radiated, cholesterol-fed rats not administered this supplement.

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Molecular Weight of Human Renin.* (31636)

BERT WARREN AND MARION DOLINSKY (Introduced by S. Levine)
Department of Pathology, New York Medical College Center for Chronic Disease, Bird S. Coler Hospital Division, Welfare Island, New York City

Whitaker first noted a linear correlation between the logarithm of the molecular weight of a protein and the ratio of its elution volume to the column void volume using G-75 and G-100 Sephadex(1). This work was extended by several investigators using G-75, G-100 and/or G-200 Sephadex to determine the molecular weights of proteins ranging from 13,000 to 225,000(2,3).

As renin has not been obtained in pure form, classical molecular weight (m.w.) determinations have not been possible. However, with the introduction of gel-filtration it proved possible to determine the molecular weight of a protein in a crude preparation (3). Gel-filtration has already been applied to *hog* renin, which had an elution volume between that of ^{125}I labelled human albumin (m.w. 69,000) and pepsin (m.w. 35,000) and was estimated as having a molecular weight between 42,000 and 49,000(4). The present study applied the method of Whitaker(1) toward the determination of the molecular weight of *human* renin.

Methods. Proteins of known molecular weight. Thyroglobulin: porcine, water soluble, lot 1923-60. Catalase: beef liver, slightly soluble, lot 45B-0440. Gamma-globulin: bovine, Cohn fraction II, lot 15B-2920. Serum albumin: human, grade III, lot 65B-1630. Ovalbumin: grade V, lot A102B-250. Pepsin: 2× crystallized, lot 15B-1370. All proteins used were obtained from the Sigma Chemical Co., St. Louis, Mo.

Human renin. The dog unit (DU) of renin is defined as that quantity of renin required to raise the mean femoral blood pressure of an unanesthetized dog by 30 mm Hg(5). Renin, of a specific activity of 0.5 DU/mg protein was prepared from fresh-frozen human kidneys by the method of Dexter, Haynes and Bridges(6), followed by lyophilization and gel filtration on G-75 Sephadex. Final preparations were lyophilized and stored un-

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til used. Assay was performed in dogs as previously described(7).

Column preparation. Sephadex G-200, lot To 5822, was obtained from Pharmacia, Uppsala, Sweden. Chromatography was performed in a sintered glass bottomed column that had an inner diameter of 1.85 cm and was packed to a height of 105 cm using approximately 9 g of G-200 Sephadex. A citrate buffer was used to develop the column. (21 g of citric acid, $C_6H8O_7 \cdot H_2O$, dissolved in 200 ml of N NaOH and diluted with 500 ml of distilled water, was diluted with an equal volume of H_2O before use and was adjusted to exactly pH 5.0 with small amounts of N HCl or N NaOH.) After removal of fine particles by decantation, the gel was again suspended in excess citrate buffer and was permitted to stand for 3 days at 4°C to allow the gel to swell. The slurry was then stirred to remove entrapped air and an initial quantity of suspended gel was carefully poured down the side of the plugged column. 5-10 cm of gel was permitted to settle before the plug was removed and the remaining slurry was added. Chromatographic development was performed at 4°C. The flow rate was less than 0.1 ml/min per cm^2 cross-section of the column (about 12-14 ml/hour).

Sample preparation. 1-10 mg of each protein was dissolved in 1-2 ml of buffer and was filtered if necessary. Beef liver catalase was found to be poorly soluble. Therefore, catalase preparations were mixed with buffer, stored overnight at 4°C, stirred for 15 minutes and centrifuged to remove insoluble particles before being utilized. The concentration that could be obtained for catalase was very low. Therefore, elution peaks for catalase were determined by spectrophotometry and verified by observing the tubes that evolved oxygen most rapidly when treated with 1% H_2O_2.

Chromatography. 1-2 ml of protein solution was slowly added down to the column surface to minimize disturbance of the gel. After the solution entirely entered the gel and before establishing a column head and connecting the buffer reservoir, the surface was washed with 2 additional ml of buffer. Fractions of 2-3 ml were collected by means of a Misco fraction collector. The volume of each fraction was measured in order to determine accurately the elution volume of each protein. Protein concentration of the fractions was determined at 280 mμ in a Hitachi-Perkin Elmer 139 spectrophotometer. Elution volumes were obtained by graphic interpolation. Thyroglobulin which is excluded by G-200 Sephadex was used to establish the void volume of the packed column. The void volume was determined before, once in between and after the other proteins were run.

TABLE I. Protein Molecular Weights as Determined by Gel-Filtration on G-200 Sephadex.

Protein	V/V_o	m.w. calculated from equation	m.w. from literature*
Pepsin	2.511 2.490	35,700	35,500
Human renin	2.421 2.412 2.409 2.409	42,300	—
Ovalbumin	2.380 2.375	45,300	45,500
Human serum albumin	2.165 2.154	70,000	68,900
Bovine gamma globulin	1.680 1.666	175,000	170,000
Catalase	1.556 1.538	223,000	225,000
Thyroglobulin	1.000	638,000	650,000

Elution at 4°C with pH 5.0, 0.1 M citrate buffer.

* The molecular weights of the reference proteins used were those applied by Leach and O'Shea (8).

Results. The molecular weights of the reference proteins used were those applied by Leach and O'Shea(8), (Table I). As expected from previous studies using this method(1,2, 3,8), a straight line was obtained when the logarithms of the molecular weights of the reference proteins were plotted against the ratios of their elution volumes, V, to the void volume V_o. The linear plot used in Fig. 1 was determined by the method of least squares. The slope of the line and the intercept were then used in the form of the equation determined by Whitaker(1). The equation found for this system was: log m.w. $=$ $-0.834\ (V/V_o - 1) + 5.805$. The greatest difference in duplicate determinations of values for $V/V_o - 1$ of the proteins used

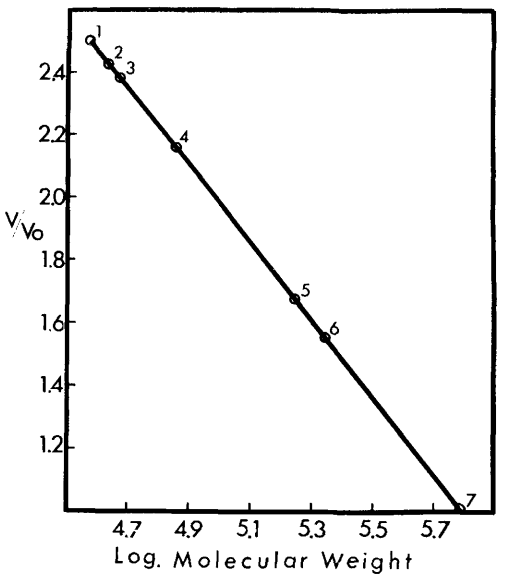

FIG. 1. Relationship between the ratio of the protein elution volume (V) to column void volume (Vo) and the log. of the protein molecular weight. 4°C, pH 5.0, 0.1 M citrate buffer. 1 = pepsin; 2 = human renin; 3 = ovalbumin; 4 = human serum albumin; 5 = bovine gamma globulin; 6 = catalase; 7 = thyroglobulin.

was about 2%. The elution volume of thyroglobulin (m.w. 650,000), that was used to determine the void volume of this system, was 88.2 ml and ranged from 87.8-88.4 ml (S.D. ± 0.28 ml).

The elution volume of renin was determined 4 times. Peak activity of the enzyme was found at an elution volume of 212.8 ml and ranged from 212.5-213.5 ml (S.D. ± 0.41 ml). The molecular weight of human renin as determined by this method was 42,300 (Table I, Fig. 1).

Assuming that this method of molecular weight determination is valid for renin, a close correlation was found between the molecular weights of human renin (m.w. 42,300) as was determined in this study and hog renin (m.w. 42,000-49,000) as determined by Kemp and Rubin(4).

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Effects of Lanthanide Chlorides on Selected Enzymes. (31637)

VIRGINIA ZEWE HOLTEN, GRANVIL C. KYKER, AND MARY PULLIAM*
Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge Associated Universities, Inc., Oak Ridge, Tenn., under contract with Atomic Energy Commission

For the past several years the physiological effects of intravenous injection of rare-earth chlorides into rats have been investigated in this laboratory. The most prominent biochemical effect is the reversible development of the fatty liver, which reaches a maximum in 48-72 hours and returns to normal within a week. The increased hepatic fat is essentially due to neutral triglycerides, while phospholipids remain normal(1). Cholesterol and plasmologen levels parallel the increase in neutral lipids(2). In general, female rats appeared to be more susceptible than males but hormonal stresses such as testosterone injection into females, hypophysectomy in both males and females, and adrenalectomy in males can prevent fatty infiltration of the liver(1). This fatty liver is also prevented in susceptible species(3) by chelation (EDTA) or aggregation (hydroxyl ion) of

* Student trainee from Birmingham Southern College, Birmingham, Ala.