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Single Injection of Testosterone to 7 Volunteers: Results from this Study

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Introduction

Testosterone is on the doping list. Endogenously synthesized androgens can be discriminated from that exogenously administered. Donike et al have shown that there is a difference in metabolism between endogenous and exogenous testosterone (1). There is practically no formation of epitestosterone from exogenous testosterone and furthermore the formation of epitestosterone is depressed by the exogenous testosterone. As a consequence of this finding the testosterone doping can be expressed as a testosterone to epitestosterone ratio (T/ET ratio).

Even if the statistical evidence provided by Donike et al is accepted, it may always be argued that exceptions may occur and that there is a lack of knowledge about all factors that may possibly affect the ratio.

According to our opinion there is a definite need for additional criteria in order to confirm a case of testosterone doping.

In order to find supplementary tests for testosterone doping, we have repeatedly followed a number of serum and urinary androgens and androgen precursors, estrogens and LH in seven healthy volunteers during 35 days after an intramuscular injection of 250 mg of testosterone enanthate (Carlström et al, accepted for publication). Each subject received an i. m. injection on day 0. Samples of venous blood and urine random samples were collected on the days 1, 2, 4, 7, 9, 11, 17, 25 and 35 after injection.

All samples from the same subject were analyzed in the same single assay in order to avoid between assay variation.

Among urinary analyses, only the T/ET ratio could be judged as a suitable marker of testosterone doping.

Of the serum assays, 17 α -OH-progesterone (17OHP), T/17OHP ratio, LH and T/LH ratio were fair to good markers of testosterone doping.

For calculation of T/ET ratio new synthesized deuterated testosterone (D-T) and deuterated epitestosterone (D-ET) were used (Garle et al, to be published).

Determination of Testosterone/Epitestosterone ratio in urine samples

The lack of appropriate method to determine the amount of testosterone and epitestosterone in order to find the ratio for confirmation of the positive cases of testosterone, makes the existing procedure for calculation the T/ET ratio complicated and time consuming. We believe that a use of deuterium-labeled internal standards for epitestosterone and testosterone should increase the accuracy of the T/ET ratio measurements.

We succeeded to produce both deuterated testosterone and epitestosterone in the same procedure.

Androst-4-ene-3,17-dione was refluxed with a solution of KOH in MeOD and D₂O (10:1,v/v). The products of this reaction, deuterated-androst-4-ene-3,17-diones were dissolved in dry toluene and treated with aluminium-tert-butoxide in sec-butanol by reflux. As a result of this reaction deuterated testosterone and deuterated epitestosterone were obtained. For separation of the products HPLC technique was used.

The mass spectra confirm the presence of both deuterated testosterone and deuterated epitestosterone.

The amounts of testosterone and epitestosterone in our study were calculated from standard curves, which were prepared from 6 standard solutions containing both testosterone and epitestosterone. Ethanolic solutions of various amounts of testosterone and epitestosterone were added to 5ml 0.26M sodium acetate buffer pH 5.2. To the standard curve (duplicates) and to the urine samples (triplicates) internal standard solution was added

This solution contains deuterated epitestosterone and deuterated testosterone in ethanol at ratio of 1 to 6.

All samples were then worked up according to the screening procedure for anabolic steroids in total fraction. Samples were analysed on MS-Engine.

The standard curves obtained with this method shows very good linearity.

Results

The urinary testosterone and epitestosterone amount (ng/ml) and T/ET ratios in the 7 volunteers are presented in the figures 1 to 7. One of the subjects shows T/ET ratio below 6, two of them between 6 and 10 and four of them higher than 10. These results show that there is a great variation of T/ET ratios after the single injection of testosterone enanthate.

The number of abnormal values for the individual serum analytes recorded at the different test occasions are given in table 1. Statistical analysis was performed by Wilcoxon's signed rank test.

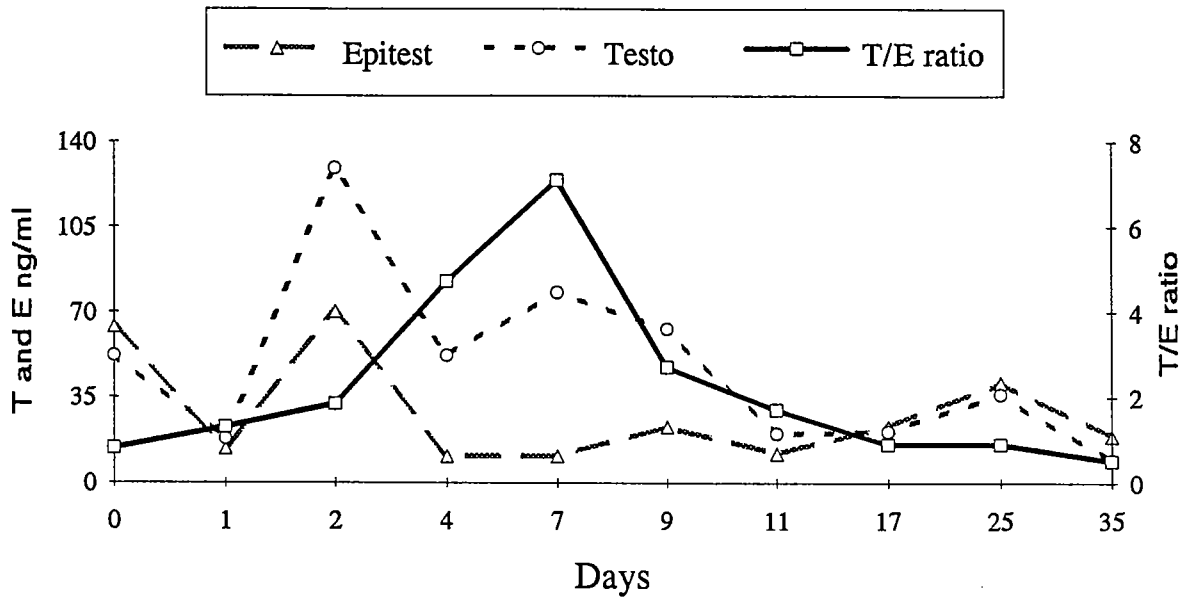
Injection of testosterone enanthate caused significant changes from the day 0 concentrations for all variables at one or several test occasions. However, only T/17OHP-ratio shows abnormal values for all 7 subjects at the same test occasion. Serum 17OHP was analyzed radioimmunologically after extraction with diethyl ether by a method developed at the Department of Obstetrics and Gynecology (2-4), using the antibodies specified in the paper of Stege et al (4).

As could be expected, the ratio between serum T and LH was about as sensitive marker for testosterone doping as the T/ET ratio. Measurement of LH might give additional valuable information in suspected cases of testosterone doping. In view of this, we believe that both the T/17OHP and T/LH ratios in serum should be measured in all suspected cases of testosterone doping. However, since there are no reference methods available for measurement and identification of the peptide hormone LH, we believe that LH measurements may have less impact in connection with legal challenges. On the other hand it is most probably possible to quantitate 17OHP in serum by isotope dilution mass spectrometry.

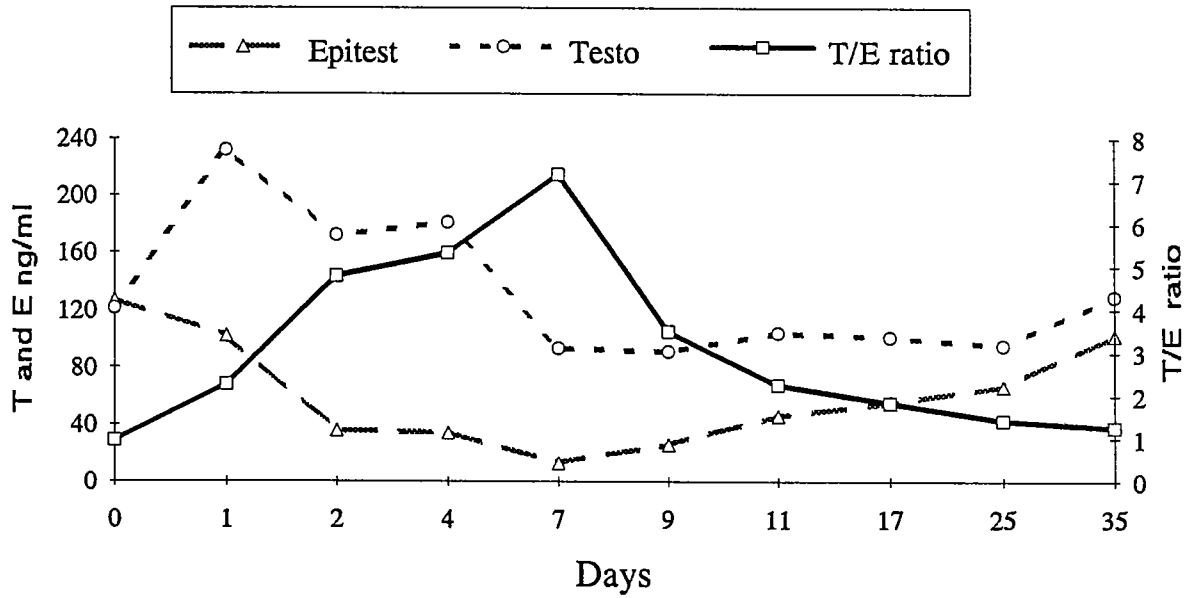
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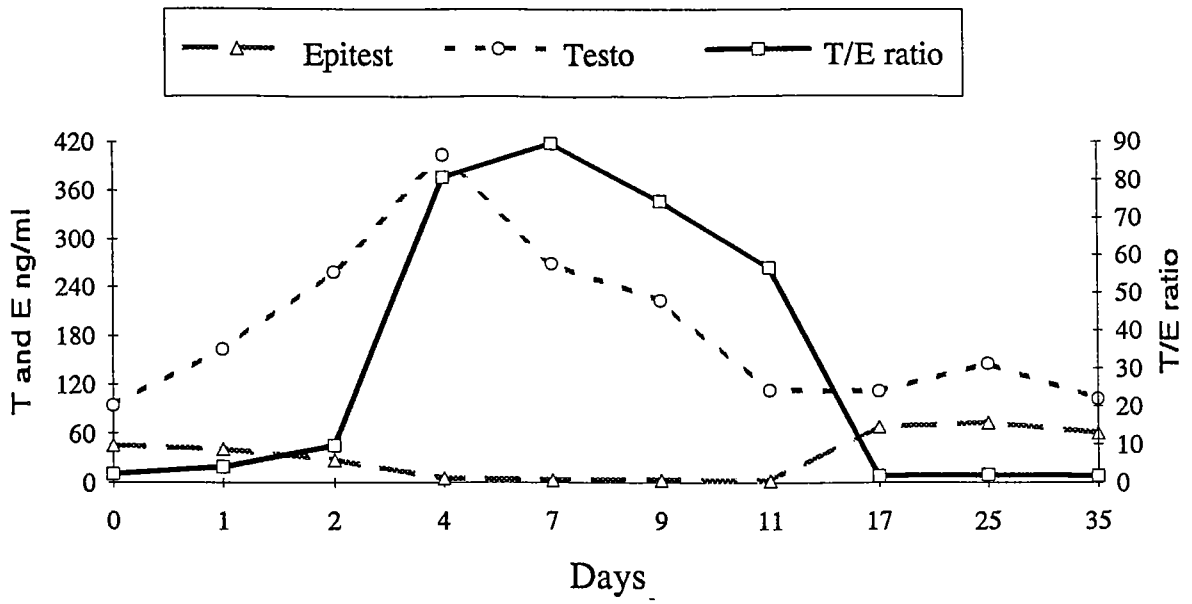
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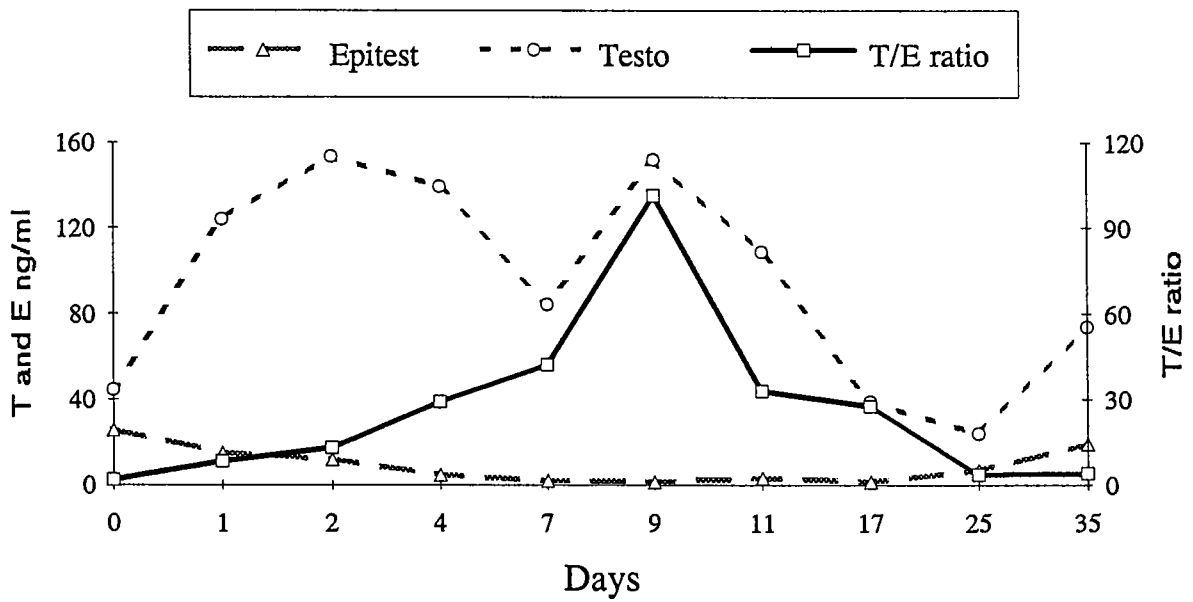
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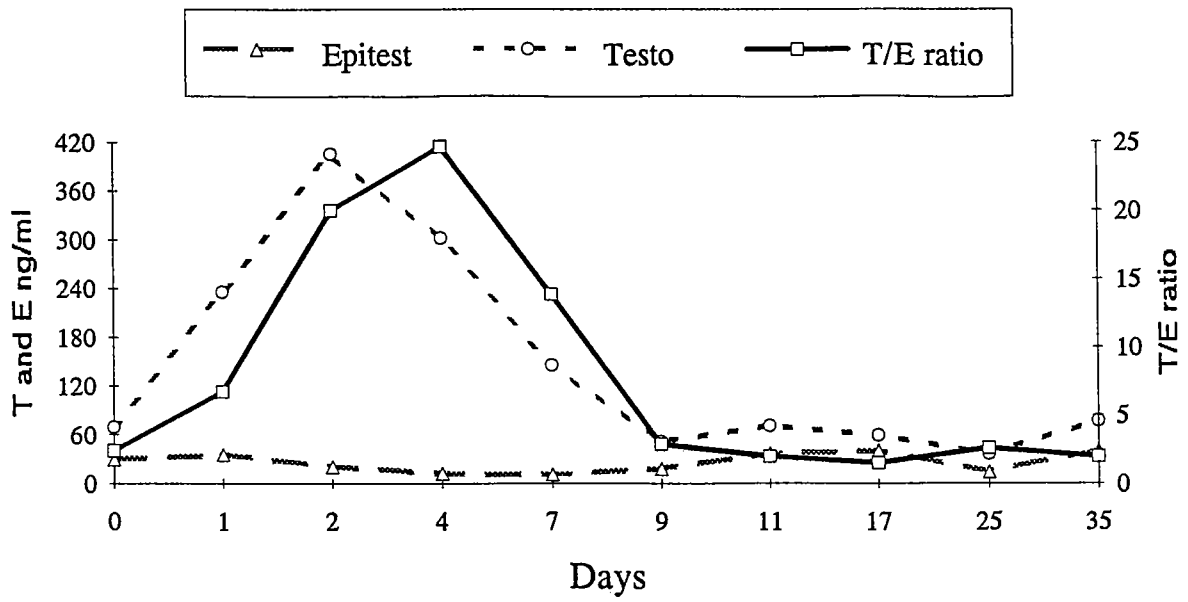
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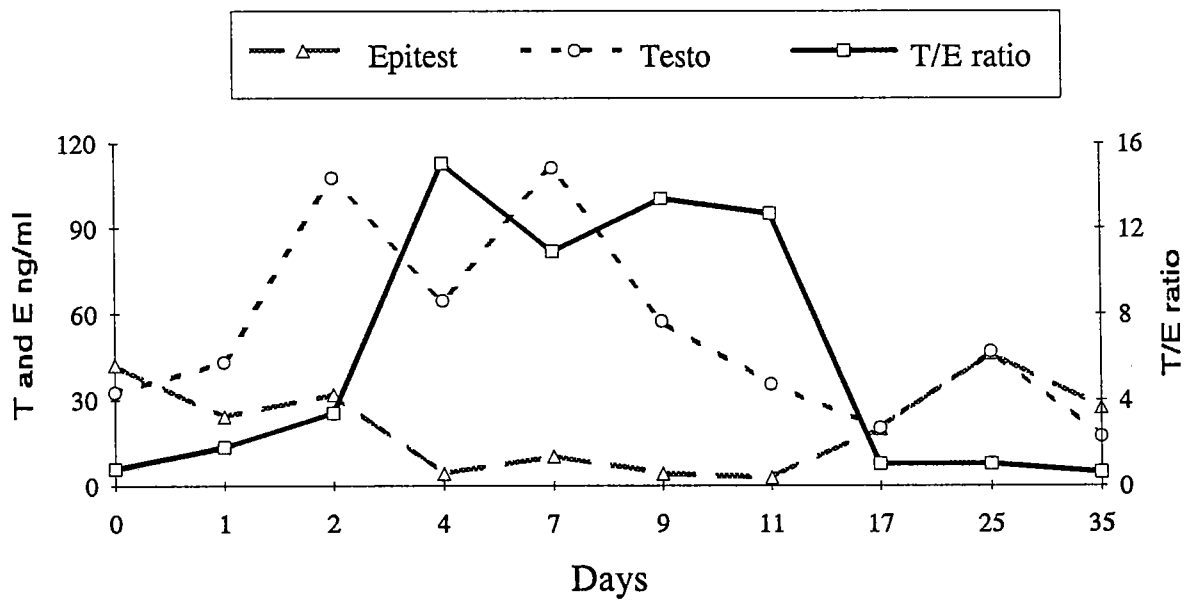
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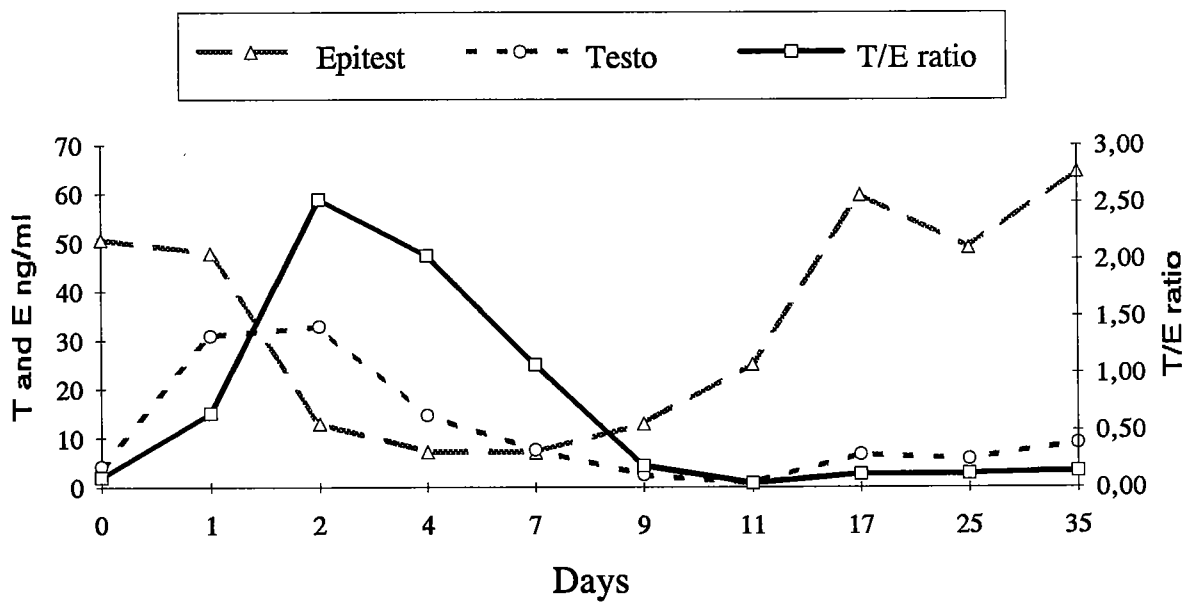
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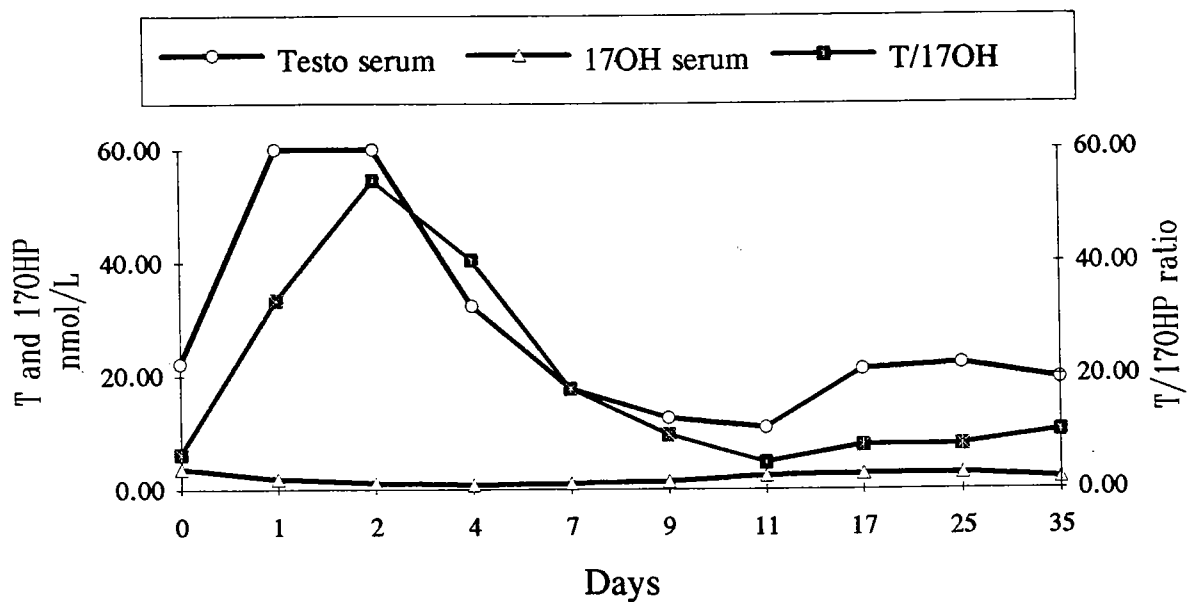
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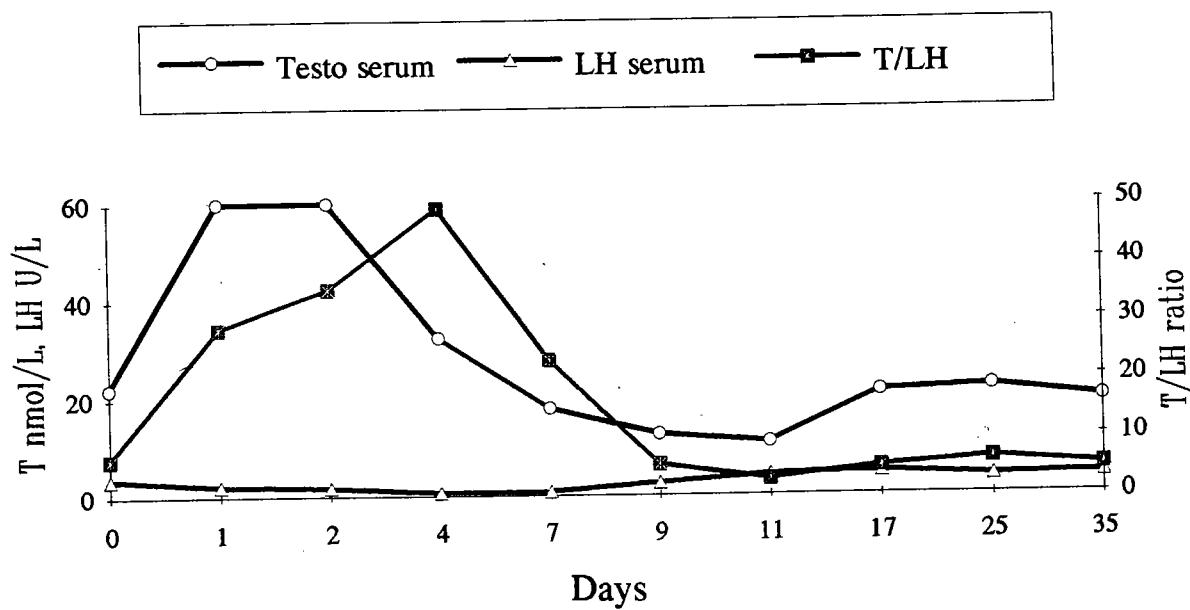
Volunteer CS



Volunteer CS



Volunteer CS



NUMBER of the seven test subjects with ABNORMAL
 SERUM HORMONE VALUES after i.m. injection of
 250 mg TESTOSTERONE enanthate

Statistics by WILCOXONS rank test

DAY after inj.	1	2	4	7	9	11	17	25	35
S-T	5/7	2/7	2/7	1/7	0/7	0/7	0/7	0/5	0/6
S-17OHP	0/7	3/7	6/7	5/7	5/7	4/7	1/7	1/5	1/6
S-T/17OHP	7/7	7/7	7/7	6/7	6/7	5/7	1/7	1/5	3/6
S-LH	0/7	0/7	4/7	5/7	4/7	4/7	0/7	0/5	0/6
S-T/LH	4/7	6/7	6/7	6/7	4/7	4/7	0/7	0/5	0/6