40 ml of 0.1 N hydrochloric acid and 0.2 g of 10 % Pd on carbon powder catalyst was hydrogenated, and V isolated as described under (a). Hydrogen absorption: 1 mole. Yield: 0.62 g; m.p. 124.5–125.5°, unchanged after recrystallization from isopropanol. The IR spectrum in KBr pellets was identical with that of IV prepared as described under (a). (Found: C 72.19; H 8.35; N 5.97).

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Molecular Weight of Renin Determined by Sephadex Gel-filtration

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The molecular weight of the kidney enzyme renin is unknown. It has not been possible to determine the molecular weight because renin has still not been prepared in a purity allowing "classical" molecular weight determination. With the introduction of Sephadex, molecular weight determination by gel-filtration seems possible, even when the substance (*i.e.* renin) for which the molecular weight is sought, is applied to the column in a crude preparation.[1]

In order to correlate the molecular weight of renin with other substances with known molecular weight, three "tracer" substances were employed: (1) ^{131}I-labelled human γ-globulin with a molecular weight of 160 000,[2] (2) ^{125}I- or ^{131}I-labelled human albumin with a molecular weight of 69 000,[3] and (3) recrystallized pepsin with a molecular weight of 35 000.[4]

Two preparations of renin were applied to gel filtration: (1) Goldblatt-renin, step V (Biochemical Nutritional Company), and (2) a hog renin preparation made by acetone-extraction of freeze-dried, ether fractionated kidney powder followed by kaolin adsorption and ammonium sulphate fractionation. Renin was estimated according to the method of Skeggs, Kahn, and Marsh.[5]

Two types of Sephadex were employed: G-200 and G-100, and two different column sizes, *viz.* one with a diameter of 21 mm and a length of 800 mm and another with a diameter of 13 mm and a length of 1050 mm. Elution was performed with a 0.5 M phosphate buffer, pH 6.0. Most of the runs were performed at 4°C but some at 20°C.

Until now 17 runs have been done. In all these experiments we have found the same pattern of elution of renin (and "tracer" elements), independent of renin preparation (Goldblatt-renin or our own preparation), type of gel, column size, number of "tracers" in one run *etc.* Renin is eluted between albumin and pepsin.

On the assumption that this method is applicable to renin preparations the molecular weight of renin is — determined by interpolation — between 42 000 and 49 000.

We are now performing experiments with other tracer substances in order to make a more detailed study of the molecular weight of renin.

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