[CONTRIBUTION FROM THE RESEARCH AND BIOLOGICAL LABORATORIES OF E. R. SQUIBB AND SONS]

THE RATE OF THERMAL DECOMPOSITION AT 100° OF THE OXYTOCIC PRINCIPLE OF THE POSTERIOR LOBE OF THE PITUITARY GLAND. I. THE EFFECT OF HYDROGEN-ION CONCENTRATION[1]

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The rate of thermal decomposition of the oxytocic principle of the posterior lobe of the pituitary body is highly desired information for the manufacturer in selecting proper conditions of extraction and stabilization of pituitary solution and in furnishing data which will assist the investigator in the differentiation of the active principles. Since the advent of this powerful smooth muscle stimulant in obstetrics, very little work has been recorded which will adequately express its stability or rate of thermal decomposition in acid solution despite the varied and voluminous experimentation.

The purpose of this work was to determine the rate of thermal decomposition at 100° of the oxytocic principle of pituitary extracts in acid solution ranging from P_H 2.0 to 5.1. These results, for the first time, furnish a complete picture of the effect of hydrogen-ion activity on the rate of thermal decomposition and thereby render more intelligible obscure features in previous experimental work. Such features will be brought out in some detail in the following résumé of the more important experiments on this phase of the pituitary investigations.

According to Guggenheim,[2] Dudley,[3] Abel and Nagayama[4] and Abel, Rouiller and Geiling,[5] the pressor and oxytocic principles are both destroyed when exposed to 1–2 *N* sodium hydroxide for about two hours at room temperature. The destruction takes place regardless of the method of preparation or purity of the active material.

In strong acid solution the action is not so drastic, therefore the temperature must be elevated to obtain rapid change. Boiling under reflux in 0.5% hydrochloric acid for half an hour abolished the pressor response and destroyed 80% of the total oxytocic activity of the commercial pituitary extracts and the trinitro-aniline salts of "pituitary tartrate" of Abel, Rouiller and Geiling. Under these conditions it appears that the pressor principle is less resistant to change than the oxytocic and, since

[1] Presented before the joint meeting of the Medicinal and Biological Divisions of the American Chemical Society at St. Louis, Missouri, April, 1928.

[2] Guggenheim, *Biochem. Z.*, **65**, 189 (1914).

[3] Dudley, *J. Pharm. Exptl. Therapy*, **14**, 295 (1919).

[4] Abel and Nagayama, *ibid.*, **15**, 347 (1920).

[5] Abel, Rouiller and Geiling, *ibid.*, **22**, 289 (1923).

the active material employed in the various investigations was of such a wide degree of purification, it would seem that purity is not a major factor in regulating the rate of decomposition.

Abel and Nagayama stated that 0.25% hydrochloric acid did not appreciably impair the activity of the commercial extract employed when boiled for one hour, and expressed the opinion that the residual 20% of oxytocic activity remaining in pituitary solutions after treatment with 0.5% hydrochloric acid for half an hour, which appeared to induce maximum reduction in oxytocic value, was due to the formation or the presence of histamine or some histamine-like cleavage product. Dudley, Dale and Dudley, as well as Hanke and Koessler,[6] were unable to accept Abel's explanation and in later investigations Abel and co-workers have concluded that histamine is not a normal constituent of the active material. Dale and Dudley carried the experiment a step farther and found that boiling the active material for six hours in 0.5% hydrochloric acid reduced the oxytocic strength to less than $^1/_{200}$ of its original value. Therefore the simplest explanation is that of incomplete decomposition of the oxytocic principle rather than assigning a special destructive action to 0.5% hydrochloric acid.

While hydrochloric acid in relatively low concentrations quickly destroys the active principle at 100°, acetic acid solutions on the other hand are comparatively stable in wide range of concentrations. According to Stasiak[7] the yield of the oxytocic principle is not impaired by boiling 0.50 g. of acetone defatted posterior lobe in 100 cc. for a "short time" in 0.05 to 6% acetic acid. The same yield was obtained by employing 0.05% (0.0137 *N*) hydrochloric acid as the extracting solvent. However, with 0.5% hydrochloric acid as the solvent, a loss of 90% of the activity was recorded, which when compared with the data presented herein is indeed a very striking observation because the recorded P_H's of Stasiak's 0.5% hydrochloric and 6.0% acetic acid extract were of a similar magnitude, which would not allow such a difference in the rate of destruction to occur.[8]

On adjustment of the reaction of an acetic acid extract of the wet posterior lobes to approximately P_H 3.0 (colorimetric) Adams[9] found no detectable loss at the end of fifty-five minutes at about 100°. Histamine

[6] Hanke and Koessler, *J. Biol. Chem.*, **43**, 557 (1920).

[7] Stasiak, *J. Pharm. Exptl. Therap.*, **28**, 1 (1926).

[8] It has been our experience that colorimetric measurements of P_H of solutions of this type are widely divergent from the values obtained by the hydrogen electrode. A rough comparison to Stasiak values can be seen in Table I. Stasiak determined the P_H of his extracts colorimetrically and they ranged from P_H 2.6 for his 0.50% hydrochloric acid and P_H 3.4 for his 6% acetic acid extract to P_H 7.0 for his 0.05% acetic acid extract.

[9] Adams, *J. Biol. Chem.*, **30**, 235 (1917).

was employed as standard. When the P_H was set at 5.0 (colorimetric), the system lost its oxytocic activity very rapidly at the same temperature and the rate of thermal decomposition was found to conform to that calculated for a first-order reaction; 80% of the total activity was lost in fifty-five minutes. Abel and Nagayama claim to have confirmed Adams' finding as to the amount lost in this region of acidity, but direct comparison cannot be made because of the different decomposition conditions. The P_H of their pituitary solution was not measured. They further state that when an extract is made just acid to litmus, two hours of boiling does not alter its oxytocic or pressor content.

Experimental

Unless otherwise stated all pituitary solutions studied for the rate of destruction were made by extraction of the acetone dehydrated posterior lobe of pituitary gland of cattle with diluted acetic acid—a procedure employed by many manufacturers and investigators. The method of preparation of the active powder[10] was essentially that of Smith and McClosky[11] with minor modifications. The dried powders represent, consistently, close to 16% of the original weight of fresh posterior lobes.

Extraction of Active Posterior Lobe Powder.—For the study of the rate of thermal decomposition of the oxytocic principle it was desired to have the extracts freed as nearly as possible from inert protein by the simple coagulation that heat affords and still obtain the full oxytocic activity of the powder. The wide divergence of extraction conditions recorded and the absence of definite published data on the P_H at which the heat coagulable protein is most effectively removed, made it necessary to subject this step to a close examination to insure reproducibility. Extractions were made with varying concentrations of acetic acid, acetic acid in saline salt and hydrochloric acid, and analyses made of each solution for total nitrogen and the nitrogen in the filtrate after precipitation with tungstic acid. The oxytocic value of the solution extracted at P_H 3.2 $\pm$ 0.2 and 4.4 $\pm$ 0.2, respectively, for each type of extraction was also determined.

The actual extraction was conducted as follows: 1.00 g. of finely divided active powder, which had been sifted through a 40-mesh sieve, was placed in suitable hard glass flasks. Part of the desired extraction solution was added and the mixture allowed to stand for ten to fifteen minutes with frequent agitation. The remainder of the 100 cc. was then added, the flask plugged with cotton and placed in a boiling water-bath for twelve to fourteen minutes, then withdrawn, filtered or centrifuged and the extract analyzed. The final temperature reached was 96–98°.

[10] Aldrich, THIS JOURNAL, 37, 203 (1915).

[11] Smith and McClosky, U. S. P. H. Bull. Hyg. Lab. No. 138 (1924).

The relationship between the concentration of the extracting acid[12] and the resulting P_H and nitrogenous matter of the extracts is shown in Table I and the data are correlated graphically in Fig. 1. The percentage of acetic acid is given in the first column of Table I for convenient comparison with the work of other investigators. Column 5 gives the mg.

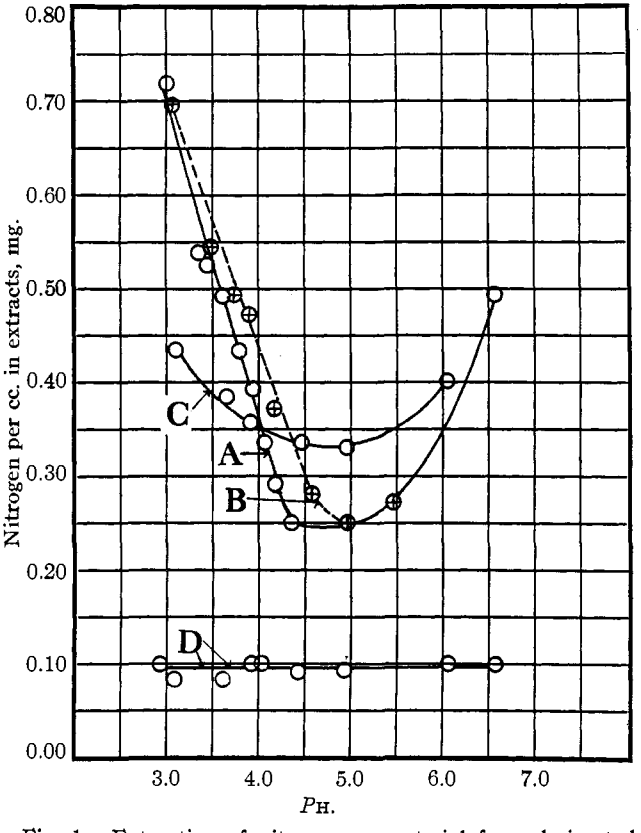


Fig. 1.—Extraction of nitrogenous material from desiccated posterior lobe No. 10941 by (A) acetic acid, (B) hydrochloric acid and (C) No. E9984 by acetic acid in 0.85% sodium chloride. (D) Extracts after precipitation with tungstic acid.

of nitrogen per cc. found in the filtrate after precipitation of the extract with tungstic acid by the method of Folin and Wu.[13] These values are fairly constant and not influenced by the P_H of extraction. Considerable

[12] For a given concentration of acid the resulting P_H of the extract is a function of the quantity or concentration of the powder extracted. For example, 4.00, 2.00, 1.00, 0.400, 0.200 and 0.100 g. of powder No. 9767 were extracted as above with 100 cc. of 0.20% acetic acid for twenty minutes and the resulting P_H's of the extracts at 25° were, respectively, 4.54, 4.24, 3.89, 3.57, 3.42 and 3.28.

[13] Folin and Wu, *J. Biol. Chem.*, **38**, 81 (1919).

oxytocic activity, about 30–40% of the total, was found in the tungstic acid filtrate of extracts previously extracted at P_H 4.3. This point was not investigated on extracts prepared at other acidities. Column 6 gives mg. of nitrogen per cc. after the reaction of the more acid extract was adjusted back at room temperature to the "isoelectric zone." The nitrogen values are not entirely reduced to the level found after coagulation at elevated temperature. The other columns are self-explanatory.

Under the condition of extraction outlined practically the same oxytocic value was obtained for the extracts of P_H 3.2 ± 0.2 as for those extracted at P_H 4.4 ± 0.2 regardless of the type of solvent. Comparison on a weight basis with the U. S. P. X. Standard Powdered Pituitary, which is referred to as 100% activity, showed our experimental solutions to be on an average of 50–60% activity. Selection of optimum extraction conditions with respect to the elimination of excessive inert protein readily can be judged by inspection of Fig. 1. Keeping in mind that the rate of decomposition is rapid enough at 100° to produce considerable loss if the reaction is P_H 5.0 to 6.0, then losses can be minimized and maximum coagulation of inert protein still obtained if the reaction of the resulting extract is P_H 4.4 ± 0.2 with acetic acid. The final temperature as previously mentioned was 96–98°. The zone of maximum coagulation in the case of hydrochloric acid was approximately P_H 4.7–5.2. In this region of acidity the rate of decomposition becomes a more important factor and is apt to produce measurable losses if the extracting system is boiled at 100°, 0.85% sodium chloride in acetic acid holds too much protein in solution to yield the desired condition although its coagulation zone is broad enough to allow easily filtered extracts to be made in more acid solution.

The extraction results as shown in Table I were reproduced on a number of other samples of acetone defatted posterior lobe.

Method of Conducting Destruction.—The total acetic acid concentration of the extracts was increased after filtration to 0.20% and further adjustments of P_H were made with hydrochloric acid or sodium hydroxide. The hydrogen-ion activity was then determined by the hydrogen electrode at 25°. The solutions were sealed in special 1.0-cc. hard glass ampules and placed in streaming steam, the temperature of which was 99.8–100°, and withdrawn at times designated in the tables. The P_H was again measured and it did not deviate after any heating period more than 0.10 unit of P_H from the original value. Assay of oxytocic strength was promptly made—particularly on those solutions whose P_H was close to 2.0 or 5.0—by checking against the control solution. The control solution was finally assayed against the U. S. P. X. standard extract. All controls and ampules were properly preserved or sterilized and held at ice-box temperature.

TABLE I

EXTRACTION OF NITROGENOUS MATERIAL FROM 1.00 G. OF DESICCATED POSTERIOR LOBE POWDER WITH ACETIC AND HYDROCHLORIC ACIDS

A. Acetic acid: Powder E-9984

HAc, %	HAc, mole/liter	P_H of extr.	Total nitrogen, mg./cc.	Nitrogen in filt. after pptn. with tungstic acid, mg./cc.	Nitrogen in filt. after adjust. of extr. to P_H 5.0, mg./cc.
0.00	0.000	6.58	0.49	0.10	..
.025	.0042	4.82	.28	.10	..
.05	.0083	4.46	.27	.11	..
.10	.0166	4.17	.28	.10	..
.25	.0416	3.77	.43	.08	0.28
.40	.0666	3.58	.48	.09	.37
.60	.1000	3.50	.52	.10	.37
.60 + 1 cc. of N HCl		3.00	.72	.10	.40
		Powder No. 10941			
0.070	0.0116	4.35	0.25	..	..
.100	.0166	4.22	.29	..	..
.200	.0333	3.94	.39	..	..
.300	.0499	3.79	.43	..	..
.500	.0833	3.62	.49	..	..
.750	.125	3.48	.53	..	..
1.000	.166	3.38	.54	..	..

B. Acetic acid in 0.85% sodium chloride: Powder E-9984

0.00	0.0000	6.09	0.40	0.10	..
.024	.0040	4.93	.33	.09	..
.050	.0083	4.49	.34	.09	..
.100	.0166	4.06	.34	.10	..
.200	.0333	3.90	.36	.10	..
.350	.0580	3.69	.38	.08	..
.600	.100	3.10	.43	.09	.39
.60 + 1 cc. of N HCl		2.94	.54	.10	.46

C. Extraction of active powder by hydrochloric acid: Powder 10941

(HCl)				
0.0020	5.48	0.27	..	..
.0030	4.92	.25	..	..
.0040	4.56	.28	..	0.30
.0050	4.14	.37	..	.36
.0060	3.87	.47	..	.35
.0070	3.68	.49	..	.35
.0085	3.48	.54	..	.35
.0110	3.08	.69	..	.50
	Powder E-9984			
0.0035	4.55	0.27	..	..
.0050	4.30	.41	0.08	0.30
.0070	3.60	.50	.08	.33
.0100	3.18	.69	.10	.37

The Oxytocic Assay.—The method of assaying the oxytocic strength was essentially that of Dale and Laidlaw,[14] which we have used successfully for a number of years. Needless to say, it required several months of

TABLE II

RATE OF DESTRUCTION OF THE OXYTOCIC PRINCIPLE OF THE POSTERIOR LOBE

t, min.	$a - x$	$K = 1/t \ 2.3 \text{ Log } a/(a-x)$
	D-8889—P_H 2.03	
0	1.00	
60	0.82	0.0033
120	.67	.0033
240	.40	.0038
480	.18	.0036
960	.05	.0031
		———
	Av.	0.0034
	C-8889—P_H 2.26	
0	1.00	
120	0.76	0.0023
240	.65	.0018
480	.43	.0018
960	.19	.0017
1500	.04 (about)	.0021[a]
		———
	Av.	0.0019
	9188—P_H 2.41	
0	1.00	
60	0.91	0.0016
180	.80	.0012
300	.65	.0014
540	.49	.0013
1020	.26	.0013
		———
	Av.	0.0014
	E-8889—P_H 2.72	
0	1.00	
120	0.93	0.00060
240	.86	.00063
480	.71	.00071
960	.50	.00072
1500	.35	.00070
		———
	Av.	0.00067

t, min.	$a - x$	$K = 1/t \ 2.3 \text{ Log } a/(a-x)$
	0-1224—P_H 3.06	
0	1.00	
240	0.86	0.00063
480	.76	.00057
1440	.47	.00052
		———
	Av.	0.00057
	10122—P_H 3.25	
0	1.00	
420	0.80	0.00053
960	.57	.00058
		———
	Av.	0.00055
	E-8889—P_H 3.82	
0	1.00	
60	0.91	0.0016
120	.86	.0012
240	.71	.0014
480	.53	.0013
960	.33	.0011
1500	.19	.0011
		———
	Av.	0.0013
	B-8889—P_H 4.32	
0	1.00	
60	0.78	0.0041
120	.67	.0033
240	.41	.0037
480	.17	.0035
960	.05	.0031
		———
	Av.	.0035
	A-8889—P_H 5.11	
0	1.00	
35	0.65	0.012
60	.50	.011
120	.28	.011
240	.09	.010
		———
	Av.	0.011

[a] Not included in average.

[14] Dale and Laidlaw, *J. Pharm. Exptl. Therap.*, **4**, 75 (1912).

TABLE III

SUMMARY OF THE RATE OF DESTRUCTION OF OXYTOCIC PRINCIPLE IN PITUITARY SOLUTION[a]

Serial no.	PH	K_{100}	Log $1/K$	Remarks
D-8889	2.03	0.0034	2.47	
C-8889	2.26	.0019	2.72	
9188	2.41	.0014	2.85	Contains 0.5% of phenol
E-8889	2.72	.00067	3.17	
O-1224[b]	3.06	.00057	3.24	
10122[c]	3.25	.00055	3.26	Contains 0.5% of phenol
F-8889	3.82	.0013	2.88	
B-8889	4.32	.0035	2.46	
A-8889	5.11	.0110	1.96	
8902-10	4.92	.0067	2.17	$a-x$ = 0.20 in 240 minutes
8902-9	4.21	.0028	2.55	$a-x$ = 0.51 in 240 minutes
8902-11	3.57	.00097	3.01	$a-x$ = 0.79 in 240 minutes
8902-13	3.21	.00050	3.31	$a-x$ = 0.88 in 240 minutes
8902-C	3.15	.00058	3.23	$a-x$ = 0.87 in 240 minutes
8902-14	2.64	.00093	3.03	$a-x$ = 0.80 in 240 minutes
8902-15	2.15	.0037	2.43	$a-x$ = 0.41 in 240 minutes

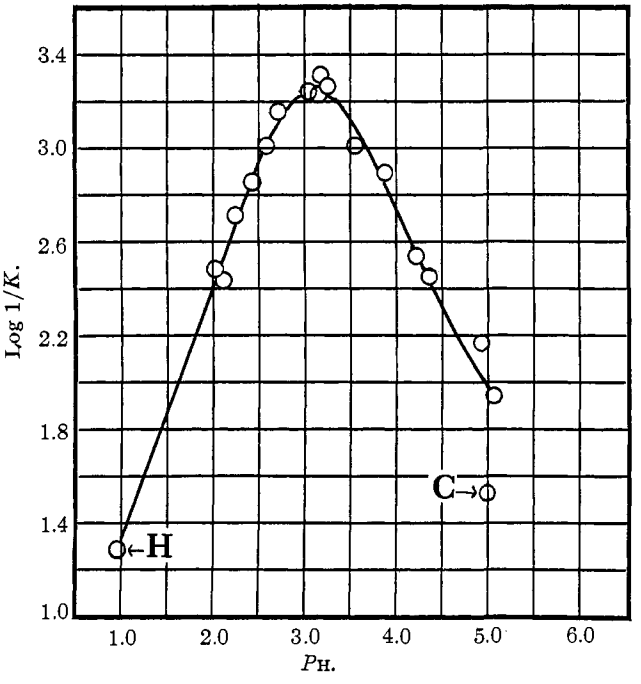
[a] Initial strength of all solutions, 55% U. S. P. X. [b] Method of preparation unknown, 110 U. S. P. X. [c] Initial strength, 100% U. S. P. X.

continuous testing to obtain the results shown in this paper and it is obvious that the actual kymographic records of contractions are too voluminous to be incorporated herein. As a general procedure the equivalent dose for each solution was determined two or three times against the control or standard. Several uterus muscles were usually required. The oxytocic strength was averaged and rounded to the nearest percentage. Under favorable conditions the oxytocic assay method is capable of a relative variation of less than 10%.

Experimental Results

The oxytocic activity remaining after time, t, in minutes has elapsed is expressed as $a-x$ in Tables II and III. K_{100} is the constant for the rate of thermal decomposition at 100° at a given PH and is calculated from the equation $K = (1/t\ 2.3 \log [a/a - x])$. The equation is that of a first order reaction and the terms have their customary significance. A is unity for the control solution. For convenience in plotting Fig. 2, K_{100} is expressed as log $1/k$, increase of which means greater stability. Maximum stability occurs in a rather limited range of PH, *viz.*, 3.0–3.4, or, more specifically stated, pituitary solutions adjusted to PH 3.0–3.4 at 25° are most stable at 100°. These results were again checked on another series of solutions made from a different powder by determining the activity left after 4.0 hours of heating at 100° and the data recorded in Table III under Serial No. 8902–9, etc. All solutions of the series contained 0.01

M NaH_2PO_4 in addition to the usual buffer, acetic acid. The sharp maximum again clearly showed the regulation of the rate of decomposition by the hydrogen-ion concentration. This is the essential factor of stability of pituitary extracts and it is important to note that the oxytocic principle of extracts made from three active powders (see solutions under serial numbers of 8889, 8902, 10122 and a commercial preparation 0–1244 of unknown history and purification) have practically the same stability at P_H 3.0–3.4.



H, Dale and Dudley, Abel and co-workers; C, Adams.

Fig. 2.—Rate of thermal decomposition of the oxytocic principle.

Other factors such as the presence of inert protein material and salts may perhaps alter the rate of decomposition somewhat, although definite information on these factors is not available. We have fragmentary evidence that 0.5 N sodium chloride accelerates at P_H 3.2 the rate of decomposition of the oxytocic principle of the extracts.

0.5 Per cent. phenol evidently does not produce any detectable alteration in the rate of destruction. Chloretone (trichloro-*tert.*-butyl alcohol, $CCl_3COHCH_3CH_3$), however, causes a marked change in the rate as a consequence of formation of free hydrochloric acid from chloretone.[15] To

[15] Chloretone solutions saturated at room temperature were found to be completely hydrolyzed in seventy-two hours at 100° in sealed glass tubes.

illustrate this point: a pituitary solution (about P_H 3.3) which assayed 80% of U. S. P. X. strength and contained 0.5% of chloretone and 0.16 mg. of nitrogen per cc. was heated in sealed ampules for seven and sixteen hours. The oxytocic activity remaining was 53 and 38%, respectively. During the decomposition the reaction of the solution heated for sixteen hours was now found to be about P_H 2.0. As a check, solution No. 10122 (See Table II) was run simultaneously.

Solutions with the initial oxytocic strength of 55, 100 and 110% U. S. P. X. showed the rate of destruction to be independent of concentration or oxytocic strength at a given P_H. This, of course, was predicted if the order of reaction is correct. The same relationship holds for solutions refluxed in 0.5% hydrochloric acid for 0.50 hour in the experiments described by Abel and co-workers and by Dale and Dudley and others. Under these conditions 20% of the total oxytocic strength was still present regardless of the original strength or purity of their preparations.[16] Since there is no indication at present that the order of reaction changes when decomposition occurs in hydrochloric acid, then the constant for the rate of change in 0.50% hydrochloric acid is computed as 0.054 and log $1/k$ = 1.28. The P_H of the acid at 25° is close to 0.95. These values conform nicely to the data of this paper.

On the basis of hydrogen-ion activity these data explain the rapid destruction in relatively low concentrations of hydrochloric acid and relatively high degree of stability in a weak acid like acetic in concentration as high as 6.0%.[7,17] Sharp breaks in the degree of stability, like those shown by Abel and Nagayama, are not to be expected. Several hours of heating at 100° is sometimes necessary to destroy sufficient active material to produce easily detectable differences. This is probably the situation in Adams' work conducted at approximately P_H 3.0 (colorimetric), in which he found no detectable loss after fifty-five minutes of heating at 100°.

In conclusion this paper suggests the importance of investigating the rate of destruction of the pressor principle. Although it seems settled that the rate of decomposition is faster for the pressor principle than the oxytocic in 0.50% hydrochloric acid and at P_H 6.0,[15] yet these data are not sufficient to venture a prediction as to its stability at intermediary acidities or give an indication of the P_H of maximum stability of the pressor principle.

[16] It would be interesting to note the extent to which the rate of thermal decomposition is governed by the removal of inert material. This study has heretofore been handicapped by the lack of suitable methods of purification which would insure reproducible material. A feasible method for purification and practically complete separation of the oxytocic and pressor principle is now available through the splendid work of Kamm and co-workers, THIS JOURNAL, **50**, 573 (1928).

[17] Tate, *Pharm. J.* (*London*), **106**, 486 (1921).

Summary

1. The rate of thermal decomposition at 100° of the oxytocic principle of pituitary solution has been determined between P_H 2.0 and 5.1.

2. The rate conforms to that calculated for a first order reaction.

3. A sharp reproducible maximum in stability occurs at P_H 3.0–3.4.

4. Details of the simple extraction process have been worked out for the oxytocic principle only. Maximum coagulation occurs at P_H 4.8–5.0. The zone is wide enough to allow routine and effective extraction at P_H 4.2–4.5 under the conditions described.

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[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF TRINITY COLLEGE, CONNECTICUT, AND THE UNIVERSITY OF MISSISSIPPI]

THE REDUCTION OF MONONITROPYROCATECHOLS[1]

BY JOHN E. FOGLESONG AND I. L. NEWELL

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The results presented in this communication represent an outgrowth of a project to prepare monohalogen derivatives of pyrocatechol by nitration, reduction, diazotization and substitution. The nitration of pyrocatechol results in two isomeric mononitro compounds

$$1,2\text{-}(OH)_2\text{-}C_6H_3\text{-}NO_2\text{-}3 \quad \text{and} \quad 1,2\text{-}(OH)_2\text{-}C_6H_3\text{-}NO_2\text{-}4$$

which upon reduction form the corresponding amines. It can be readily seen that this offers the possibility of obtaining certain pyrocatechols substituted in either the 3- or 4-position. Since 3-aminopyrocatechol had not been prepared, it seemed profitable to study it and some related compounds in detail.

In their work on aromatic amines, Jacobs and Heidelberger[2] prepared 4-aminopyrocatechol hydrobromide, 4-aminopyrocatechol, and several compounds derived from them. They arrived at the hydrobromide through sulfanilic acid, *p*-sulfophenylazoguaiacol, 4-aminoguaiacol, and finally 4-aminopyrocatechol hydrobromide by demethylation with hydrobromic acid.

Our work will show that the same results can be obtained easily and in good yield by the direct nitration of pyrocatechol, the separation of the isomers and subsequent reduction.

Nitration of Pyrocatechol.—Pyrocatechol was first nitrated by Benedikt[3] using a nitration mixture of potassium nitrate and sulfuric acid. It was not recognized that two isomers were formed and the melting point

[1] Part of the material of this paper has been taken from a thesis submitted by I. L. Newell to the faculty of Trinity College (Connecticut), in partial fulfilment of the requirements for the degree of Master of Science.

[2] Jacobs and Heidelberger, THIS JOURNAL, **41**, 467 (1919).

[3] Benedikt, *Ber.*, **11**, 362 (1878).