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.

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A Simple Method for the Bioassay of Renin.

OTTO SCHALES AND FLORENCE W. HAYNES. (Introduced by Soma Weiss.)

From the Medical Clinic, Peter Bent Brigham Hospital, and the Department of Medicine, Harvard Medical School, Boston.

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.

training they would sit quietly. The left ear was placed between the capsule and light of the manometer without forcing it away from its natural position. Measurements of the blood pressure were taken at about ½-minute intervals; after a few minutes the readings were constant, fluctuating not more than one millimeter from the average level. After a constant level had been maintained for at least 3 minutes, the extract was injected intravenously in the right ear. The introduction of the needle had no effect on the blood pressure, as the right ear had previously been denervated. Injection of 5 cc of physiological saline solution also had no effect on the blood pressure.

The injection time chosen was 60 seconds, but practically the same results were obtained if the injection was made in 30 seconds. When the injection was started, measurements of the blood pressure were done in rapid succession, about 3 per minute. The maximum response to renin was usually obtained about 3 minutes after the start of the injection. For each extract 4 animals were used and amounts of renin were injected which would cause a rise in blood pressure of 20 to 40 mm mercury. If the potency of an extract was unknown, this amount was first determined approximately by an experiment with one animal. The volume of isotonic solution injected (0.9% NaCl) was kept between 1 and 2 cc per rabbit.

From the data on 4 animals the amount of renin was calculated (as micrograms nitrogen) which had to be given per kilogram of rabbit to cause an average rise in blood pressure of 30 mm. We call this amount one rabbit unit (R.U.). It is the number derived from the average response of 4 animals and its calculation is justified by our finding that the response in the range of 20 to 40 mm pressure rise is approximately proportional to the amount of renin given. This straight linearity is not present in the range above 40 mm as one can see in the dose-response curve by Pickering and Prinzmetal.[2] With increasing purity of an extract the amount of nitrogen equivalent to one rabbit unit will decrease as less inert proteins are present and the method offers therefore a simple way to judge the success of any attempt to purify renin. The degree of purity can be expressed as the number of rabbit units per milligram of nitrogen. Independent of its purity the concentration of renin in an extract may be expressed as the number of units per cc.

The following data illustrate the assay procedure. Table I shows the bioassay of an extract, repeated after an interval of 2 days. To calculate one rabbit unit, the sum of micrograms of nitrogen given

[2] Pickering, G. W., and Prinzmetal, M., *Clin. Sc.*, 1938, **3**, 211.

TABLE I.
Extract N55 (diluted 1:25, 1 cc equals 41.6 μg nitrogen).

Rabbit No.	μg N_2 given per kg 2/18/41	Rise in B.P. mm Hg	μg N_2 given per kg 2/20/41	Rise in B.P. mm Hg
42	50.8	27		
59			46.0	18
64			50.3	37
65	30.0	18	48.5	33
66	45.5	28		
68	39.6	27	44.4	30
	165.9	100	189.2	118

$$1 \text{ R.U.} = \frac{165.9 \times 30}{100} = 49.8 \ \mu g \ N_2/kg. \qquad \frac{189.2 \times 30}{118} = 48.1 \ \mu g \ N_2/ \ kg.$$

$$1 \text{ cc (undiluted)} = \frac{1040}{49.8} = 20.9 \text{ R.U.} \qquad \frac{1040}{48.1} = 21.6 \text{ R.U.}$$

per kilogram is divided by the sum of the rises in blood pressure and multiplied by 30.

Table II shows the result of the bioassay of 2 preparations of renin, C_1 and C_2, made according to the method of Helmer and Page[3] for their fraction C. From the statement of the authors one can conclude that an amount of this fraction containing 93 μg of nitrogen caused a rise in blood pressure of 30 mm or more per kilogram of dog. The findings in Table II confirm the statement of Hessel[4] that about the same amount of renin was necessary per kilogram to produce the same rise in blood pressure in rabbits as in dogs.

The accuracy with which this method can be used to determine yields can be seen from the following example:

TABLE II.
Extract C_1 (diluted 1:50, 1 cc equals 78.4 μg nitrogen).
" C_2 (" 1:50, 1 " " 93.6 " ").

Rabbit No.	Extract C_1: μg N_2 given per kg	Extract C_1: Rise in B.P. mm Hg	Extract C_2: μg N_2 given per kg	Extract C_2: Rise in B.P. mm Hg
52	125	34	127	42
55	127	40	127	36
56	130	21	126	32
57	130	31	130	20
	512	126	510	130
1 R.U.	122 μg N_2		118 μg N_2	

[3] Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.
[4] Hessel, G., *Klin. Wchnschr.*, 1938, **17**, 843.

A solution containing 12400 rabbit units was fractionated and the resulting precipitate was found to contain 650 R.U. The assay of the filtrate showed it contained 11700 R.U. This is a total recovery of 99.6%. In working with small quantities of concentrated solutions, small unavoidable losses are incurred in the various procedures. For example, a solution containing 3100 R.U. gave 2 fractions with 2740 and 10 R.U. The recovery in this case was 88.7%.

The simple method for the bioassay of renin, suggested in this paper, will make it possible to give data concerning yield and degree of purity for each step of the various chemical procedures involved in the preparation of renin.

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Distribution of Body Weight in the Organs and Tissues of the Rabbit.*

CHARLES J. LEVINE, WALTER MANN, HAROLD C. HODGE, I. ARIEL AND O. DUPONT.

From the Departments of Radiology and Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

In the study of the distribution of radioactive isotopes in rabbits, it is usually convenient to prepare for the counting procedures, 1 or 2 g or less of the various tissues. From this sample, the percentage of the isotope dose per gram of tissue may be calculated. However, to account for all of the isotope dose, it becomes necessary to know the total amount of muscle, brain, bone and other tissues in the experimental animal. Such data are not available in the literature.

Procedure. The animals were adult, male, New Zealand albino rabbits in good health which had been for several months on our stock ration of oats, alfalfa and lettuce supplemented by chow.

Immediately upon sacrifice, the skin was removed from the body. Considerable subcutaneous fat adhered to the skin and was not separated from it.

The viscera weight included the usual viscera, the salivary glands, larynx, perirenal fat and also the weight of blood removed in the blood volume determinations.

All the adhering soft tissues were removed from the skeleton. As

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