rectal carcinoid tumors (11) by this method but has not yet been fully characterized by radioimmunoassay. The variations in cross reactivity that can arise on using these techniques may result from the use of higher concentrations of antisera for immunohistochemical studies. Solcia et al. (12) proposed that sub-populations of cross-reacting antibodies, which are undetectable at dilutions used for radioimmunoassay, can give rise to specific immunohistochemical staining.

Lack of cross reactivity of peptides with antibodies raised to PYY and NPY for radioimmunoassay and immunohistochemistry studies cannot be assumed because of the results obtained in this study. Appropriate specificity studies will be required as the antisera become available.

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Ionized Calcium and the Donnan Effect

To the Editor:

In a recent letter, Butler and Payne (1) conclude that "much of the published work on ionized calcium must be re-examined in the light of protein interference." This conclusion is based on a previous finding of a higher concentration of ionized calcium in the original serum than in an ultrafiltrate of it, and an even higher ionized calcium concentration in the retentate after ultrafiltration (2). Payne (2) discards the Donnan theory (3) as an explanation of these results, referring to the fact that the ionic composite of the ultrafiltrate of a protein solution is found to be constant regardless of the duration of filtration.

We have re-examined the ionic distributions during ultrafiltration by theoretical calculation (4) as well as experimental measurement (5). The theoretical calculations based on the Donnan theory (see Figure 1) indicate an increase in the molality of CaCl₂ in the retentate but fairly constant molality in the ultrafiltrate. This is consistent with the experimental data of Butler and Payne and also consistent with our own experimental results.

Our experiments included a study of the effect of large variation in the salt concentration in the original protein-containing solution (5). Again, the experimental data were consistent with the Donnan theory rather than with a protein interference on the measurements.

So there is no need to postulate a protein effect on the liquid-junction potential.

We have previously noted a protein effect on the calcium electrode (6), namely, an irreversibility when measurements are made alternately in pure aqueous and protein-containing solutions. However, by covering the calcium selective membrane with a cellophane membrane—as with the Radiometer ICA 1 (7)—this effect was eliminated.

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Correction Factors for Hemoglobin Derivatives in Fetal Blood, as Measured with the IL 282 CO-Oximeter

To the Editor:

For fetal blood measured with an IL 282 CO-Oximeter (Instrumentation Laboratory Inc., Lexington, MA 02173) Zwart et al. (1) reported a false increase in the percentage of HbCO with increasing oxygen saturation. This phenomenon, which we confirmed (2), is not observed for blood from adults.

Here we report a practical procedure for correcting the measured percentages of oxyhemoglobin (%HbO2) and carboxyhemoglobin (%HbCO). We transformed the hemoglobin of venous blood samples obtained from adults, and blood from umbilical veins and arteries, into Hb, HbO2 and HbCO by tonometry. These samples were mixed in different ratios and the results given by the CO-Oximeter were compared with the calculated ratios. The correctness of the mixing-technique used was checked by isotope- and dye-dilution methods.

Figure 1 shows the results for the two-component system (Hb/HbO2), when no HbCO was added. Even though no HbCO was added, as much as 5% HbCO was found in fetal blood, a percentage linearly related to the percentage of HbO2. As can be seen from Figure 1, the differences between the measured and calculated %HbO2 for adult and fetal blood are identical to the %HbCO for fetal blood as measured with the IL 282 CO-Oximeter.

Next we made a three-component system (Hb/HbO2/HbCO) by adding various quantities of HbCO to the mixtures described above. The results are given in Figures 2 and 3. Parallel shifts were obtained for both adult and fetal blood; the slopes did not show significant differences (p > 0.05). The y-intercepts were identical to the calculated quantities of added HbCO. No significant differences (p > 0.05) were seen in the slopes of fetal blood from smoking mothers as compared with nonsmoking mothers, and the following averaged figures were found: slope m = 0.0541, SDm = 0.00091, CV = 1.7% (n = 9; number of measurements = 169). The averaged y-intercept for fetal blood from nonsmoking mothers (n = 11) was 0.24%.

The linear relationship between HbCO and HbO2 for fetal blood represents the fictitious percentage of HbCO, which depends on the measured %HbO2:

%HbCO (fictitious) = 0.054 · %HbO2 (measured) + 0.24%

This correction factor depends on the Hbf/Hba ratio, as shown by the effect of mixing different percentages of fetal and adult blood. The slope, m, changed in proportion to the percentage of Hbf (m = 0.065 · %Hbf + 0.005; r = 0.986). The slopes for nine different blood samples from mature infants had a CV of 2.8%, which indicates a fairly constant Hbf/Hba ratio. We found a mean %Hbf of 82.0, SD = 3.8, CV = 4.6%, determined according to the method of Wimberley (3).

To obtain the correct %HbCO, the calculated fictitious %HbCO must be subtracted from the measured %HbCO:

%HbCO (correct) = %HbCO (measured) − %HbCO (fictitious)

To obtain the correct %HbO2, one must add the calculated fictitious %HbCO to the measured %HbO2:

%HbO2 (correct) = %HbO2 (measured) + %HbCO (fictitious)

Prolonged tonometry of fetal blood with oxygen did not alter the results given by the IL 282 CO-Oximeter, which led us to conclude that the %HbCO was artificially high. When we excluded any influence of plasma

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