It is assumed that pK' and S do not change significantly in the particular patient's blood with change in $p_{CO_2}$. With $[HCO_3^-]$ determined, S, and then pK', for this particular blood can be readily calculated by substituting in equations 1-4.

When techniques were not available for direct measurement of certain parameters, there was some justification for approximating the concentration of certain blood components by calculation from those that could be measured. Where convenient procedures are available to measure the component directly it should be apparent that this is the method of choice.

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A Modified Retainer for Prolonging Uninterrupted Use of the Oxygen Sensor in the Beckman Glucose Analyzer

To the Editor:

Although the Beckman Glucose Analyzer is a valuable instrument in both the emergency and the pediatric clinical laboratory, the construction of the oxygen sensor limits its uninterrupted use and, therefore, the assurance of 24-h emergency glucose determination. According to the operating manual the oxygen sensor must be recharged once a week to ensure a minimum of down time. The reason for this frequent recharging is that the electrolyte gel soon dries because of the limited and relatively small volume (one droplet) of it that must be introduced. This may be the reason that this oxygen sensor often functions for only a few days in our hands. Therefore we constructed an alternative retainer that can contain more than 400 µl of electrolyte solution.

Figure 1 shows the construction of the modified retainer, made of methyl methacrylate and installed in our analyzer (type ERA 2002, Beckman Instruments, Inc.) with use of the original sensor body. The O-ring, which tightly fits the sensor body (11) by an extra pressure of the sleeve (2) initiated by the screw cap (1), prevents the presence of electrolyte solution at the rear end of the sensor body. This construction prevents false contact between the electrical connections of the anode and the cathode at the rear end of the sensor body, caused by the electrolyte solution. The O-ring (8), combined with the sleeve (7), ensures a perfect fixing of the original Teflon membrane, while the O-ring (9) is used for outside sealing of the oxygen sensor in the sample cup. The small wedge originally present at the rear end of the sensor body is removed.

To recharge the oxygen sensor, sleeve 2, O-ring 3, and retainer nut 5 are put into place. Screw cap 1 is loosely put into place and the sensor body is pushed gently from the rear end into the container just as far as past O-ring 3. About 0.5 ml of a nonvacuous electrolyte solution (for this we use $p_{CO_2}$-electrolyte supplied by Instrumentation Laboratory) is brought into the container and the sensor body is pushed into position as depicted in Figure 1. The Teflon membrane is put into place by means of sleeve 7 and O-ring 8.

Check the recharged sensor tip for air bubbles; if any are present, hold the oxygen sensor (including the electrical connections) in one hand and tap the sensor firmly with the tip downwards. Once a week the sensor should be inspected. If air bubbles are observed these are removed in the same manner.

During nearly three years we have had to recharge our modified oxygen sensor only six times.

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Effect of Posture of Subject on Reference Values

To the Editor:

In an attempt to provide an on-going physician educational service, we publish a small Pathology Newsletter in which we address, succinctly, a single topic of general interest. In one of our future issues we were going to discuss the topic of reference values vs. hospital population values while simultaneously publishing revised reference values for several analytes. These analytes were determined on a sample of 115 volunteer Red Cross blood donors. The combination of these circumstances made the article (1) by Humphrey et al. extremely interesting and led to the discovery of an oversight on the authors' part. I find no fault with the manipulation of the data or the overall philosophy of the article; however, I must question the procurement of the specimens from the "normal reference group." As is well known, blood donors donate in the supine position with a time variable for completion of the donation. In our experience, the donation, itself, would have a median time interval of 15-20 min. In the article the authors have neglected to define the time at which the samples were procured. Obviously, if the samples were procured at the same time as the pilot tubes, post donation, then their own work indicates that the serum protein and calcium concentrations are in a state of flux rather than equilibria as the article indicates. If this is so, then the deviation from "Reference Group" to "Hospital Population" is even more profound.

It is apparent that in this particular article the referent values (2) should have been more rigidly defined concerning posture and time of specimen procurement.
References

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Two of the authors respond:

To the Editor:
It has been standard procedure in our laboratory to verify normal reference ranges each time when methods were introduced or revised, and to compare these ranges with those cited in the literature. The blood bank provided us with a sample from the donors, which was taken for hemoglobinometry either before or after a posture-dependent normal reference range.

Our findings demonstrated that universally accepted normal reference ranges, including our own, may be inconsistent with the population—i.e., a supine patient population—one wants to study. With our described protocol for the student group, we have shown that posture at the time of sampling can have a significant effect on the reference group and that this protocol describes a reasonable approach for the evaluation of a posture-dependent normal reference range.

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Attempts to Characterize the Fluorescent Compound(s) in Serum of Patients with Chronic Renal Failure

To the Editor:
Galen reported the frequent presence of creatine kinase isoenzyme BB (CK-BB) in serum of patients with renal diseases (1). All the patients who had the alleged CK-BB (demonstrated by electrophoretic separation of serum) also had increased total CK activity. Subsequently, McKenzie and Henderson described a fluorescent substance in serum of patients with end-stage renal disease requiring maintenance hemodialysis (2). This substance appeared as an artefact during the LDH isoenzymes separation on thin-layer agarose gel, even when the specific substrate was not added. For the fluorescent spectrum of the sera, these authors concluded that the natural fluorescence seen in their patients was attributable to an abnormal albumin.

We have also seen a natural fluorescent substance in sera of all renal failure patients on either hemo- or peritoneal dialysis maintenance, and in sera of some patients with myocardial infarction who were tested for CK isoenzymes. The unexpected finding of a natural fluorescence substance was always associated with chronic renal failure, and this fluorescence appears to be a marker for chronic renal diseases. We confirmed that the fluorescence was seen at the point of application on the thin-layer agarose (Corning ACI, Palo Alto, Calif. 94306) and that a narrow fluorescent band appeared in the albumin region after electrophoresis in bivalent buffer (pH 8.6, 60 mmol/liter). In contrast, the CK-BB band migrated faster, in the prealbumin region. This band was seen without the addition of CK substrate, had a stronger fluorescence after concentrating the serum, and was more sharply defined than the CK-BB band. Similar findings were reported in this journal by Coolen and Herbstman (3) and recently rediscovered by Aleyassine et al. (4).

We are aware that the clinical differentiation between acute and chronic renal failure is sometimes difficult; however, this differentiation is of particular importance. Since the presence of a naturally fluorescent substance seems to be ubiquitous in patients with chronic renal failure, the isolation and characterization of this substance could provide a diagnostic tool and a parameter for monitoring the treatment of chronic renal failure, because the intensity of the fluorescence seems to decrease with the improvement of the disease (2, 3, 5).

It is our impression that the natural fluorescent substance, which has a maximum emission near 340 nm, is not a protein; however, due to its great electrophoretic mobility it appears to be bound to a fast-moving serum protein such as albumin or, less likely, prealbumin. Albumin is of course known to bind various molecules. We incubated various amounts of serum from patients with chronic renal failure with anti-albumin (Cappel Laboratories, Inc., Downington, Pa. 19335) and anti-prealbumin (Behring Diagnostics, Somerville, N. J. 08876) sera. The sera were applied to the agarose gel after 1–16 h of incubation at 37 or 4 °C, before and after centrifugation to remove the antigen–antibody precipitates. Surprisingly, the fluorescent band was still seen after incubation with antibodies, suggesting that either the fluorescent substance was not bound to albumin or prealbumin or the ratios of antigen to antibody were not appropriate.

We cannot now ascertain the nature of the fluorescent substance. The absorption–emission spectrum (2) is not similar to that of riboflavin. Because patients with chronic renal failure very often receive supplemental vitamins and anabolic steroids, we added these substances to serum but could not reproduce the same fluorescence. Retinol-binding protein is attached to prealbumin, but we did not show an increased concentration of retinol in serum of patients with chronic renal failure. We concentrated the serum by positive pressure through a Diaflo membrane XM 100A (Amicon Corp., Lexington, Mass. 02173) and obtained a stronger fluorescence, suggesting that the fluorescence is bound to a substance with a molecular weight in excess of 100 000. However, the intensity of the fluorescent band on thin-layer agarose gel electrophoresis, as measured with an ultraviolet densitometer (Model 740; Corning ACI) seemed somewhat to decrease in the same patient after hemodialysis. This is a puzzling finding, because the dialysis membrane only passes molecules with a relatively low molecular weight. Finally, to rule out some fluorescent contaminants in the hemodialysis machine which could remain in the serum after dialysis, we investigated patients on peritoneal dialysis maintenance and found the same fluorescent substance in their sera.

The nature of this substance remains an enigma. Since we could not find it in sera of patients with acute renal failure on hemodialysis, similar to McKenzie and Henderson (2), it appears that the chronicity of the disease rather than the dialysis itself is responsible for the natural fluorescence of the sera. It can be due to an endogenous metabolite but exogenous factors have not been ruled out. Attempts to isolate it by starch–block electrophoresis were so far unsuccessful.

Further studies are in progress for isolation and characterization of this substance whose monitoring promises to be of clinical value.

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References