Table 1. Study of Linearity with Gornall's Biuret Reagent[a]

Sample	Protein, mg/5.1 ml 2.0	4.0	6.0	8.0	10.0	12.5	15.0
1	0.1136[b]	0.2286	0.3422	0.4476	0.5458	0.6140	0.6555
2	0.1121	0.2286	0.3415	0.4460	0.5448	0.6144	0.6567
3	0.1131	0.2278	0.3412	0.4468	0.5445	0.6140	0.6553
$\bar{X}$	0.1129	0.2283	0.3416	0.4468	0.5450	0.6141	0.6558
$A^{1g/liter}_{540\ nm}$	0.288	0.291	0.290	0.285	0.278	0.250	0.223

[a] Reagent volume: 5.0 ml; sample volume (bovine serum albumin): 100 μl; absorbance measurements at 540 nm in Cary 16 Spectrophotometer after a 1-h incubation at 25 °C.

[b] Net absorbance values.

Table 2. Effect of Dextran on the Determination of Total Serum Protein with Three Biuret Reagents

Dextran, g/liter	Protein, g/liter Magid		Gornall		Doumas	
0	57.2	(58.4)[a]	64.0	(64.1)	64.2	(64.3)
0.5	—	—	—	—	65.9	(64.5)
1.0	58.2	(58.8)	67.0	(64.1)	67.0	(64.3)
5.0	68.1	(62.6)	82.8	(64.0)	82.1	(64.2)
10.0	90.6	(59.0)	105.6	(63.9)	101.3	(64.2)
20.0	134.8	(57.9)	158.8	(61.6)	127.6	(64.3)

[a] Protein values for the centrifuged reaction mixture.

of visible precipitation required an explanation. We supplemented portions of a fresh serum pool with dextran (av mol wt, 40 000) to produce concentrations between 0.5 and 20 g/liter, while maintaining the protein concentration constant. The samples were analyzed with Gornall's (*3*), Magid's, and our reagents (*1*). Absorbance was measured before and after centrifugation, after an incubation of 1 h at 25 °C. It is obvious (Table 2), that dextran precipitates with all reagents at concentration of 5 g/liter or higher.

With Magid's reagent the reaction mixture appears opalescent to turbid but the precipitate does not sediment spontaneously; this is because of the low concentration of NaOH in his reagent. When the incubation time with Magid's reagent was shortened to 20 min, no dextran interference was encountered up to a concentration of 5 g/liter.

Finally, we have tried to explain the lower protein value of the pool obtained with Magid's reagent. The absorbance values of the sample blanks remained constant throughout the incubation period with Gornall's and our reagents. However, with Magid's blank biuret reagent there was a 10-fold increase in the absorbance of the sample blanks between the beginning and the end of the incubation period. Sample blanks appeared turbid after 1 h while test solutions (in the absence of dextran) were clear. The resulting over-correction may be restricted to the serum pool used but it might be interesting to examine if this is the case with individual serum samples.

References

1. Doumas, B. T. and the Study Group on Proteins, Standards for total serum protein assays—a collaborative study. *Clin. Chem.* 21, 1159 (1975).

2. Reinhold, J. G., Total protein, albumin and globulin. *Stand. Methods Clin. Chem.* 1, 88 (1953).

3. Gornall, A. G., Bardawill, C. J., and David, M. M., Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177, 751 (1949).

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More on Urinary Protein Determination Technique

To the Editor:

The chromatography columns for use in the Selected Method for urinary total protein determination (*1*) are available from the vendor (Oxford Laboratories, San Mateo, Calif. 94401) by direct special order. We report two alternative columns with adaptations of volumes used for gel filtration. Additionally we analyzed five protein calibration standards and a peptide, the molecular weights of which range from 4000 to 68 000.

1. Columns similar to the ones originally reported (2) and which have a 3.0-ml bed volume are "Q-S" polypropylene columns with porous polyethylene bed support, cat. No. QS-A. Extension funnels accommodate 12 ml, cat. No. QS-R (Figure 1) (Isolab Inc., Akron, Ohio 44321). The first column, for desalting, is made up to a bed height of 44 mm. The second column, for separating free from bound copper, is made up to a bed height of 39 mm. (This allows 5 mm for swelling when the gel is made alkaline.) Use a 1.0-ml sample volume, 2.0-ml eluent volume, and 0.5 ml of biuret reagent. Analytical recovery of bovine serum albumin from gel filtration is 95 ± 5%.

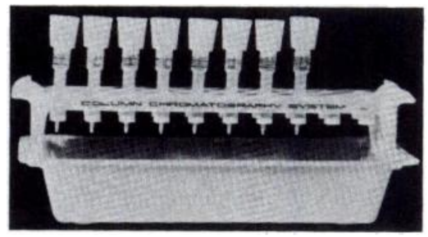

Fig. 1

2. Smaller columns (2.1-ml bed volume) are polyethylene column elements with porous polyethylene bed support, cat. No. 731-1110 (Bio-Rad Laboratories, Richmond, Calif. 94804). The chromatography system, consisting of two circular polystyrene platforms with stands and elution trays (cat. No. 183-1110), is convenient to use with an AutoAnalyzer sampler wheel (cat. No. 127-0162-01; Technicon Instruments Corp., Tarrytown, N. Y. 10591). The first column is made up to a bed height of 40 mm. The second column is made up to a bed height of 35 mm. Use a 0.6-ml sample volume, 1.2-ml eluent volume, and 0.3 ml of biuret reagent. Analytical recovery of bovine serum albumin from gel filtration is 95 ± 5%.

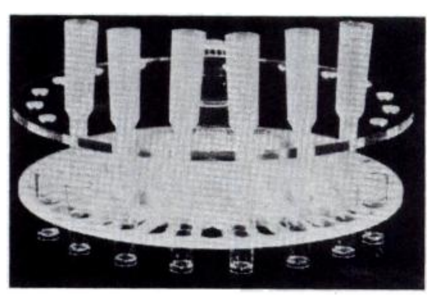

Fig. 2

Table 1. Results of Analysis for Some Proteins and Peptides by the Selected Method (*1*)

	Mol wt	Present (mg/liter)	Found (mg/liter)	Anal. recovery, %
A. Protein Calibration Kit				
Lysozyme	14 500	290	250	86
Beta-lactoglobulin	18 000	610	500	82
Carbonic anhydrase	32 000	680	570	83
Ovalbumin	45 000	401	385	95
Bovine serum albumin	68 000	410	390	95
B. Commercially available control material				
	Labeled value, mg/liter			
Bovine serum albumin (Sigma, Fraction V)		740	690	93
Bovine serum albumin Control	860 ± 100	—	880	—
Hyland Urine Control	450 ± 100	—	460	—
Ortho Urine Control	420 ± 100	—	400	—
C. Purified corticotropin (porcine)				
	Mol wt			
	4000	400	52	13
	4000	600	84	14

3. Because the molecular weight cut-off value for Sephadex G-50-80 (Pharmacia Fine Chemicals, Piscataway, N. J. 08854) is 30 000, we assessed the analytical contributions of a series of proteins including and smaller than serum albumin (Isolab. Inc., cat. No Code GE-66; protein calibration kit, low range). We weighed the crystalline proteins separately into cold physiological saline and let the mixtures stand at 4 °C overnight to dissolve.

These solutions were assayed for total protein concentrations as described previously (*1*). Human Protein Standard (lot No. PRS-416; Dade Division, American Hospital Supply Corp., Miami, Fla. 33152) with manufacturer's reference assay value of 79 g/liter was diluted with physiological saline to give concentrations of 395 and 790 mg/liter for use as reference material. Four controls were included: (*a*) bovine serum albumin (BSA), Cohn Fraction V, lot No. 114C-0008, containing 96–99% albumin (Sigma Chemical Co., St. Louis, Mo. 63178), was weighed into cold physiological saline and treated as the samples; (*b*) a previously analyzed BSA control, 860 ± 100 mg/liter; (*c*) Hyland "Q-PAK" Chemistry Urine Control Supplemental, lot No. 0521L002AA, with manufacturer's reference assay value of 900 ± 200 mg/liter was used at a twofold dilution with physiological saline to give a target value of 450 ± 100 mg/liter. (Hyland Laboratories, Costa Mesa, Calif. 92626); and (*d*) Ortho Control Urine II, lot No. 12N204A with manufacturer's assay value 420 ± 100 mg/liter (Ortho Diagnostics Inc., Raritan, N. J. 08869).

Our results (Table 1) indicate that the color yield for proteins with a mass as small as 14 500 daltons is 80% as great as that for albumin. Loading the gel column with a sample volume equal to the void volume of the gel results in desalting with practically no separation according to size of the protein molecule. We cannot explain the failure of lysozyme to fall into the expected pattern of decreasing recovery with decreasing molecular size of the protein, but this pattern was demonstrated on five separate trials.

Small proteins, those approaching the 10 000-dalton limit, are determined with an efficiency similar to that for larger serum proteins (*2*) and urinary mucoprotein (*3*). Amino acid polymers with a mass of less than 10 000 daltons are termed peptides (*4*); based on this study a peptide with a molecular weight of 4000 contributes 14% to the total protein assay value (Table 1). These data indicate that the Selected Method for urinary protein (*1*) provides an accurate index of proteinuria and in nephron pathology may include some contribution from peptides if they are present (*5*, *6*).[1]

[1] *Ed. note*: Peptides and glycopeptides are plentiful in normal urine [*cf. J. Clin. Invest.* 37, 1658 (1958); 38, 1927 (1959)], but still have no recognized clinical significance.

References

1. Doetsch, K. and Gadsden, R. H., Determination of urinary total protein by use of gel filtration and a modified biuret method. *Clin. Chem.* 21, 778 (1975).

2. Doetsch, K. and Gadsden, R. H., Determination of total urinary protein combining Lowry sensitivity and biuret specificity. *Clin. Chem.* 19, 1170 (1973).

3. Doetsch, K. and Gadsden, R. H., Further on urinary protein determination. *Clin. Chem.* 20, 1384 (1974). Letter to the Editor.

4. Peters, T., Jr., Proposal for standardization of total protein assays. *Clin. Chem.* 14, 1147 (1968).

5. Doetsch, K. and Gadsden, R. H., Selectivity of proteinuria: Origin of the proteins and potential diagnostic aid. *J. S. C. Med. Assoc.* 71, 83 (1975).

6. Rennie, I. D., Proteinuria. *Med. Clin. North Am.* 55, 213 (1971).

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Improvement in a Gas-Chromatographic Assay for Anticonvulsant Drugs

To the Editor:

The method of Roger et al. (*1*) is one of the few procedures available for the simultaneous gas chromatographic assay in blood plasma of the four most-prescribed anticonvulsant drugs: diphenylhydantoin, phenobarbital, primidone, and carbamazepine. We found this method to be reliable and accurate, but somewhat lengthy and cumbersome for routine use. Relatively large quantities of plasma sample (2 ml) and extraction solvent are required, with lengthy extractions on a mixing machine and lengthy solvent-evaporation steps.

I wish to report some modifications that considerably shorten the time required to process plasma samples for subsequent derivitization and chromatography. With these modifications, eight samples can be processed in 45 min, as compared with 2.5–3.0 h by the original procedure. This was done by decreasing the quantities of plasma sample and extraction solvents and by using smaller extraction tubes so that mixing by hand could be substituted for the slower mixing by machine. The solvent evaporation steps were accelerated, partly because of the smaller volumes involved, but also because the extracts were warmed on a heating block during evaporation under a stream of air (instead of nitrogen as in the original method).

Procedure. To Pyrex extraction tubes (12 mm o.d. × 125 mm long) with Teflon-lined screw caps, add, in the order