	180
3.1	0.30	0.03	24	30% sulfuric	4300	180

Table VIII.

Experiments with Adenine Sulfate and Guanine Hydroch

Material treated	Wt. of material (grams)	Wt. of HgCl (mgs.)	Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatmer
Adenine sulfate	0.3887	1068.5	1068.5	104.28	26.80	24
Adenine sulfate	0.4682	1132.4	1132.4	110.41	25.58	24
Adenine sulfate	0.0293	105.3	105.3	10.3	35.0	36
		111.7	111.7	10.9	37.2	53
Adenine sulfate	0.0293	63.8	63.8	6.22	21.2	36
		68.4	68.4	6.67	22.7	60
		76.0	75.6	7.37	25.1	84
		75.9	75.5	7.37	25.1	132
		81.7	80.9	7.90	26.9	156
Adenine sulfate	0.0293	76.6	76.2	7.45	25.5	24
		99.7	98.9	9.66	33.1	60
		117.4	116.2	11.33	38.9	156
Adenine sulfate	0.0293	78.2	77.8	7.62	26.0	24
		87.2	86.4	8.46	28.9	60
		92.3	91.1	8.92	30.5	156
Guanine hydrochloride	0.4832	60.3	60.3	5.88	1.22	24
Guanine hydrochloride	0.5005	64.8	64.8	6.32	1.26	24
Guanine Hydrochloride	0.0286	17.5	17.5	1.71	5.96	60
		27.5	27.1	2.65	9.27	84
		38.8	38.0	3.71	12.9	132
		44.2	43.0	4.20	14.7	156
		51.2	49.6	4.84	16.9	199
Guanine Hydrochloride	0.0288	15.0	15.0	1.46	5.08	60
		23.2	22.8	2.22	7.71	84
		30.6	29.8	2.90	10.1	132
		35.9	34.7	3.38	11.7	156
		42.3	40.7	3.97	13.8	204
		50.2	48.2	4.70	16.3	300

Table VIII.

s with Adenine Sulfate and Guanine Hydrochloride.

of HgCl blank mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatment	Acid used	Vol. of distillate (milliliters) total	per hr.
068.5	104.28	26.80	24	30% sulfuric	5100	210
132.4	110.41	25.58	24	30% sulfuric	5100	210
105.3	10.3	35.0	36	30% sulfuric	4600	130
111.7	10.9	37.2	53	30% sulfuric	7900	150
63.8	6.22	21.2	36	30% sulfuric	5000	140
68.4	6.67	22.7	60	30% sulfuric	8300	140
75.6	7.37	25.1	84	30% sulfuric	13200	155
75.5	7.37	25.1	132	30% sulfuric	22800	170
80.9	7.90	26.9	156	30% sulfuric	27600	180
76.2	7.45	25.5	24	30% sulfuric	4800	200
98.9	9.66	33.1	60	30% sulfuric	12000	200
116.2	11.33	38.9	156	30% sulfuric	31200	200
77.8	7.62	26.0	24	30% sulfuric	4800	200
86.4	8.46	28.9	60	30% sulfuric	12000	200
91.1	8.92	30.5	156	30% sulfuric	31200	200
60.3	5.88	1.22	24	30% sulfuric	4600	190
64.8	6.32	1.26	24	30% sulfuric	4600	190
17.5	1.71	5.96	60	30% sulfuric	8500	140
27.1	2.65	9.27	84	30% sulfuric	13400	160
38.0	3.71	12.9	132	30% sulfuric	23000	175
43.0	4.20	14.7	156	30% sulfuric	27800	180
49.6	4.84	16.9	199	30% sulfuric	36400	180
15.0	1.46	5.08	60	30% sulfuric	8500	140
22.8	2.22	7.71	84	30% sulfuric	13400	160
29.8	2.90	10.1	132	30% sulfuric	23000	175
34.7	3.38	11.7	156	30% sulfuric	27800	180
40.7	3.97	13.8	204	30% sulfuric	37400	180
48.2	4.70	16.3	300	30% sulfuric	55400	185

Table IX.

Formic Acid Production from Nucleic Aci

Material treated	Wt. of material (grams)	Wt. of HgCl (mgs.)	Wt. of HgCl - blank (mgs.)	Wt. of HCOOH (mgs.)	% of HCOOH	Hours of treatment	A
Yeast nucleic acid	0.3787	148.6	148.6	14.49	3.826	24	30%
Yeast nucleic acid	0.3793	127.5	127.5	12.43	3.277	24	30%
Sodium thymo-nucleate	0.2124	33.1	31.7	3.09	1.46	3	30%
Sodium thymo-nucleate	0.2000	60.8	60.4	5.90	2.95	24	30%
		90.7	89.9	8.78	4.39	66	30%
Sodium thymo-nucleate	0.2000	80.8	80.4	7.74	3.87	24	30%
		106.4	105.6	10.20	5.100	60	30%
Sodium thymo-nucleate	0.4058	128.9	128.5	12.53	3.087	24	30%
		200.7	199.9	19.49	4.803	48	30%
Sodium thymo-nucleate	0.4010	118.2	117.8	11.49	2.864	24	30%
		179.6	178.8	17.43	4.347	48	30%
Sodium thymo-nucleate	0.4038	36.3	34.9	3.40	0.842	3	30%
		80.5	77.7	7.58	1.88	6	30%
		137.1	132.9	12.96	3.209	12	30%
		185.1	179.5	17.50	4.334	18	30%
		224.0	217.0	21.16	5.240	24	30%
Sodium thymo-nucleate	0.4046	41.3	39.9	3.89	0.961	3	30%
		85.5	82.7	8.18	2.02	6	30%
		136.4	132.2	12.89	3.186	12	30%
		200.3	194.7	18.98	4.691	18	30%
		241.4	234.4	22.85	5.648	24	30%
Sodium thymo-nucleate	0.2012	48.0	46.6	4.54	2.26	3	65%
Sodium thymo-nucleate	0.4098	87.8	87.8	8.56	2.09	24	40%
Sodium thymo-nucleate	0.4024	76.7	76.7	7.48	1.86	24	40%
Sodium thymo-nucleate	0.2073	15.5	14.1	1.37	0.661	1.5	30%
		54.6	51.8	5.05	2.44	3	30%
		61.6	57.4	5.60	2.70	,6	30%
		62.0	56.6	5.52	2.66	12	30%

Table IX.

cid Production from Nucleic Acids.

. of OOH gs.)	% of HCOOH	Hours of treatment	Acid used	Vol. of distillate (Milliliters) total	per hr.	Remarks
.49	3.826	24	30% sulfuric	4400	185	
.43	3.277	24	30% sulfuric	4500	190	
.09	1.46	3	30% sulfuric	1200	400	
.90	2.95	24	30% sulfuric	4800	200	
.78	4.39	66	30% sulfuric	13200	200	
.74	3.87	24	30% sulfuric	4800	200	
.20	5.100	60	30% sulfuric	12000	200	
.53	3.087	24	30% sulfuric	8400	350	
.49	4.803	48	30% sulfuric	16800	350	
.49	2.864	24	30% sulfuric	8400	350	
.43	4.347	48	30% sulfuric	16800	350	
.40	0.842	3	30% sulfuric	800	270	
.58	1.88	6	30% sulfuric	1700	280	
.96	3.209	12	30% sulfuric	3700	305	
.50	4.334	18	30% sulfuric	5900	325	
.16	5.240	24	30% sulfuric	8300	350	
.89	0.961	3	30% sulfuric	800	270	
.18	2.02	6	30% sulfuric	1700	280	
.89	3.186	12	30% sulfuric	3900	320	
.98	4.691	18	30% sulfuric	6100	340	
.85	5.648	24	30% sulfuric	8500	350	
.54	2.26	3	65% sulfuric	1200	400	
.56	2.09	24	40% phosphoric	5100	210	
.48	1.86	24	40% phosphoric	5100	210	
.37	0.661	1.5	30% sulfuric	600	400	Removed purines
.05	2.44	3	30% sulfuric	1300	435	Ag and $(NH_4)_2SO_4$ present
.60	2.70	,6	30% sulfuric	2200	365	Ag and $(NH_4)_2SO_4$ present
.52	2.66	12	30% sulfuric	4000	335	Ag and $(NH_4)_2SO_4$ present

in a volume of 20 ml. and the analysis continued. The results are included at the end of Table IX.

Nothing definite can be concluded from this experiment for the small amounts of silver nitrate probably hastened the oxidation of the material yielding some formic acid.

III. Discussion

Hydrolysis with 30% sulfuric acid was chosen because, (A), practically all the earlier workers used sulfuric acid and usually of this strength to produce levulinic acid (and formic acid) from thymonucleic acid, thus affording comparable results; (B), it is active enough to split the pyrimidine-sugar bonds in the nucleic acids within a few hours; (C), it is non-volatile with steam; (D), the rate at which it decomposes formic acid is slow enough to allow good recovery of small amounts of formic acid; and, (E), it does not produce appreciable amounts of formic acid from levulinic acid.

The apparatus and procedure adopted have in their original form been repeatedly tested for over twenty years and their reliability is assured. But as the experimental results show, the use of 30% sulfuric acid in a small volume (20 ml.) entails hazards and inaccuracies not found in the original procedure.

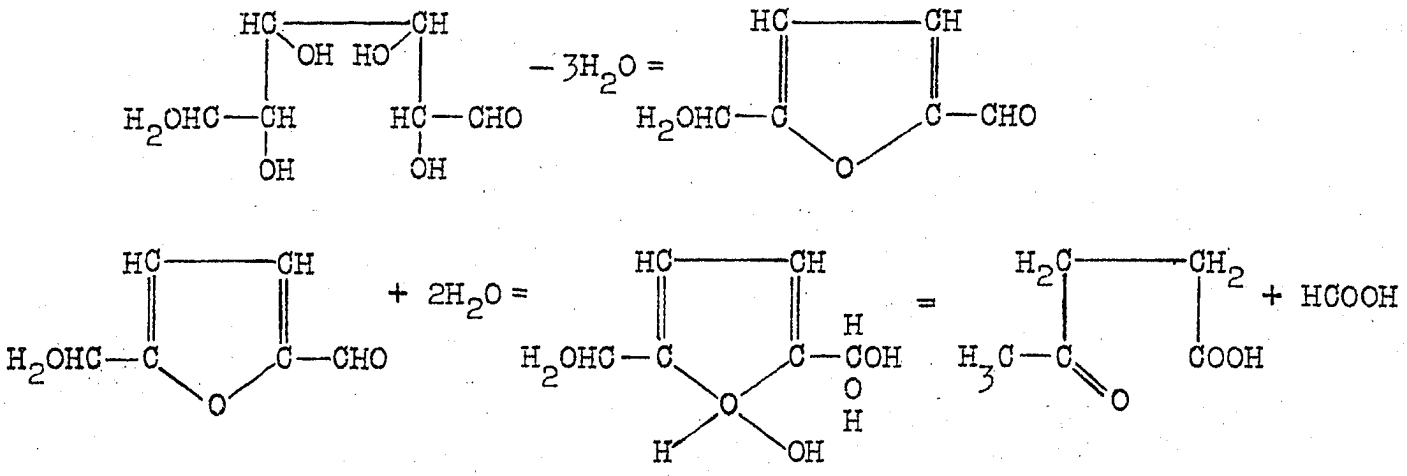
The recoveries of formic acid from sodium formate placed in 2% tartaric acid solution amounted to 94%, confirming the findings of Seeker and his many colleagues (62). In 65% sulfuric acid much of the formic acid was quickly decomposed. From 30% sulfuric acid 90% of formic acid was recovered, but the variations among the determinations show there is danger of decomposing part of the formic acid under these conditions if more than ten milligrams are present at one time. This condition was probably avoided in the experiments other than those on sodium formate, as consideration of the tables shows.

The blank analyses of tartaric and of sulfuric acid solutions indicate something of the error of the method of determination.

The results of analyses made with levulinic acid in 2% and in 5% tartaric acid solutions confirm those of Seeker (62) in that the amount of levulinic acid which may contaminate the determinations, while small, is quite variable. The amount of contamination was the same when 30% sulfuric acid was used in place of tartaric acid, indicating that levulinic acid probably did not yield an appreciable quantity of formic acid under this treatment.

The analyses of formic acid produced from hexoses by acid hydrolysis in part confirm the work of Conrad and Guthzeit (6, 7), although their methods were so

different from these as to render comparisons inexact. The method used here gave somewhat higher yields of formic acid than their method, but still under one mol of formic acid per mol of sugar. The formic acid is thought to be produced principally in this manner (70):



The amount of formic acid produced on acid hydrolysis of pentoses was less than one-fourth the amount of formic acid reported by Conrad et al. (6, 7). Although there is no doubt that small quantities (about .5%) of reducing volatile substances, presumably formic acid, may be produced on prolonged acid hydrolysis of pentoses, the mechanism of its production has not been worked out. And the quantity is less than 1/20th that from the same weight of hexoses.

The results of the experiments with uracil, thymine, cytosine and uric acid are in consonance with previous

work that they do not decompose readily in acid and show that the substances produce no appreciable amounts of formic acid under the treatment given. The small quantities of calomel produced in some cases indicate something of the error of the method of determination. Evidently a volatile reducing material of some kind is at times formed. It might or might not be formic acid. Since methyl cytosine is converted to thymine by deamination and thymine produces only very minute amounts of formic acid, if any, it (methyl cytosine) probably would not yield any formic acid on similar treatment.

Adenine, and to a much less extent guanine yield formic acid when hydrolyzed with sulfuric acid. The present experiments are the first, we believe, in which the amount of this formic acid has been determined.

Treatment of hypoxanthine with water in a sealed tube heated to 200° C. produced a small amount of formic acid, as Kossel reported in 1882 (24a). Adenine and hypoxanthine heated to 180 - 200° C. with concentrated HCl decompose according to Krüger (32) in this way:

$$C_5H_5N_5 + 8H_2O = 4NH_3 + CO_2 + C_2H_5NO_2 + 2HCOOH$$

$$C_5H_4N_4O + 7H_2O = 3NH_3 + CO_2 + C_2H_5NO_2 + 2HCOOH$$

In the present experiments between one and two mols of formic acid were produced per mol of adenine. It is possible that one of these comes from carbon atom 8, as it possibly occurs with guanine and xanthine. The source of the other mol of formic acid may be any of the other carbon atoms. Professor Mathews has suggested carbon atom 6 as a possible source. The relations of carbon atoms 4, 5 and 6 to the rest of the molecule are similar among hypoxanthine, xanthine and uric acid which yield two, one and no mols of formic acid, respectively. In all three instances carbon atom 6 is bound to oxygen, nitrogen and carbon. If it gave rise to formic acid from hypoxanthine and from xanthine, it might be thought that it would give rise to formic acid from uric acid. But this did not occur. On the other hand carbon atom 2 is differently bound in hypoxanthine than in xanthine and uric acid. It might be the source of the second mol of formic acid. No suggestion as to the exact source of the formic acid from adenine or hypoxanthine has been found in the literature.

As Fischer showed (11), the main reaction of guanine with boiling 25% HCl is

Guanine + water = xanthine + ammonia.

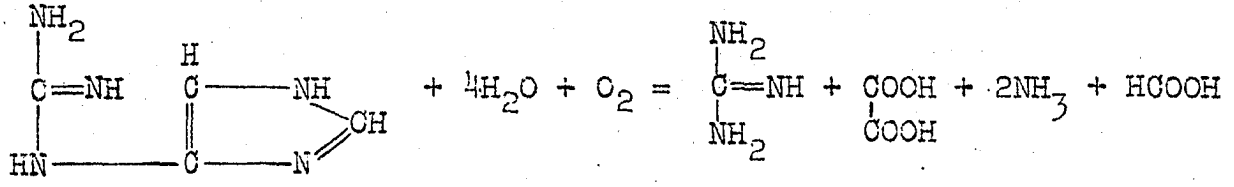
On heating with concentrated HCl under pressure, it was early found that xanthine decomposed in this way (61):

$$C_5H_4N_4O_2 + 6H_2O = 3NH_3 + C_2H_5NO_2 + 2CO_2 + HCOOH$$

Similarly, Wulff (72) found guanine decomposed in this way:

$$C_5H_5N_5O + 7H_2O = 4NH_3 + C_2H_5NO_2 + 2CO_2 + HCOOH$$

Hunter (18) has recently shown that simultaneously with xanthine production, guanine may yield 4- (or 5-) guanidinoglyoxaline. On oxidation of this glyoxaline he found:



After quantitative determination of a number of the reaction products, Hunter concluded the formic acid came from carbon atom 8 of the guanine.

Whether the reactions proceed through xanthine or through this glyoxaline, one molecule of formic acid is produced from one molecule of guanine according to these equations. Somewhat less formic acid than this was found in the present experiments, but the production of formic acid had not stopped after some hundreds of hours of treatment. This is not surprising in view of the stability of xanthine in acid, and the comparatively small quantity of guanidinoglyoxaline probably involved.

From the work of Kossel and Neumann (30) we know that thymonucleic acid yields formic acid on acid hydrolysis. Brown and Johnson (4) have shown that the nucleic acid of the tubercle bacillus also yields formic acid on acid hydrolysis. But so far as is known this is the first report of the production of formic acid by acid hydrolysis of yeast nucleic acid, as well as the first report on the quantities of formic acid produced on acid hydrolysis of nucleic acids.

The average yield from yeast nucleic acid was 3.6% of formic acid in 24 hours of treatment by 30% H_2SO_4. By calculating from the formic acid production of the individual substances, one finds:

1 mol of adenine yielded 52 g. HCOOH in 24 hours.
1 mol of guanine yielded 2.6 g. HCOOH in 24 hours.
4 mols of xylose yielded 3.6 g. HCOOH in 24 hours.

Total yield = 58 grams HCOOH in 24 hours.

While the expected yield is thus 58 grams of formic acid from one mol of yeast nucleic acid, that found was 47 grams, the difference possibly being due to the different reactivities of ribose, or of the different substances when in combination, or to the impurities of the nucleic acid, or to the errors of the method.

The sodium salt of thymonucleic acid gave an average yield of 3.2% of formic acid in 24 hours of

treatment, or 43 grams of formic acid per mol of sodium nucleate. From the above calculation, it is seen that the adenine and guanine in the nucleic acid molecule should have given 55 grams of formic acid. Much of the sugar of the nucleic acid was converted to humus, although the yellow color of the calomel precipitates indicated an indeterminate amount of levulinic acid contaminated the formic acid determinations. The difference between the calculated and found percentages of formic acid must be explained similarly to the case of yeast nucleic acid.

IV. Summary and Conclusions

The chief source of formic acid obtained by acid hydrolysis of thymonucleic acid is adenine, while guanine contributes a minor portion, about 1/20th as much as the adenine.

Yeast nucleic acid also yields formic acid on acid hydrolysis and to the same amount as thymonucleic. The chief source of this formic acid is adenine, while guanine and ribose contribute minor portions, together about 1/8th as much as the adenine.

It is probable that the chief sources of formic acid produced on acid hydrolysis of the nucleic acid of the tubercle bacillus are its adenine and, to a

much less extent, guanine radicles.

Since Levene has shown that desoxyribose on acid hydrolysis forms levulinic acid it thus happens that these two substances, levulinic acid and formic acid, are now accounted for. This is an illustration of how easily an erroneous conclusion may be drawn as to the nature of a substance from a qualitative study of its decomposition products. For twenty years or more the fact that nucleic acids yielded these two substances on acid hydrolysis was considered evidence that the carbohydrate in them was a hexose, since hexoses also show this peculiarity. We now know the sugar of thymonucleic acid was really a desoxypentose and the formic acid came almost entirely from adenine.

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