proteinase properties, for example, alpha,-antitrypsin. On the possibility that the additional isoenzyme, or one or more of these proteins, has the effect just described, we are carrying out further investigations to try to elucidate this.

References

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Radioimmunoassay Used to Monitor Therapy with Testosterone

To the Editor: Radioimmunoassay (RIA) for testosterone is used in many laboratories to monitor therapy with testosterone. Traditionally, a preliminary extraction into organic solvent is required, to isolate the steroid and separate it from its binding proteins. Kits not requiring such extraction are now being marketed. We compared two such assays, BIO-RIA's "125I-Testosterone" and Mallinckrodt's "RIA-mat Testosterone" with a method that requires extraction, Amersham's "Testosterone/Dihydrotestosterone RIA" kit. The assays were all done according to the manufacturers' instructions. In investigating the patients being treated with testosterone, we also performed the BIO-RIA assay on plasma extracted with dichloromethane in a manner identical to that used for the Amersham method.

Although we found good intercorrelation of the assay results when measuring endogenous testosterone (correlation coefficients of 0.90 to 0.94), results disagreed if the patients were being treated with testosterone analogs, methyl testosterone or testosterone oenanthate (Table 1).

| Table 1. Data on Six Patients Being Treated with Testosterone Analogs |
|-----------------|-----------------|
| BIO-RIA        | Amersham        |
| Plasma          | Extract          | (extract)         |
| Testosterone, nmol/L   |                  |
| Methyl testosterone |                  |
| >69>69          | 4.0             |
| >69>45          | 4.9             |
| Testosterone oenanthate |                  |
| >69>5.4         | 14.9            |
| >69>21          | 32.3            |
| >69>1.8         | 29.3            |
| 18.3>3.2        | 19.1            |

Plasma from patients receiving methyl testosterone gave high values with BIO-RIA reagents. The fact that the values for extracted plasma were of the same order as the direct values shows that the drug was extracted. Hence, the low values found with the Amersham reagent must be related to a lower affinity of the Amersham antibody for methyl testosterone.

The case of testosterone oenanthate is more complicated. Very high values were obtained with the direct BIO-RIA assay, but low values were obtained with the same reagent for extracted plasma. Evidently the plasma contains non-extractable metabolites with an affinity for the BIO-RIA antibody.

The Amersham kit values for extracted plasma were higher than the BIO-RIA values for extracted plasma. This shows a greater cross reactivity of the Amersham antibody for extracted metabolites. However, the Amersham results were lower than the direct BIO-RIA values for plasma. Thus there are both extraction and antibody cross-reactivity problems with testosterone oenanthate. We do not know which of these metabolites, extractable or non-extractable (or both), are biologically active, and to what extent each cross reacts in the assay.

Further studies with testosterone oenanthate, testosterone β-glucuronide, and 17α-methyl testosterone (obtained from Sigma Chemical Co.) showed that the presence of the large oenanthate and glucuronide groups on the molecule blocked the reaction with the antibodies from the three sources we examined. The 17α-methyl group hindered the reactivity of the antibodies to different extents.

The curves obtained with BIO-RIA reagents for testosterone and methyl testosterone were superimposable. The binding curve for methyl testosterone with Amersham reagents was almost horizontal, showing low antibody affinity and confirming the patients' data. The binding curve for methyl testosterone with Mallinckrodt reagents paralleled that of testosterone but showed lower affinity for the drug. This means that measured results for testosterone in the plasma of patients treated with methyl testosterone would differ among the three different sets of reagents used.

We conclude that the values for testosterone obtained by RIA for patients on replacement therapy may not have the same clinical significance as the values for endogenous testosterone.

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Lactate Dehydrogenase and Its Isoenzyme, LDH-1, in Serum Are Markers of Testicular Germ Cell Tumors

To the Editor: The observations of Vladutiu (1) do not allow any conclusions as to the histological pattern of testicular germ cell tumors from the finding of increased LDH (1.1.1.27) and (or) LDH-1 (only in serum. This agrees with our experience (2). We found no statistically significant difference between LDH activity in the serum of 11 patients with lesions from seminomas, five patients with embryonal carcinoma, or 21 with other nonseminomatous testicular germ cell tumors. Of 11 patients with seminomas, five had increased LDH but only one had increased LDH-1 (the only increased isoenzyme, 72% of the 920 U/L total) in the serum. Of 26 patients with nonseminomatous testicular germ cell tumors, 10 had increased LDH and, of these, only three had increased LDH-1 only. One of these with seminoma + embryonal carcinoma + yolk sac tumor had 63% LDH-1 of the total 610 U of LDH per liter, and one with seminoma + embryonal carcinoma + immature teratoma had 59% of the 680 U/L total. The third patient, with lesions from only yolk sac tumor of the testis, had LDH-1 that was 87% of the 570 U/L total LDH in serum collected from a peripheral arm.