TABLE III. Contrast Between IC Injections at Two Dose Levels.

Rabbit	Maximum T_r (°C) .1 ml	.2 ml	Fever index 300 min (cm²) .1 ml	.2 ml
1	2.2*	2.6	114*	128
2	2.3	2.0	119	104
4	2.2	2.5	106	124
6	2.0†	2.5	89†	130

* Mean of 5 injections—see Table II.
† Mean of 2 injections—see Table II.

(Table III), and we suggest that the log dose fever response curve for LP is dependent upon route of administration. Since similar i.v. doses produced similar fevers in our study (Table I) and the one cited(4), it is unlikely that the higher fevers found with i.c. injection are due to a greater pyrogenicity of our LP.

Although our data clearly indicate that LP has a site of action somewhere in the CNS, they do not locate the site more precisely. The observation that inhibition of heat loss mechanisms precedes the initial rise in rectal temperature is consistent with a direct effect of pyrogens on the medullary-pontine motor centers(5,6), but is also compatible with an indirect effect on these centers secondary to a direct effect on the hypothalamic temperature regulating centers or on postulated pyrogen "receptors"(10) in the CNS.

Summary. Intracisternal injection in rabbits of endogenous (leucocytic) pyrogen prepared from rabbit leucocytes *in vitro* causes a high and sustained elevation of body (rectal) temperature, accompanied by prolonged depression of heat-loss mechanisms (panting and ear blood flow). In paired experiments, the same dose of endogenous pyrogen always caused a much greater increase of body temperature when given intracisternally than when given intravenously. The evidence strongly supports an hypothesis that endogenous pyrogen has a primary site of action in the central nervous system.

We wish to record our gratitude to Dr. Jean Mayer, in whose laboratory this work was done, for his guidance and support and to Dr. Ronald Grant for reviewing manuscript.

1. Atkins, E., Physiol. Rev., 1960, v40, 580.
2. Bennett, I. L., Jr., Beeson, P. B., J. Exp. Med., 1953, v98, 477.
3. Bennett, I. L., Jr., Petersdorf, R. G., Keene, W. R., Trans. Assn. Am. Physicians, 1957, v70, 64.
4. Bornstein, D. L., Bredenberg, C., Wood, W. B., Jr., J. Exp. Med., 1963, v117, 349.
5. Chambers, W. W., Koenig, H., Koenig, R., Windle, W. F., Am. J. Physiol., 1949, v159, 209.
6. Grant, R., ibid., 1949, v159, 511.
7. Grant, R., Whalen, W. J., ibid., 1953, v107, 291.
8. King, M. K., Wood, W. B., Jr., J. Exp. Med., 1958, v107, 291.
9. Petersdorf, R. G., Bennett, I. L., Jr., ibid., 1957, v106, 293.
10. Sheth, U. K., Borison, H. L., J. Pharmacol. and Exp. Therap., 1960, v130, 411.

Received March 1, 1965. P.S.E.B.M., 1965, v119.

Molecular Size of Rat Urinary Protein. (30265)

J. S. FINLAYSON AND H. P. MORRIS
Division of Biologics Standards and Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Since the observation that most of the protein of normal rat urine has the electrophoretic mobility of serum globulin(1), many publications have contained the assumption that the normal urinary proteins of the rat have molecular weights equal to or greater than that of serum albumin(2-5), which is 65,000(6,7). The present work demonstrates that a considerable portion of the normal rat urinary protein has a lower molecular weight.

Methods. Urine was collected by bladder massage from adult rats of 3 inbred strains, Buffalo, ACI/N, and Wistar, as well as from Sprague-Dawley non-inbred rats, all of which

TABLE I. Characteristics of Rat Urinary Protein.

Strain	Sex	Unfractionated urine		Region 4*	
		s_{20}	Main electrophoretic component(s)	s_{20}	Main electrophoretic component
Buffalo	♂	2.26 (1.00)†	α_2-globulin‡	2.06 (.65)†	α_2-globulin‡
	♀	2.29 (.87)	β-globulins		
ACI/N	♂	2.36 (1.00)	α_2-globulin	2.01 (.56)	"
	♀	2.30 (.60)	β-globulins		
Sprague-Dawley	♂	2.33 (1.00)	α_2-globulin	2.12 (.80)	"
	♀	2.34 (1.00)	α-globulin; β-globulins		
Wistar	♀	2.46 (.75)	β-globulins		

* See Fig. 1.

† Sedimentation coefficients, reported as s_{20} values, are in S units; figures in parentheses indicate protein concentration at which ultracentrifugation was done.

‡ Designations are based on electrophoretic similarity to serum components.

had been fed National Cancer Institute pelleted stock ration containing approximately 20% protein.

The urine was kept at 5°C under a layer of toluene until a suitable amount for processing had been obtained. It was then filtered through Whatman No. 42 filter paper and dialyzed exhaustively at 5°C against 0.9% NaCl. The protein was concentrated by negative-pressure dialysis against 0.9% NaCl at 2°C. Protein concentration was estimated by differential refractometry at 546 mμ with a Brice-Phoenix instrument; an assumed specific refractive increment of 0.186 ml/g was used. Values obtained in this manner for dialyzed, unfractionated urine agreed closely with those determined by a biuret method(8) using a bovine plasma albumin standard.

Ultracentrifugal analyses were carried out in a Spinco model E ultracentrifuge. A synthetic boundary cell was employed when diffusion coefficients were measured. The solvent was phosphate-buffered NaCl, pH 7.2, $\Gamma/2$ 0.154. All experiments were done at 20°C.

Paper electrophoresis was done at room temperature in a Spinco model R cell on Whatman No. 3MM paper. Barbital buffer, pH 8.6, $\Gamma/2$ 0.075, was used in all experiments; staining was done with bromphenol blue.

Gel filtration was carried out on a 1.8 × 76 cm column of Sephadex G-100 (140-400 mesh). The eluant was 0.2 M NH_4HCO_3. Fractions of 3-4.5 ml each were collected. Ion exchange chromatography was carried out on a 1 × 20 cm column of DEAE cellulose (0.78 meq N/g) prepared in our laboratory by the method of Peterson and Sober(9). A linear gradient was used from "starting buffer" [0.005 M H_3PO_4, 0.039 M tris(hydroxymethyl)aminomethane, pH 8.6] toward "starting buffer" which was 0.3 M with respect to NaCl. Fractions of 4.2-4.5 ml were collected. Gel filtration and ion exchange chromatography were done at 6°C. To locate the protein peaks, the absorbances of the fractions were measured at 280 mμ in a Beckman DB spectrophotometer.

Results and discussion. Ultracentrifugation of dialyzed, unfractionated urine revealed a single boundary in every case. The sedimentation coefficients ranged from 2.26 S to 2.46 S (Table I).

Upon gel filtration, urine from Buffalo and ACI/N males gave elution patterns such as that shown in Fig. 1, as did the urine of Sprague-Dawley males, which had a small additional peak in region 2. The patterns given by urine from Buffalo and ACI/N females consisted of 4 peaks of approximately equal height, whereas those of urine from Sprague-Dawley and Wistar females had relatively less material in regions 2 and 4, respectively. Thus region 3 was a major peak in the patterns of the females of all strains.

The presence of numerous peaks in the elution patterns suggested a wide range of molecular sizes. For example, the position of region 1 was the same as that at which 7 S γ-globulin and larger molecules were eluted from this column, suggesting the presence of

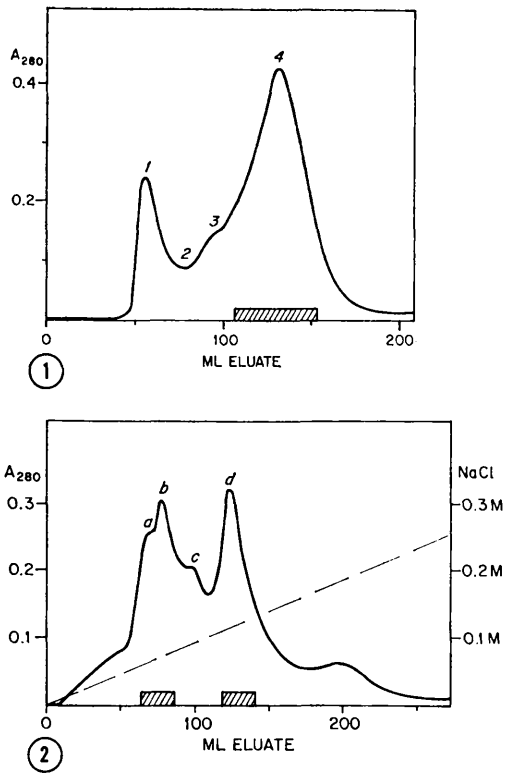

FIG. 1. Gel filtration of male rat urinary protein on Sephadex G-100. Hatched region indicates material subjected to rechromatography.

FIG. 2. Cromatography of region 4 on DEAE cellulose. Solid line = absorbance at 280 mμ; broken line = NaCl concentration of eluting buffer. Hatched regions indicate material subjected to rechromatography.

very large molecules in this fraction. Though ultracentrifugation confirmed this, components with sedimentation coefficients as high as 12 S being detected, a considerable portion of region 1 consisted of material of approximately 2.7 S. The ultraviolet spectrum of this region showed a maximum at 277 mμ, indicating the presence of protein; electrophoresis revealed a faint band in the β- to γ-globulin region. Several explanations might be offered for the ultracentrifugal heterogeneity; however, because of the complexity of this material and the small amounts of it available, region 1 was not examined further. Similarly, little of region 2 or 3 could be accumulated, owing to their relative absence in most male samples and to the lower concentration of protein in female rat urine.*

Attention was directed to region 4 (Fig. 1), which constituted the major portion of the urinary protein of the males of all strains examined. Ultracentrifugation of this fraction yielded, in each case studied, a single peak of 2.01-2.12 S (Table I). Electrophoresis revealed a broad band in the α_2-globulin region and 2 very faint bands at the leading edge of and faster than albumin, respectively.

The center portion of region 4 (Fig. 1) from Buffalo male rats was concentrated by freeze drying after dialysis against distilled water and again subjected to gel filtration. Most of the material emerged at the position of the original region 4, but a small sharp peak in region 1 was observed. The peak of this region 4 appeared at an elution volume between the elution volumes determined on this column for the major urinary protein complex of mouse urine(10), molecular weight 17,800(4), and bovine pancreatic ribonuclease, molecular weight 13,683. By means of either a standard curve consisting of a simple plot of the molecular weights of a series of proteins against their elution volumes or by logarithmic interpolation(11,12) the molecular weight was estimated at 15,000.

The center portion of this "rechromatographed region 4" from Buffalo male rats was then subjected to ion exchange chromatography on DEAE cellulose. The pattern revealed 2 major peaks and several shoulders (Fig. 2). After dialysis against "starting buffer," each of the regions indicated was rechromatographed in the same system. Subsequently the tubes corresponding to fractions b and d, respectively (Fig. 2), were pooled, concentrated by negative pressure dialysis and ultracentrifuged at a protein concentration of 0.5%. In each case, the sedimentation coefficient was found to be 1.9 S. Moreover, in each case the apparent diffusion coefficient, calculated according to the method described by Schachman(13), was 11×10^{-7} cm^2/sec. Assuming a partial specific

* When sufficient protein from region 2 could be obtained for study, this fraction was found by paper electrophoresis to be a mixture of albumin and some β_2-globulin; the sedimentation coefficient was 3.7 S (protein concentration, 0.59%). A sample of region 3 from female urine consisted primarily of material with β-globulin mobility.

volume of 0.72 ml/g, these values give a molecular weight of 15,000.

It is currently thought that the normal urinary protein of man arises from both the plasma and other sources(14). This view is consistent with the observations that, in the case of the rat, serum protein or fractions thereof differ from urinary protein with respect to the associated sterols(15), carbohydrates(16), and esterase activity(17). Though King(14) has pointed out that such differences may have several bases, the hypothesis that rat urinary protein can result from a filtration-resorption process† is consonant with the suggestion that the normal rat could be a suitable animal for experimental studies bearing on nephritis(4,18). The present demonstration that a substantial fraction of the urinary protein is of lower molecular weight than serum albumin may alter, but does not vitiate, this suggestion. Creeth *et al*(19) found that human patients with glomerular disorders excreted urinary proteins with sedimentation coefficients of 4.0-4.4 S, whereas patients with tubular disorders had urinary proteins of 2.2-2.8 S, consisting largely of α- and β-globulins.

Summary. The normal urinary protein of rats of 3 inbred strains and one non-inbred stock was examined by gel filtration and ultracentrifugation. A considerable portion of the total urinary protein was found to be of low molecular weight, especially in the males. In the case of male rats of the Buffalo strain, the major urinary protein fraction isolated by gel filtration and two subfractions of it prepared by ion exchange chromatography had molecular weights of the order of 15,000.

† More direct evidence indicating the plasma as a source of rat urinary protein includes the observation, made in association with Dr. M. Potter of the National Cancer Institute, that upon immunoelectrophoresis Buffalo male rat serum formed several precipitin arcs with rabbit antiserum prepared against the non-dialyzable fraction of Buffalo male urine. One of these arcs occurred at the same position as that formed with the urinary α_2-globulin.

1. Sellers, A. L., Roberts, S., Rask, I., Smith, S., Marmorston, J., Goodman, H. C., J. Exp. Med., 1952, v95, 465.

2. Sellers, A. L., Griggs, N., Marmorston, J., Goodman, H. C., ibid., 1954, v100, 1.

3. Rumsfeld, H. W., Jr., Am. J. Physiol., 1956, v184, 473.

4. Finlayson, J. S., Baumann, C. A., ibid., 1958, v192, 69.

5. Wakim, K. G., in Biochemical Clinics, No. 2, The Kidney, R. H. Donnelley Corp., New York, 1963, p5.

6. Charlwood, P. A., Biochem. J., 1961, v78, 163.

7. Jungblut, P. W., Turba, F., Biochem. Z., 1963, v337, 88.

8. Johnson, B. C., Swanson, A. M., J. Dairy Sci., 1952, v35, 823.

9. Peterson, E. A., Sober, H. A., J. Am. Chem. Soc., 1956, v78, 751.

10. Finlayson, J. S., Potter, M., Runner, C. C., J. Nat. Cancer Inst., 1963, v31, 91.

11. Whitaker, J. R., Anal. Chem., 1963, v35, 1950.

12. Andrews, P., Biochem. J., 1964, v91, 222.

13. Schachman, H. K., in Methods in Enzymology, S. P. Colowick, N. O. Kaplan, ed., Academic Press, Inc., New York, 1957, v4, 59.

14. King, J. S., Jr., in Biochemical Clinics, No. 2, The Kidney, R. H. Donnelley Corp., New York, 1963, p171.

15. Finlayson, J. S., Baumann, C. A., Am. J. Physiol., 1957, v190, 297.

16. Hardy, R. W. F., Ph.D. Thesis, Univ. of Wisconsin, 1960.

17. Hermann, G., Talal, N., de Vaux St. Cyr, C., Escribano, J., J. Immunol., 1963, v90, 257.

18. Addis, T., Proc. Cal. Acad. Med., 1931, v2, 38.

19. Creeth, J. M., Kekwick, R. A., Flynn, F. V., Harris, H., Robson, E. B., Clin. Chim. Acta, 1963, v8, 406.

Received March 2, 1965. P.S.E.B.M., 1965, v119.