11363

A Modified Method for the Preparation of Renin.

W. D. COLLINGS,* J. W. REMINGTON, H. W. HAYS AND V. A. DRILL. (Introduced by W. W. Swingle.)

From the Biological Laboratory, Princeton University, Princeton, N. J.

Partially purified renin has been prepared in several laboratories from NaCl or cold acetone extracts of the kidney cortex.[1-4] Recently modifications in our original extraction procedure[2] have been introduced which eliminate many of the more cumbersome manipulations involved, with considerable saving of time, without sacrificing the relative high potency of the final product.

Extraction Procedure. Demedullated kidneys are ground, frozen, and, before thawing, reground into 2% NaCl solution (10 liters to 3 kg tissue). It is possible to use fresh, unfrozen kidneys, but the extraction appears to be less complete. After the salt extract has been stored for 24 hours under toluene, the meat sludge is removed by straining and centrifuging (Sharples). The pH is then lowered to 4.5. After an interval of 12 to 24 hours to insure complete precipitation, the heavy precipitate is removed by centrifuging (Sharples) and filtering through Hyflo Super-Cel.† For easier handling in subsequent procedures, the filtrate, adjusted to pH 6.8, is concentrated *in vacuo* (maximum temperature 45°C) to a volume of 1 liter. The concentrate is filtered, 100 g NaCl are added and the pH is lowered to 2.0. The heavy precipitate is removed on a filter cake of Hyflo which is then suspended in 2 liters of water. After the pH is raised to neutrality and the suspension thoroughly mixed by a motor stirrer for about 30 minutes, Hyflo and any insoluble precipitate are filtered off and discarded. The filtrate is saturated with solid NaCl and the pH again lowered to 2.0. The precipitate is removed on a filter cake and redissolved in 500 cc N/10 acetate buffer at pH 5. As before, insoluble residue is discarded. At this stage two lots of extract (each representing 3 kg kidney cortex) are combined in the same 500 cc of buffer solution. Precipitation with

* E. R. Squibb and Sons Fellow in the Biological Sciences.

1 Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.

2 Swingle, W. W., Taylor, A. R., Collings, W. D., and Hays, H. W., *Am. J. Physiol.*, 1939, **127**, 768.

3 Hessel, G., *Klin. Woch.*, 1938, **17**, 843.

4 Hill, J. R., and Pickering, G. W., *Clin. Sci.*, 1939, **4**, 207.

† Celite Filter Aid, Johns-Manville Company, New York.

solid ammonium sulfate at 0.4 saturation is made 5 successive times, with the volume of buffer solution reduced at each step, *e. g.,* 500, 400, 300, 200, and 100 cc. The first 4 precipitates are removed on filter cakes, the 5th is removed by centrifuging. The discarded supernatant should be color-free. When the final precipitate is not easily soluble in 40-70 cc water, to give a clear, light amber solution, ammonium sulfate precipitations are repeated from a volume of 50-100 cc. It is important that the volume of buffer solution be kept small, since inactive less soluble globulins are left behind at each filtration. The extract is now dialyzed at 6°C against distilled water. We have found that any precipitate forming in the dialyzer can be discarded as inactive. The final volume of 60-100 cc should have 10-15 mg solids per cc. Where the total solids are higher, further ammonium sulfate precipitations from the dialyzed solutions are usually made. The recovery is not quantitative, but inactive solids are often eliminated by this step.

All pH adjustments are made by 10% HCl or 10% NaOH and are measured with a glass electrode. Stock reagents are kept in the refrigerator, and care is taken to keep the extract chilled as much as possible throughout its preparation.

Dialysis. A circulating dialyzer[5] in the refrigerator is usually employed. However, no difference in the activity of the final product has been observed when the slower, standing dialysis (cellophane bag in a cylinder of water) is substituted. There is apparently no loss of potency, in the cold, even though dialysis is prolonged 3 to 4 days.

Stability. The extract is stored either frozen or in the lyophile state. The latter material is prepared by the Cryochem process[6] and stored in the refrigerator. It has shown full activity when tested as much as 10 months after preparation. Frozen extract, especially if melted and refrozen repeatedly, shows a gradual loss of activity.

Preparation of Sterile Renin. The extract can be sterilized by filtering through a Jena glass filter and then rendered lyophile in sterile vials. A Seitz filter adsorbs all the active protein from the acid solution.

Chemical Properties. Renin, as prepared by the method described above, is a pseudo-globulin and is precipitated by 0.38 to 0.41 saturated ammonium sulfate at pH 5, by saturated NaCl, by 0.7 to 1.0 saturated magnesium sulfate and by the various protein precipitants.

[5] Taylor, A. R., Parpart, A. K., and Ballentine, R., *Ind. and Eng. Chem.*, 1939, **11**, 659.

[6] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1938, **34**, 469.

TABLE I.
Some Color Reactions of Renin.

Reaction	Reactive group	Result
Biuret	peptide linkage	+
Millon	tyrosine	+
Xanthoproteic	benzene nucleus	+
Hopkins-Cole	tryptophane	+
Sullivan's	cysteine, cystine	—
Ehrlich-diazo	histidine, tyrosine	+
Sakaguchi	arginine (guanidine)	+
Molisch	carbohydrates	—
Benzidine	pentoses	—

It will form a picrate and is destroyed rapidly by boiling and by protein denaturants. It is free from carbohydrate and responds to routine color tests as shown in Table I. The test for –SH groupings was negative in both native and denatured (boiled) protein and was absent also after treatment with NaCN. However, renin shows the presence of sulfur after sodium fusion.

Yield. The yield in total solids per kg kidney cortex is fairly constant at 150-180 mg. The variability in activity, however, is large, for reasons at present not known. The renin unit[2] has been defined as the amount of material, per kg body weight, which will produce a 40 mm rise in the mean blood pressure of the anesthetized dog. In the assay standard, 1 unit was the equivalent of 0.1 mg renin. In 39 successive lots of extract, the rise given by 0.1 mg renin, per kg body weight, has been between the extremes of 13 and 62 mm, with the mean at 34 mm. In other words, the average yield was 1450 units per kg fresh cortex. In our most potent extract (Table II) 1 unit represents 9.6 μg nitrogen.

TABLE II.
Renin Assay at Dosage of 0.1 mg per kg Body Weight.

Dog No.	1	2	3	4	5	6	7	8	9	10	11	12	Avg
Body wt, kg	9.7	9.5	13.1	12.5	7.5	9.5	7.6	10.7	12.5	11.6	12.6	9.4	10.5
Initial B.P.,* mm Hg	108	114	115	103	88	89	122	93	146	95	125	96	108
Peak B.P., mm Hg	175	165	177	209	174	139	178	139	199	156	172	157	170
Rise in B.P., mm Hg	67	51	62	106	86	50	56	46	53	61	47	61	62.2

*B.P. readings are mean pressures obtained by intra-arterial needle puncture.