treated serum aliquots were assayed together with matched untreated aliquots in two Amerlex FT4 assays. The results were as follows:

FT4, pmol/L	
Untreated samples	Samples + added NEFA
Non-pregnant	
14.3 ± 1.5	14.2 ± 1.4[a]
Pregnant	
10.2 ± 1.0	10.0 ± 0.67[a]

[a] Results not statistically significantly different from untreated control samples.

Clearly, the results were unaffected by added oleic acid. The absence of a statistical interaction in the analysis of variance also rules out the possibility of the lower FT4 values in late pregnancy being the result of an interaction between increased NEFA and decreased albumin.

In conclusion: we believe that the Amerlex FT4 values obtained during pregnancy are correct in showing a steady decline as gestation proceeds (*8*). This is in harmony with mass-action theory, and it follows inevitably from the increase in T4 and TBG in serum and the accompanying decline in the T4/TBG ratio. No arguments as yet advanced supply convincing reasons why this theoretical expectation and its practical support are invalid. This is not to say that if the concentration of albumin (or of any other T4-binding protein) is altered sufficiently, results with the Amerlex FT4 kit would be unaffected. If any demonstrable residual binding occurs for the T4 analog with any protein, then a large enough variation in concentration for any such species would be capable of distorting the results. This has not been shown within the clinically encountered limits of TBG and TBPA concentration in serum, but has been shown in the rare situation of familial dysalbuminemic T4 excess, in which the high capacity of albumin is accompanied by a mutation that creates a protein with greatly increased binding power (*11*), which then possesses sufficient binding potential to distort the assay. Possibly also, in the opposite direction, true analbuminemia may give somewhat distorted results for the same reason. However, it is not valid to extrapolate from these extremes and then conclude that significant distortion occurs in the much narrower range of albumin concentrations encompassing both the normal population and, indeed, pregnancy and nonthyroidal illness.

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A Defense of the Coomassie Blue Method for Urinary Protein

To the Editor:

In their report on the relative suitability of various methods for measuring urinary protein, Dilena et al. (*1*) concluded that the Coomassie Brilliant Blue (CBB) technique was the simplest but less precise than the Ponceau-S (P-S) technique. Because their findings concerning the precision of the CBB method are at variance with ours, we re-examined our previous studies (*2*).

In our routine use of the CBB method, we establish a standard curve once per month or with each batch of reagent with a human albumin standard (Dade Diagnostics, Aguada, PR). The concentrations of standard are 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 g/L.

A standard (0.8 g/L) and a quality control (0.4 g/L albumin solution) are used with each analysis to check the curve, while monitoring precision. Reagent is dispensed with a "Dispensette" (Brinkmann Instruments, Rexdale, Ontario, Canada) and samples and standards are added with an SMI pipette (Scientific Manufacturing Industries, Emeryville, CA).

We selected three patients' urine specimens with protein concentrations similar to those analyzed by Dilena et al. (*1*). Our results, shown in the following tabulation, compare favorably with those reported for the P-S method.

Mean protein concn, g/L	No. assays	CV, %
Within-day precision		
0.225	29	2.26
0.850	32	1.74
1.195	30	1.77
Between-day precision		
0.225	25	6.17
0.881	25	2.14
1.192	25	1.76

During the past three-month period, 64 individual measurements were made by different technologists, using the 0.4 g/L albumin solution as quality control. The mean was 0.39 g/L and the CV 2.54%.

We believe the results indicate that a decision between the two methods (CBB and P-S) can be made simply on the basis of the relative convenience of the assay, because the precision achieved with the two methods is similar.

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Software for Apple II Handling of Endocrine RIA Data

To the Editor:

When microcomputer systems are used in laboratories, adequate software is needed. One such field of application

is an endocrine laboratory that is using radioimmunoassays (RIA). We believe that RIA data processing should be done in the laboratory by the RIA technician, rather than depending on large, centralized computer facilities. Programs for RIA data analysis and quality control and for Scatchard plot analysis have been published (*1, 2*), intended for use with a small calculator.

Continuing along these lines of work, we devised a similar computer system for the analysis and quality control of RIA data based on the Apple II microcomputer. This system has been conceived in a modular form that includes the definition of assay parameters, preparation of working protocols and actual data processing and quality control. With reference to the latter point, an interactive operating scheme was designed. In essence the procedure is as follows. Raw counts are entered manually through the keyboard. The user can select the number of standards and the number of replicates for each set of tubes. Each set is immediately transformed, with correction for nonspecific binding and logit-log transformation (*3*). Actual standard points are both displayed on the screen and printed out in a logit-log format. Regression analysis is done by the unweighted least squares method. A deletion facility of standards is included. Goodness of fit parameters such as r and S_{yx} and curve parameters such as slope, y-intercept, effective dose 85% (ED85), ED50, and ED15 are displayed and printed out. Raw counts of the unknowns—again, as many replicates as desired—are entered manually. Single concentrations as well as mean final concentrations after transformation into the clinically useful dimension are printed out, including a parameter of random scatter of replicate measurements. Different dilutions of the same specimen can also be handled. Quality control consists of the summary of local assay quality-control parameters as well as of cumulative mean values of these quality-control parameters (*4*).

This system has proven to be versatile and useful for this type of application. Copies of the system can be obtained free of charge by sending a blank five-inch diskette.

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Ed. note: The Editors are contemplating a format for routine announcement of availability of specialized software programs such as this. If it is decided upon, an announcement will appear in The Clinical Chemist *section in due course.*

On the Presence of Cholesterol in Urinary Calculi

To the Editor:

Recently, Zoppi et al. (*1*) reported a case of cholesterol gravel in the urinary tract and mentioned that they "are investigating how this substance could be passed in urinary ultrafiltrate." They also cited a reference (*2*) pointing out that the (very rare) cholesterol urinary calculi are found only in women. A reviewer of the Letter from Zoppi et al. (*1*) furthermore suggested that the presence of cholesterol in a urinary calculi could be explained by passage of a gallstone into the urinary tract. However, another explanation should be seriously considered, as illustrated by the following case.

A 65-year-old woman with "renal colic" was admitted to a surgical clinic, where she presented what she claimed to be urinary calculi. When these were analyzed by use of a commercial kit for calculi analysis (Merckognost[R], Boehringer-Mannheim), only calcium oxalate was found. One month later the patient had another renal colic episode and now presented eight calculi, which were analyzed with the same result as earlier. She was now referred to our Department of Urology for further investigations according to the scheme for recurrent stone formers (*3*). However, results of all investigations, including an intravenous pyelography, were completely normal. Moreover, a recently developed quantitative wet-chemical method (*4*) showed that commonly occurring urolithiasis components including calcium oxalate were absent from the patient's urinary calculi. The calculi were insoluble in nitric acid but soluble in chloroform, a behaviour that prompted us to suspect the presence of cholesterol. The Liebermann–Burchard reaction was positive, and the presence of cholesterol was confirmed by gas chromatography/mass spectroscopy.

A check-up of the patient's history revealed that she had undergone an operation for gallstone 15 years ago, with removal of about 250 small gallstones. The latter were handed over courteously to the patient by the surgeon. Although she initially presented more calculi as inpatient no calculi were obtained when she had an indwelling catheter. Thus strong evidence was obtained that the pretended urinary calculi were gallstones, although the patient never admitted forgery. The patient was then referred to the Department of Psychiatry for further investigation where a disturbed social situation was found to explain her desire for hospital care. The present case thus represents a variant of the Münchausen syndrome (*5*) belonging to clinical chemistry. We suggest that many if not all of the reported cholesterol urinary calculi are of a similar nature.

In fact we claim that formation of cholesterol calculi in the urine is unlikely, requiring supersaturation of the urine with the compound in question. Cholesterol is present in plasma in form of lipoprotein particles, and these will not pass the glomerular membrane in normal subjects. When extensive damage of the glomerular membrane is present—for instance, in the nephrotic syndrome—lipoproteins may appear in urine (*6*), but precipitation of cholesterol from lipoprotein particles in urine appears very unlikely and has to our knowledge never been documented.

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