They appeared as round blebs of cytoplasm containing a few ribosomes and a single compact bundle of smooth endoplasmic reticulum (SER), sometimes enveloping a microbody (Fig. 2). Similar blebs were occasionally observed in control animals and treated males, but these usually contained degenerating mitochondria and cytosomes. Isolated, spherical masses containing a single bundle of SER were noted in the apical cytoplasm of pars recta cells from affected kidneys (Fig. 3).

In this study, the glomeruli of all animals appeared structurally normal. No alteration of epithelial cell foot processes, basal lamina, or capillary endothelium was noted. This observation indicates that the animals were not losing abnormal amounts of serum protein; rather, it suggests that any increased protein excretion was the result of cytoplasmic blebs extruded from proximal tubule cells.

The presence of these cytoplasmic blebs in this portion of the nephron is significant in light of the known conversion of organo-mercury compounds to inorganic mercury in the kidney (*1*) and the described toxic effect of inorganic mercury (that is, mercuric chloride) on the pars recta (*8*). Mercury derived from CH_3Hg has been reported to concentrate in the microsomal fraction of rat kidney (*9*). Microsomal enzyme systems are closely associated with the SER (*10*). The SER is a logical site for the conversion of CH_3Hg to inorganic mercury because of its detoxification activity. Cleavage of the carbon-mercury bond would release inorganic mercury, which could react with microsomal enzymes as a noncompetitive inhibitor. Mercurials strongly inhibit liver enzymes of sterol biosynthesis and these enzymes are present in the microsomal fraction (*11*).

If mercury inhibits these enzyme systems in the kidney the selective extrusion of SER bundles by pars recta cells of female animals may represent the removal of nonfunctional organelles through the process of potocytosis (exocytosis) (*12*). The more prominent effect of CH_3Hg on the pars recta of female rats in comparison to males is probably due to known sex differences in the activities of kidney enzymes (*13*). Enzymes responsible for the metabolism of organic or inorganic mercury in female rat kidneys may be less efficient than those of males.

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Improved Flow Rates with Porous Sephadex Gels

Abstract. *The use of an internal support of siliconized glass beads 6 millimeters in diameter was found to improve markedly the flow rates obtainable with porous Sephadex gels without significant alteration of other properties of the gels. The method may be generally applicable in situations where rapid separations on Sephadex are required.*

The speed with which separations can be performed on porous Sephadex gels is severely limited by the development of compression in the gel bed and the consequent diminution of flow rate when the operating pressure head is increased (*1*). We report here a simple modification of column design which greatly increases the maximum flow rates obtainable with such gels without significant impairment of resolution. The modification consists in providing the gel with an internal support of glass beads which enables the gel to withstand much greater operating pressures without being compressed. The "bead column" thus operates in a fashion similar to a series of very short columns linked end to end.

Any conventional column used for gel filtration can be converted to a bead column provided that the column diameter is greater than 1.5 cm (a limit imposed by the diameter of the glass beads). One fills the column about half full with solvent and then adds a sufficient number of glass beads to fill the column to the desired height, taking care to add the beads slowly enough so as not to trap any air between the beads as they settle through the solvent. Solid 6-mm glass beads (Propper Manufacturing Company) provide the optimum support. Smaller beads cause difficulties in the packing of the gel, whereas larger sizes cause impaired resolution and are not as effective in preventing gel compression. The beads are siliconized with Siliclad (Clay Adams) before use.

We then allowed the swollen Sephadex gel to pack between the glass

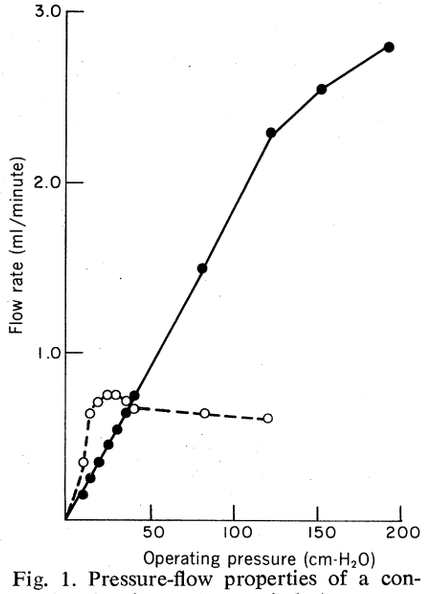

Fig. 1. Pressure-flow properties of a conventional column (open circles) as compared to those of a bead column (solid circles); both columns were of the same diameter and were packed with the same quantity of Sephadex G-200 (the bed of the bead column being approximately twice as long as that of the conventional column).

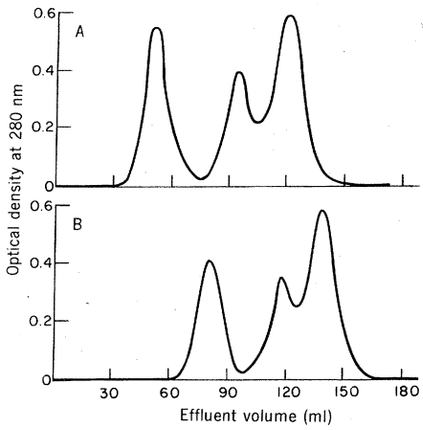

Fig. 2. Separation rates of the solution components (from left to right) goat gamma globulin, bovine pancreatic ribonuclease, and riboflavin on (A) a conventionl column and (B) a bead column. The absorbance (optical density at 280 nm) of the column effluents were monitored continuously with a Uvicord and recorder (LKB). Both flow and chart speed for the bead column were set at three times the corresponding values for the conventional column.

beads by adding it in portions to a reservoir vessel attached to the top of the column. The column is packed with gel to approximately 2 cm above the level of the glass beads. The most uniform packing is obtained by the use of a constant flow pump attached to the effluent line from the column and set to run at the desired operational flow rate for the particular column being prepared. In general, the flow can be set at a value three times the maximum flow that would be obtainable by conventional operation of a column of the same diameter containing the same quantity of gel. Uniform packing can also be obtained without the use of a pump, but this requires careful attention to the height of the outflow line so as to maintain a reasonably constant flow throughout the packing operation. Sephadex G-100, G-150, and G-200 and Sepharose 2B and 4B (Pharmacia Fine Chemicals, Inc.) have all been used successfully in bead columns. Once packed, the bead column can be used in the same manner as conventional columns but with greater operational pressure heads.

In order to compare the properties of the bead column to a conventional column, two columns of the same diameter (2.5 cm) were each packed with the same amount of swollen Sephadex G-200 (130 ml). The bead column contained an internal support of 6-mm glass beads whereas the conventional column was packed in the same fashion but without glass beads. In Fig. 1 the presure-flow properties of these two columns are compared. The flow through each column was allowed to stabilize for several hours after each increment of pressure head before flow was measured. At the highest pressure head employed (limited by the height of our ceiling) the flow rate through the bead column was still rising with pressure increments while the conventional column had long since reached its maximum flow rate and had begun to compress.

Two milliliters of a solution containing goat gamma globulin (4 mg/ml), bovine pancreatic ribonuclease (6 mg/ml), and riboflavin (0.1 mg/ml) were applied to each of these columns in order to compare their separative properties. The pressure head of the bead column was set to give a flow rate approximately three times the maximum flow rate of the conventional column. The separations achieved on the two columns are shown in Fig. 2. The void volume of the bead column was greater than that of the conventional column by about 30 ml, indicating tighter packing of the gel in the conventional column. As seen in Fig. 2, the resolutions achieved on both columns were similar.

Another Sephadex G-200 bead column of smaller dimensions (diameter, 1.5 cm; bed volume, 35 ml) was calibrated with a series of molecular weight standards. The results, plotted in Fig. 3, are analogous to those that have been reported for conventional columns (*2*), again indicating that there is no qualitative difference in the function of the gels when used in bead columns. At an operating pressure head of 50 cm of water the flow rate of this column was 1.1 ml/min, thus making possible a molecular weight determination for an unknown in about 30 minutes.

In addition to saving time in routine separations and in molecular weight determinations, the bead columns have facilitated work with labile proteins for which the time needed for separation becomes a critical factor in final yield (*3*). The method has also proved applicable to long preparative Sephadex and Sepharose columns and to columns of chemically modified Sepharose 4B used for affinity chromatography (*4*).

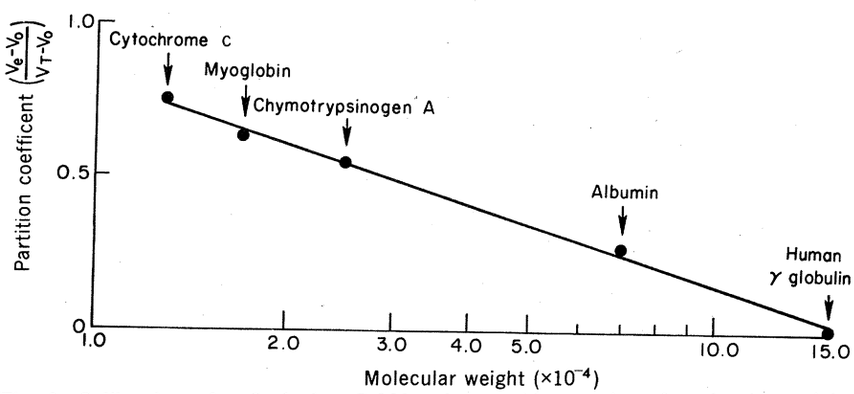

Fig. 3. Calibration of a Sephadex G-200 column with a series of molecular weight standards; 1 ml of a solution (5 mg/ml) of each of the proteins shown was passed through the same bead column in separate runs. The partition coefficient for each protein is plotted against the logarithm of its known molecular weight; V_e, effluent volume of a partitioning solute; V_o, void volume of the column; V_T, total volume of the gel bed.

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