TABLE I.
Coagulation Time of Blood.

Case	Type of surface: Glass min	Methyl methacrylate min	Paraffin min
1	7	13	10
2	9	20	27
3	5	9	11
4	5	12	18
5	5	11	19
6	5	14	16
7	7	19	16
8	6	12	18
9	6	11	22
10	7	18	26

Summary. The blood coagulation time in methyl methacrylate (boilable "lucite") tubes was found to be twice as long as the coagulation time in glass tubes. The blood coagulation inhibiting effect of this material follows Lampert's rule of surface adhesion.

13123

Cleavage of the Imidazole Ring of Histidine and Carnosine by Bromine.*

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The literature records no chemical reagent capable of rapidly splitting the imidazole ring of histidine with the liberation of ammonia. This cleavage is readily accomplished by an enzyme, histidase, found in liver.[1, 2] The following data show that a buffered solution of bromine rapidly splits the imidazole ring of histidine, carnosine and other imidazole compounds.

Experimental Procedure. A citrate buffer solution is prepared by dissolving 352.8 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ in ammonia-free water to a final volume of 1200 cc. The pH is adjusted to 1.0 (glass electrode) by adding approximately 315 cc of C. P. HCl, sp gr 1.18.

* Contribution from the Department of Agricultural Chemistry, Missouri Agricultural Experiment Station, Journal Series No. 732.

1 Edlbacher, S., and Kraus, J., *Z. Physiol. Chem.*, 1930, **191**, 225; 1931, **195**, 267.

2 Edlbacher, S., and Neber, M., *Z. Physiol. Chem.*, 1934, **224**, 261.

Liquid C. P. bromine is then added in excess to 100 cc of the buffer solution, the mixture is shaken, and allowed to stand for 12 hours. This saturated bromine solution (approximately 1.0 N bromine) is kept in the dark, and is prepared fresh every fourth day.

To determine the ammonia liberated by bromination, 1 cc of a solution containing 0.5-2.0 mg of imidazole nitrogen is placed in a 25 x 200 mm test tube with 9 cc of the bromine buffer solution. The tube is covered with a 40 x 60 mm test tube and protected from light for a period of 2 hours.† An excess of 20% $Na_2S \cdot 9H_2O$ solution‡ is then added. At the end of 10 minutes a few drops of motor oil are added, the tube is connected with an aeration apparatus, an equal volume of approximately 15 N NaOH solution is introduced, and the alkaline imidazole solution is aerated until it is free of ammonia (30-60 minutes). The ammonia is trapped in 2% H_3BO_3 solution containing methyl red-methylene blue indicator, and is titrated with 0.01 N HCl.[3]

Results. The volatile amine obtained by the above procedure was identified as ammonia by converting it to benzamide; 0.24 g of pure benzamide was recovered from 0.5 g of histidine monohydrochloride. The ammonia was also identified by quantitative colorimetric comparisons with nesslerized ammonium sulfate.

The yield of ammonia from brominated histidine is dependent upon 4 factors: pH of the solution at the time of bromination, concentration of bromine, the period of bromination, and the period of reduction. The influence of each factor was determined separately and the optimum conditions were found to be as follows: pH: 1-1.5, bromine concentration: 0.5-1.0 N, period of bromination: 2 hours or more, and period of reduction: 3 minutes or more. Under these *optimum* conditions, 80(±2)% of the total nitrogen of histidine was recovered as ammonia at every level of histidine monohydrochloride tested (3, 6, 9, and 12 mg).

The ammonia recoveries obtained under these optimum conditions from related imidazole compounds are listed in Table I.

The extent of liberation of ammonia from the imidazole ring is affected by the nature of the side chains attached to the ring. Imidazole itself does not yield all of its nitrogen as ammonia. Methylation

† Tests on histidine show that under these conditions the bromination reaction is 90% complete in 5 minutes, 95% complete in 15 minutes, and 100% complete in 1 hour. At a concentration of 0.5 N bromine, the reaction is 100% complete in 2 hours.

‡ Excess bromine can be removed equally effectively with Na_2S, or by aeration. The use of Na_2S is more convenient.

[3] Sobel, A. E., Yuska, H., and Cohen, J., *J. Biol. Chem.*, 1937, **118**, 443.

TABLE I.
The Ammonia Recovery from Various Imidazole Compounds.

Compound	Quantity used mg	Total nitrogen (theory) mg	Ammonia nitrogen found mg	Recovery of imidazole nitrogen %
Imidazole	4.84	2.00	1.096	54.7
			1.094	
2-methyl imidazole	3.41	1.16	0.632	54.8
			0.640	
4 (or 5)-methyl imidazole oxalate	6.65	1.12*	0.348	30.6
			0.338	
Imidazole lactic acid monohydrate	6.00	0.96	0.962	99.7
			0.973	
			0.937	
Histamine dihydrochloride	4.38	1.00	0.083	13.0
			0.091	
l-carnosine	8.07	2.00	0.897	89.8
			0.899	
l-carnosine + 4.2 mg $CuCO_3 \cdot Cu(OH)_2$	8.60	2.13	1.062	100.6
l-carnosine + 50 mg $CuCO_3 \cdot Cu(OH)_2$	8.70	2.16	1.097	
Cu-carnosine	10.91	1.87†	0.955	100.2
			0.926	
			0.931	
Cu-anserine	11.41	1.88‡	0.273	28.4
			0.261	
Acetyl l-histidine	10.70	2.09	1.286	93.4
	10.90	2.13	1.325	
	9.60	1.87	1.178	
Acetyl l-histidine + 50 mg $CuCO_3 \cdot Cu(OH)_2$	10.10	1.97	1.298	98.8
	10.55	2.06	1.355	
l-histidine monohydrochloride monohydrate	10.10	2.02	1.600	118.9
l-histidine monohydrochloride monohydrate + 50 mg $CuCO_3 \cdot Cu(OH)_2$	10.30	2.06	1.640	118.6
	10.00	2.00	1.580	
			1.570	
Ergothionine	5.45	1.00	0.198	31.2
			0.219	

*Total nitrogen found (micro-Kjeldahl). Theory for $C_4H_6N_2 \cdot H_2C_2O_4$ = 1.08 mg.

†Total nitrogen found (micro-Kjeldahl). Theory for $C_9H_{14}O_3N_4 \cdot CuO$ = 2.00 mg.

‡Total nitrogen found (micro-Kjeldahl). Theory for $C_{10}H_{16}O_3N_4 \cdot CuO$ = 2.00 mg.

of the 2-position has no apparent effect on ammonia recovery, whereas methylation of the 4 (or 5) position decreases it. A marked decrease is also observed when the β-ethyl amine group is in the 4 (or 5) position of the ring (histamine). On the other hand, all of the ring nitrogen is recovered as ammonia when a lactic acid group is in the 4 (or 5) position of imidazole (imidazole lactic acid).

l-Carnosine and acetyl l-histidine yield 7-10% less ammonia than the values calculated for complete cleavage of the imidazole ring.

However, if cupric ion is added at the time of bromination, the ammonia recovery is 100%. The action of cupric ion may be similar to its well-known catalytic effect on Kjeldahl digestions.

Under optimum conditions of bromination, histidine yields 2.4 moles of ammonia per mole of compound. Attempts to modify the experimental conditions so as to recover either exactly 2 or 3 moles of ammonia have never been successful, and cupric ion has no effect upon ammonia recovery (Table I). The additional 0.4 mole of ammonia obtained from one mole of histidine may be derived from a partial cleavage of the alpha-amino group.

Summary. When histidine and related compounds are treated with bromine at pH 1 to 1.5, the excess bromine removed, and the solution alkalinized and aerated, significant amounts of ammonia are recovered from the imidazole ring. Copper carnosine, copper acetyl histidine, and imidazole lactic acid yield amounts of ammonia which correspond to a complete cleavage of the ring nitrogen. Histidine yields sufficient ammonia to account for all of the ring nitrogen plus almost one-half of the alpha-amino nitrogen. Other compounds tested gave 13-55% of their imidazole nitrogen as ammonia.

13124

A Simple Method for the Bioassay of Renin.

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In connection with work on hypertension it was of importance to have a simple method for testing a large number of extracts for their renin content. Rabbits were used for the test as their blood pressure is conveniently measured in the artery of the ear by a membrane manometer.[1]

After several preliminary trials a procedure was standardized, which, despite its simplicity gives reliable and reproducible results, as accurate as one would expect from a bioassay.

The test was carried out in a warm room. The unanesthetized animals were placed in a box with open front and top; after some

[1] Grant, R. I., and Rothschild, P., *J. Physiol.*, 1934, **81**, 265.