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 assumed a semi-supine position or within 5 min after they had settled down on the donor couch. In either instance, the sample was obtained before a donation.

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 hemo-

dialysis (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. From 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 barbital 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 rediscov-
ered 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 samples 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 mole-
cules with a relatively low molecular weight. Finally, to rule out some flu-
orescent contaminant in the hemodialysis machine which could remain in the serum after dialysis, we investigated patients on peritoneal dialysis mainte-
nance 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.

The excellent technical help of Miss Joyce Meinke and the support of the Junior Board of The Buffalo General Hospital are acknowledged.

References
