where stress (σ) and strain (ϵ) have units of kilograms square centimeters and microstrains $\times 10^{-6}$, respectively. The cross-sectional area (A) of the dosator, where the bonded strain gauges are located, was reported as 0.174 cm^2. Therefore, the expected sensitivity (microstrains per kilogram) from longitudinal compression can be calculated:

$$\text{sensitivity} = (E \times A \times 10^{-6})^{-1} \qquad \text{(Eq. 3)}$$

This value is 2.92 microstrains/kg.

Then, to calculate the contribution to the sensitivity from lateral strain, the Poisson ratio (μ) for steel of 0.3 is used (3). The value for the lateral sensitivity was calculated as 0.88 microstrain/kg. The total sensitivity for this instrumented dosator is the sum of the lateral and longitudinal components, which is 3.80 microstrains/kg. This value compares well with that observed (1), 3.96 microstrains/kg.

The preceding discussion does not invalidate this type of strain-gauge application, but it does clarify and explain the sensitivity obtained.

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Clarification of Nomenclature

Keyphrases □ Capsule-filling machine, automatic—strain gauges, explanation of activity □ Instrumentation—automatic capsule-filling machine, explanation of activity of strain gauges

To the Editor:

Kent and Yost (1) commented on the Wheatstone bridge design for an automatic capsule-filling machine (2). The described position of the gauges on the modified dosator piston to measure the force applied to the dosator piston and to provide temperature compensation is common in experimental stress analysis (3) and pharmaceutical research (4, 5).

Theoretical stress calculations are not required for accurate calibration of the instrumented piston. The calculations offered by Kent and Yost for the net strain, ϵ_{net}, at the strain-gauge bonding site are well known for this bridge application and may be simply expressed as (3):

$$\epsilon_{net} = (1 + \mu)\epsilon_A \qquad \text{(Eq. 1)}$$

where μ is the Poisson ratio and ϵ_A is the axial strain as calculated from Hooke's law.

Kent and Yost agree that this type of calculation does not invalidate the design of the Wheatstone bridge in question nor the validity of the work presented. The issue is the use of the term "passive" to describe the temperature-compensating gauges in Fig. 3 of Ref. 2. Other terms have been applied to these same gauges (4, 6–9). Knoechel *et al.* (4) used the term "dummy" gauges. Arthur (6) stated: "It sometimes happens that it is convenient to use 'dummy' gages for temperature compensation." Neubert (7) mentioned that the dummy gauges "should be mounted in a direction of minimum strain. . . ."

Perhaps the best term for the temperature-compensating gauges in the bridge arrangement in question is *Poisson gauges* (9), in recognition of the fact that these gauges also contribute to the total sensitivity of the piston.

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Effect of Hemodialysis on Cefazolin Protein Binding

Keyphrases □ Cefazolin—protein binding, effect of hemodialysis □ Protein binding—cefazolin, effect of hemodialysis □ Binding, protein—cefazolin, effect of hemodialysis □ Hemodialysis—effect on protein binding of cefazolin □ Antibacterials—cefazolin, protein binding, effect of hemodialysis

To the Editor:

Cefazolin, a semisynthetic derivative of 7-aminocephalosporanic acid, is indicated for use in infections caused by various Gram-positive and Gram-negative bacteria. Cefazolin is very highly bound to serum proteins; over the therapeutic range of 1–200 μg/ml, greater than 80% of the total drug in plasma is in the bound form (1–4). In the presence of renal impairment, the fraction bound

has been reported to decrease significantly. In one study, the percent bound decreased from 84% in normal patients to 63% in uremic patients (1). The effect of hemodialysis on the half-life and pharmacokinetics of cefazolin also was studied (5). However, the effects of hemodialysis on protein binding have not been reported.

Craig *et al.* (6) studied serum from uremic patients and reported that treatment with activated charcoal at pH 3 significantly increased the fraction bound for several drugs, *e.g.*, sulfamethoxazole, dicloxacillin, phenytoin, salicylate, and digitoxin. These investigators speculated that the treatment of serum with charcoal removed a protein binding inhibitor present in the serum of uremic patients, thus restoring the binding to near normal levels.

This study was conducted to determine whether the decreased protein binding capacity in uremic serum would be affected by hemodialysis. If hemodialysis removes the protein binding inhibitor present in uremic serum, the binding capacity should return to normal levels.

Twelve patients currently undergoing maintenance hemodialysis[1] were studied. All patients had serum creatinine values above 6.0 mg % (11.5 ± 3.8 mg % *SD*) and blood urea nitrogen values above 32 mg % (90.3 ± 30.9 mg %) before hemodialysis. Serum creatinine and blood urea nitrogen values decreased to 5.2 ± 2.0 and 14.2 ± 18.0 (*SD*) mg %, respectively, after the hemodialysis procedure.

Blood samples were collected both before and after hemodialysis. Hemodialysis was conducted using artificial kidneys[2] in dialysis machines[3] supplied by a central dialysis bath. Dialysis was performed with systemic heparinization for 5–6 hr. Serum was removed from all samples, frozen at −4°, and used within 3 weeks.

Cefazolin was later added to the serum to give a total drug concentration of 92 μg/ml. Seven-milliliter samples of the serum–cefazolin solution were pipetted into an ultrafiltration apparatus[4]. An ultrafiltration membrane[5] with a molecular weight cutoff of 10,000 was used to separate the free drug from the bound drug. Nitrogen gas at a pressure of 15 psi was applied to the ultrafiltration apparatus, and the protein-free ultrafiltrate was collected and frozen until assay.

The drug concentration in the ultrafiltrate samples was determined using the microbial disk diffusion assay of Grove and Randall (7). Twenty microliters of the ultrafiltrate was pipetted onto 6.25-mm disks placed on agar seeded with *Bacillus subtilis* spores. Standards were made in 0.067 *M* phosphate buffer, pH 7.4. All plates were incubated overnight at 37° before the zone sizes were measured. The drug concentration in the ultrafiltrate was determined by interpolation from the standard curve made with phosphate buffer.

The serum protein binding of cefazolin in patients with uremia averaged 72.5 ± 13.4 (*SD*) % prior to dialysis, a significant decrease from the value of 80–90% found in nonuremic patients. All values of protein binding in the uremic patients, except one, were below 86% (Table I). There did not appear to be any significant statistical correlations between the fraction bound and the values of serum creatinine, blood urea nitrogen, calculated creatinine clearance, and serum albumin concentration.

Table I—Protein Binding in Patients with Uremia

Subject	Percent Protein Bound	
	Predialysis	Postdialysis
A	70	5
B	80	15
C	72	2
D	42	6
E	61	42
F	86	30
G	65	20
H	78	26
I	76	26
J	71	0
K	73	22
L	96	75
Mean ± *SD*	72.5 ± 13.4	22.4 ± 20.9

The fraction of cefazolin bound to protein after the patients had undergone dialysis decreased significantly to 22.4 ± 20.9 (*SD*) %. The values ranged from 0 to 75% (Table I). A paired Student *t* test comparing the pre- and postdialysis values for each patient showed a significant difference in the protein binding after hemodialysis ($p < 0.005$). The amount of change in binding did not correlate with the type of artificial kidney, the duration of dialysis, or the amount of heparin used. Therefore, the dialysis procedure apparently does not remove a protein binding inhibitor from the serum; on the contrary, hemodialysis actually introduced, either directly or indirectly, a protein binding inhibitor.

Craig (8) recently demonstrated that the binding of proteins in serum decreased in patients undergoing cardiopulmonary bypass. The decrease in binding was inversely proportional to the increase in the serum free fatty acid levels, which was attributed to the use of heparin during the bypass procedure. The decrease in the binding to serum proteins in each uremic patient after hemodialysis may also possibly be attributed to an increased free fatty acid level due to the administration of heparin during the dialysis procedure.

Drugs in the protein-bound state are neither pharmacologically active (9) nor able to distribute to the tissues (10). Since few infections are located in the circulatory system, the ability of the antimicrobial to leave the circulation and to enter the tissues is critical before the infecting organism can be eradicated. Decreased binding to proteins would increase drug distribution to tissues; thus, the drug may be more effective. The decrease in protein binding also can have certain dangers. Although cefazolin is relatively safe over a wide therapeutic range of concentrations, toxic reactions are more likely with greater tissue concentrations.

Other drugs with a similar decrease in protein binding after dialysis and a narrower therapeutic margin would present a great potential for toxicity due to increased free serum and tissue drug levels. Further studies of the effect of dialysis on the protein binding of other pharmacological agents is necessary to determine whether this phenomenon is common to other highly bound drugs. Adjustment of the dosage regimen after dialysis may be necessary to compensate for the increases in tissue distribution occurring immediately after dialysis.

(1) W. A. Craig, P. G. Welling, T. C. Jackson, and C. M. Kunin, *J.*

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[2] Cobe Laboratories, Cranbury, N.J., and Gambro, Inc., Wheeling, Ill.
[3] Milton-Roy, Extracorporal Medical Systems Inc., Pinellas Park, Fla.
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Use of Trimethylanilinium Hydroxide–Tetramethylammonium Hydroxide as On-Column Methylating Agent for GLC Analysis of Phenytoin

Keyphrases □ Phenytoin—GLC analysis in plasma □ GLC—analysis, phenytoin in plasma □ Anticonvulsants—phenytoin, GLC analysis in plasma

To the Editor:

Numerous procedures for the GLC analysis of plasma phenytoin have been reported (1–7). One promising procedure was that of Orme *et al.* (7), who were able to analyze phenytoin from 100-μl plasma samples with a lower limit of sensitivity of 0.5 μg/ml. When I attempted to use this procedure, a peak for the on-column methylating agent, trimethylanilinium hydroxide, interfered with the peak for the internal standard, 5-(4-methylphenyl)-5-phenylhydantoin. This interfering peak was not an extracted plasma component or an impurity from the extracting solvent, toluene, since it was seen when just the methanolic trimethylanilinium hydroxide was injected on column as well as when the extractions were performed with water blanks.

Orme *et al.*'s procedure for phenytoin involved the extraction of phenytoin from a 100-μl plasma sample into toluene containing the internal standard. The toluene was then extracted with 25 μl of a 0.5 *M* trimethylanilinium hydroxide–50% (v/v) methanol–water mixture. One microliter of the lower aqueous layer, after aspiration of the upper toluene layer, was injected into a gas chromatograph and analyzed *via* a flame-ionization detector.

Attempts to overcome the peak interference in using Orme *et al.*'s procedure by lowering the trimethylanilinium hydroxide concentration to 0.1 *M* (concentration below 0.1 *M* led to problems with the final extraction step) and altering column conditions proved unsuccessful. The use of commercial as well as fresh batches of trimethylanilinium hydroxide, synthesized by the method of Brochmann–Hanssen and Oke (8) from trimethylanilinium chloride or iodide, was similarly unsuccessful in removing or lessening the interfering peak.

Brochmann–Hanssen and Oke (8) showed that trimethylanilinium hydroxide is a superior methylating agent compared to tetramethylammonium hydroxide and need only be present in reasonable excess to methylate barbiturates, *etc.*, quantitatively. For concentrations of up to 20 μg of phenytoin/ml in 100 μl of plasma and the internal standard used in Orme *et al.*'s procedure, the concentration of trimethylanilinium hydroxide in the final methanol–water mixture needed only to be slightly greater than 0.001 *M; i.e.*, for methylating purposes, the concentration of trimethylanilinium hydroxide of 0.5 *M* appeared to be unnecessary. Of course, the presence of trimethylanilinium hydroxide serves a secondary purpose in that the final methanol–water solution must be sufficiently alkaline to extract phenytoin and the internal standard efficiently from the toluene phase.

In the present study, sufficient alkalinity for the efficient extraction in the final extraction step and quantitative methylation of the extracted phenytoin and internal standard were achieved by the use of 0.1 *M* tetramethylammonium hydroxide–0.01 *M* trimethylanilinium hydroxide and a solvent of 50% (v/v) methanol–water. Tetramethylammonium hydroxide was present as the alkalizing agent and as a secondary methylating agent, while trimethylanilinium hydroxide was primarily present as the methylating agent.

Plasma samples, 100 μl, containing phenytoin were analyzed according to the procedure of Orme *et al.* (7) with the following modifications. To a 100-μl plasma sample was added 100 μl of freshly prepared 10% metaphosphoric acid. This addition was necessary because an interfering amine prodrug of phenytoin of low pKa was present in the plasma samples. Metaphosphoric acid also quenched the conversion of the prodrug to phenytoin. For normal samples, the addition of monobasic sodium phosphate, as suggested by Orme *et al.* (7), is sufficient.

Two milliliters of toluene containing 1.4 μg of 5-(4-methylphenyl)-5-phenylhydantoin was then added to the treated plasma sample, and the sample was vortexed vigorously for 1 min and then centrifuged. Approximately 1.8 ml of the toluene layer was removed by pipet. The toluene was then extracted with 25 μl of 50% (v/v) methanol–water that was 0.1 *M* in tetramethylammonium hydroxide and 0.01 *M* in trimethylanilinium hydroxide. This addition of the 25 μl of methanol–water *to* the toluene while vortexing was crucial for efficient extraction. After vortexing for 1 min and centrifuging, the upper toluene layer was removed by aspiration.