The statistical analyses were done by the Statistics Laboratory, Technion, Haifa, Israel.

Reference

R preceded by \( R \) in the text.

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An Alkaline Phosphatase Isoenzyme Mystery: a Challenge to the Reader

To the Editor:

Like the format of early detective novels, we present here observations on a patient with some remarkable alkaline phosphatase (EC 3.1.3.1, ALP) isoenzyme changes accompanied by a transient abnormality of serum protein electrophoresis, and we challenge the reader to provide an explanation for our findings.

A 21-month-old Nigerian boy was brought by his mother to Medical Outpatients because she thought he was bowlegged. His eight-year-old sister had been previously treated with calcium tablets in Nigeria because she was bowlegged. The boy showed no abnormality on clinical examination. A blood sample showed normal values for serum calcium and inorganic phosphate, but a markedly increased ALP (4258 U/L at 37°C; upper reference limit for this age, 1000 U/L). Because of this, the sample was submitted to ALP isoenzyme electrophoresis (1), with highly abnormal results (Figure 1, left, track 3). Normal liver- and bone-origin ALP were absent, and a single band of material staining for ALP activity was observed migrating slightly cathodal to the origin.

It was at first thought that an ALP-binding immunoglobulin might be responsible for this appearance, though all such immunoglobulins we had previously encountered had stained for ALP activity anodal to the origin, generally in the \( \beta \)-globulin position. To exclude an ALP-binding immunoglobulin, we performed enzyme-immunofixation electrophoresis (2), using rabbit anti-whole human, anti-human G, A, M, and \( D \) heavy chain, and anti-human kappa and lambda light chain antisera. No ALP immunofixation was observed. Proteolysis with pronase, to liberate any bound enzyme, was without effect. The sample was also heat-treated (56°C for 10 min) and the isoenzyme found to be completely heat labile. Neither liver, bone, nor intestinal-origin ALP was bound when sera containing these fractions were added to the heat-treated sample. Taken together, these observations strongly argue against the presence of an enzyme-binding immunoglobulin to account for the electrophoretic abnormality. By including l-homoarginine, 10 mmol/L, or l-phenylalanine, 5 mmol/L, in the staining gel, we found the abnormal ALP band to be homoarginine sensitive and phenylalanine resistant. Together with the heat lability, these are the characteristics of bone-origin ALP.

The volume of sample remaining for examination was insufficient to carry out any further analyses other than serum protein electrophoresis. These results were as remarkably abnormal as the ALP isoenzyme pattern (Figure 1, right, track 5). The sample showed a reduplication of the albumin band (bisalbuminemia), a reduplication of the \( \alpha \)-globulin, and absence or displacement of the \( \alpha_2 \)- and \( \beta \)-globulin fractions, giving rise to two dense bands, one on either side of the position normally occupied by the \( \beta \) fraction, with absence of staining in the usual \( \alpha_2 \)-globulin position.
Wishing to confirm these findings, we requested a second specimen from this child. In addition, to eliminate the possibility of a genetic abnormality, we obtained samples from both parents. The parental samples showed normal total ALP activity, normal ALP isoenzyme patterns, and normal serum protein electrophoresis patterns (Figure 1, right, tracks 2 and 3). In the second sample from the child, obtained 21 days after the original specimen, the ALP had fallen to 1000 U/L and the protein electrophoresis pattern had reverted to normal (Figure 1, right, track 4). The ALP isoenzyme pattern on the child was now quite different from that originally observed, but nevertheless abnormal (Figure 1, left, track 1). Two fractions were visible: a compact band in the a1/a2-globulin position of mobility, between the phosphatases seen in a sample containing liver-origin (a2) and high-molecular-mass "biliary" (a1) ALP (Figure 1, left, track 2), and a second diffuse band with the mobility of bone-origin ALP. These appearances are characteristic of transient hyperphosphatasemia of infancy and childhood (3). At the time of the second sample, a history was obtained of mild upper respiratory illness in two of the boy's older sisters but no serum specimens could be obtained from either, nor any further specimens from the boy.

We are quite at a loss to explain our findings. Transient bisalbuminemia is well recognized in association with pancreatitis and with antibiotic therapy (4), but neither was relevant to this patient. Also, the transient changes in the other protein bands would seem to be unique and certainly have not been reported in association with an abnormal ALP. Special search was made for any unusual factor in the collection or handling of the initial sample but none was revealed. There was no history of any drug administration. Because the second serum showed the ALP pattern typical of transient hyperphosphatasemia of infancy, one might postulate that the abnormal pattern in the first serum represented the ALP isoenzyme pattern at the very onset of the disorder. Failure to encounter this in our previous studies (3) could reflect the greater likelihood of examining sera obtained when the disorder is established rather than at its immediate onset. We have previously speculated that transient hyperphosphatasemia of infancy represents a response to an infection; perhaps the abnormal appearances seen in the first serum sample here represent the very earliest stage of this response.

We have shown that the glycation (sialylation) of ALP is increased in transient hyperphosphatasemia of infancy (3). Post-translational modification of both ALP and serum proteins might also be responsible for the unusual ALP isoenzyme and abnormal protein electrophoretic patterns in the initial sample here, although the nature of any such modification is obscure.

Because of the highly speculative nature of our suggestions, we describe our findings as a "mystery," and challenge readers to provide a (more) satisfactory explanation of our observations.

We thank Dr. D. W. Rogers for permission to investigate this patient and his family.

References

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Effect of Thyroid Hormone-Binding Proteins and Fatty Acids on Modified Analog Assays of FT4 and FT3 in Serum

To the Editor:

Although the final conclusion drawn by Tikanoja and Liewendahl (1) regarding the use of analog FT4 and FT3 assays in nonthyroidally ill patients broadly coincides with views previously expressed by most independent commentators, their note unfortunately conveys an impression regarding the effects of TBG, albumin, TBPA, and free fatty acids in these assays that may be misleading and deserves comment.

Addition of an extraneous substance (such as TBG or oleic acid) to an assay system designed to measure the total amount of an analyte (such as T4) in the test sample can legitimately be said to cause no effect if the assay result is thereby unaffected. However, as previously discussed (2), testing the specificity of free hormone assays fundamentally differs in that the ambient free hormone concentration is not a static, immutable, quantity, but may itself be modified by addition of the extraneous substance. In this circumstance, the substance's true biasing effect on the assay system comprises the difference between the assay result and the (altered) free hormone concentration actually present in the sample (see Figure 1). Thus, in contrast with conventional total analyte assays, the biasing effect of an extraneous substance in a free hormone assay is not necessarily represented by the difference in assay results obtained before and after the substance is added to the system.

Misunderstanding of this point seems to underlie Figure 1 in the report by Tikanoja and Liewendahl (1). For example, these authors apparently concur with the claim by Diagnostic Products Corp. (DPC) that the modified DPC FT4 and FT3 kits show no dependency on the TBG concentration in serum, because the kits yield essentially unchanged results after substantial in vitro addition of TBG to test samples. But addition of exogenous TBG to serum is known to profoundly alter the ambient FT4 level, causing a substantial decrease therein (2). The observation that the assay result remains relatively unaffected in these circumstances is therefore contrary to expectation, and implies that the anticipated decrease in ambient FT4 has been approximately offset by a TBG-induced biasing effect on the assay system.

One possible explanation of this observation is that the binding proteins used in Tikanoja and Liewendahl's experiments contained T4, because certain preparations of TBG (3), like those of albumin, have been shown to retain T4 through purification procedures. Likewise, the binding properties of the particular proteins used by Tikanoja and Liewendahl may have been al-