method described previously (RIANEN; New England Nuclear, Billerica, MA) (2, 5). The measurements were made in duplicate on the same day, with an intra-assay CV of 5%. Aliquots (2 mL) of plasma from each subject were stored at –30 and 4 °C, respectively, for a mean period of 19.5 weeks (SD 9.7, range 2 to 32 weeks) until assay. In addition, we measured total DLIF concentrations from aliquots of pooled plasma stored at –30 °C, for 50, 100, 120, 170, 250, 280, and 300 days. The interassay CV for these measurements (SD 62 ng/L) was 7.2%. Total (protein bound and free) DLIF was measured after pre-diluting the plasma threefold with de-ionized water and heating at 82 °C for 5 min. Heating increases measurable immunoreactivity, and this procedure is accepted as a method for rapidly estimating total DLIF (2, 4). Taking the dilution factor into account, we determined the lower limit of detection in this assay to be 150 ng/L. The free fraction of DLIF was measured directly without pre-dilution or heating; here, the lower limit of detection was 50 ng/L (2, 5).

In 88.8% (16/18) of the women's samples stored at 4 °C, the total DLIF concentrations exceeded those in corresponding samples stored at –30 °C: mean 1094 (SD 389) vs mean 773 (SD 317) ng/L, respectively (P = 0.0012). In 83.3% (15/18) of the samples this was also true for free DLIF: mean 142 (SD 92) vs mean 84 (SD 61) ng/L, respectively (P = 0.0016). The mean total DLIF concentration in the pooled plasma stored at –30 °C [862 (SD 62) ng/L] did not change significantly for up to 300 days (DLIF = 942.93 – 0.44 days, n = 7, r = 0.68, P = 0.091).

We conclude that total and free DLIF activity increases promptly and significantly in plasma samples stored under nonfreezing conditions. Storage of pooled plasma at –30 °C did not significantly affect total DLIF concentrations, confirming a previous report by Clerico et al. (4) on DLIF measurements in urine. We recommend that plasma samples for DLIF measurements be stored at –30 °C.

References

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Creatine Kinase MB Isoenzyme in Rhabdomyosarcoma

To the Editor:

We read with much interest the report by Emancipator et al. (1) of a patient with increased MB isoenzyme of creatine kinase (CK-MB) in serum, apparently deriving from a teratoma with rhabdomyosarcomatous elements. As they point out, a markedly increased proportion in serum of CK-MB of noncardiac origin is very rare. Cases in which this isoenzyme predominates are rarer still and are not associated with cardiac disorders. Therefore, we thought it appropriate to report briefly a further case that displayed this exceptional isoenzyme pattern.

The patient, a 29-year-old man, presented in February 1986 with acute left proptosis accompanied by loss of vision in the left eye. Biopsy from the orbital mass showed a small round-cell tumor, possibly an olfactory neuroblastoma. He was treated at once with radiotherapy, with excellent resolution of the proptosis and return of vision in the eye. In June 1986 he developed left cervical lymphadenopathy, and excision biopsy of a lymph node showed an alveolar rhabdomyosarcoma. On review, the histology of the original orbital tumor was identical.

The patient was treated with six courses of chemotherapy with vincristine, adriamycin, actinomycin D, and cyclophosphamide. His last course of chemotherapy was at the end of October 1986. Once again he had an excellent response to treatment. In November 1986 he complained of back pain, limb pain, and a band-like discomfort around his limbs and pelvis. His symptoms did not respond to simple analgesia, but he was admitted for investigation in December 1986. At this time his creatine kinase (CK) value was high. Bone-marrow examination showed extensive infiltration by alveolar rhabdomyosarcoma. He was treated with chemotherapy with bleomycin, etoposide, and cisplatinum. He failed to respond to this treatment and died in February 1987.

When first measured, serum CK activity was 980 U/L (by the Scandinavian recommended method at 37 °C). Electrophoresis with fluorescence scanning showed the following isoenzyme distribution: MM 637 U/L (65%); MB 274 U/L (28%); and BB 69 U/L (7%). One month later total CK was 235 U/L, of which 23 U/L (9%) was MM, 149 U/L (64%) was MB, and 63 U/L (27%) was BB.

The BB dimer is the predominant isoenzyme of CK in samples of human muscle obtained before 12 weeks of gestation. The pattern then shifts to increasing proportions, successively, of CK-MB, then of CK-MM, with a declining contribution of CK-BB. The adult pattern, with its almost exclusive expression of CK-MM, is usually established by the time of birth (2). In tumors such as that reported here, skeletal muscle loses its mature differentiation and regresses towards an embryonic character. Increasing production of B-CK subunits and declining production of M-CK subunits within the same cell will reach a point at which, by random association of subunits, the MB isoenzyme becomes the major component. The escape of CK isoenzymes from necrotic cells presumably accounts for the developing isoenzyme pattern seen in serum.

Skeletal muscle, and to a lesser extent cardiac muscle, are the only tissues that display this ontological progression from expression of mainly B to mainly M subunits. Therefore, it is only in these tissues that MB-CK is likely to become the predominant isoenzyme as the expression of subunits regresses towards its embryonic pattern. An association between an increased value for plasma CK-MB and rhabdomyosarcoma has been noted previously (3). The increasing number of reports of this association suggests that disproportionately increased CK-MB may have a potentially useful role as a tumor marker in this condition.

References
Benign Transient
Hyperphosphatasemia in an Adult with Malignant Lymphoma

To the Editor:

A transient increase in serum alkaline phosphatase (ALP; EC 3.1.3.1; orthophosphoric-monoester phosphorylase (alkaline optimum)) was first described in apparently healthy infants by Bach in 1954 (1). Posen et al. (2) called the condition "transient hyperphosphatasaemia of infancy" (TH). However, TH has also been described in two adult cases, one by Rosalki and Hurst in 1976 (3) and one by Chisholm in 1986 (4). Here we report the third case of TH in an adult, a woman with malignant lymphoma.

In 1979, a 55-year-old woman with symptoms of lymphadenopathy in the right axillary and inguinal region was admitted to the Shizuoka Red Cross Hospital and pathologically diagnosed as having lymphocytic lymphoma (small diffuse type, LSG classification) by biopsy of the lymph node. The patient was treated with cyclophosphamide, oncovin, and prednisolone, whereupon she underwent complete remission of the disease. The patient has shown no signs or symptoms of recurrence and she is still in remission. In November of 1986, however, she was admitted with a complaint of abdominal pain and macrohematuria. Intravenous pyelography and computer tomography showed no evidence of ureterolithiasis or kidney tumor. The patient's ALP was markedly increased, to 2439 U/L (adult reference interval: 100–270 U/L). Other enzyme measurements were 461 U/L for lactate dehydrogenase (220–420 U/L), 26 U/L for aspartate aminotransferase (10–30 U/L), 9 U/L for alanine aminotransferase (3–25 U/L), and 11 U/L for gamma-glutamyltransferase (0–40 U/L). Her calcium concentration was 2.15 mmol/L (2–2.5 mmol/L) and phosphate was 0.87 mmol/L (0.80–1.30 mmol/L). Bone scintiscan and scintiscan with gallium showed neither bone abnormalities nor the recurrence of lymphoma. The cause of the abdominal pain and macrohematuria was unclear, and the patient was diagnosed as having idiopathic renal hemorrhage.

The ALP activity concentrations before and after the extremely high measurements were consistent, and the increase in ALP showed a transient course (Figure 1). No other enzymes showed significant changes comparable with the increase in ALP activity.

Electrophoretic analysis of the highly increased ALP activity in the patient's serum revealed typical double peaks characteristic for TH. The fast a2 band has been reported in most cases with TH (3), and its presence suffices for a diagnosis of TH (5). The patient's ALP returned almost to the upper limit of the reference interval within five weeks.

The ALP activity in the present case is very similar to that described in most reports about TH. Such reports have predominantly dealt with young children, especially those younger than five years. Because we report the third case of TH in an adult, we prefer calling this condition "benign transient hyperphosphatasemia" (5) instead of the name "transient hyperphosphatasemia of infancy" proposed by Posen et al. (2).

References

More on Ornithine Decarboxylase

To the Editor:

Further to the comments of Carakostas (1), at least three non-isotopic methods for ornithine decarboxylase (ODC, EC 4.1.1.17) have been described. These methods involve either HPLC with fluorescence detection (2) or spectrophotometry with use of the substrates 2,4-dinitrofluorobenzene or 2,4,6-trinitrobenzenesulfonic acid (3, 4). The inconvenience of an assay is not an overriding factor in drug safety evaluation studies, but the case for assaying serum ODC is unproven. Until the current guidelines are revised, ODC remains a "suggested" rather than a "mandatory" test. Indeed, published data for tissue ODC measurements may require re-evaluation in view of the artefactual increases that follow the freezing and thawing of whole rat tissues (5).

References