β-Endorphin in normozoospermic and pathologic human semen

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Summary. β-Endorphin was estimated in normozoospermic, oligozoospermic and azoospermic human semen. The mean amount in normozoospermic specimens was 278.6 ± 43.6 (SE) pg/ml while in the others only 191.1 ± 25 pg/ml. Both values are significantly higher than those present in the blood.
Key words: Semen, human, pathologic; β-endorphin.

The interest generated by the discovery of endogenous opioid peptides was further stimulated by the evidence that these substances might be involved in a variety of physiological processes aside from pain perception and analgesia. The identification of β-endorphin (β-E) and related peptides in compartments of the mammalian male genital system and in the semen[1–3], and the postulation that β-E may have a regulating role in male reproduction[1] prompted us to study the presence of this compound in normozoospermic, azoospermic and oligozoospermic human seminal plasma as well as in sonicates of isolated spermatozoa. It was our purpose to determine a possible correlation between sperm production and the levels of β-E in the compartments of the male genital system and/or its secretion in situ, as evidenced by the concentration of this peptide in the semen.

The material studied included 43 samples of seminal plasma from semen obtained after 4 days of abstinence from patients ranging in age from 19 to 55 years. Of these 12 were normozoospermic ($> 40 \times 10^6$ sperm/ml) – group A; group B consisted of 27 oligozoospermic and 4 azoospermic patients (8 suffering from varicocele, stages I and II, one with hypogonadism, 2 with cryptorchidism in childhood and 20 with fertility disorders and oligozoospermia of unknown origin). Promptly after liquefaction the following andrologic parameters were assessed: volume, sperm count, motility (% and grade), % viability and % normal morphology. The remaining volume was centrifuged at 15,000 rpm for 15 min at 0 °C and the plasma stored. Spermatozoa from 11 normozoospermic and 4 oligozoospermic (of unknown cause) specimens were treated as follows: suspensions of twice-washed sperm (0.05 M Tris-HCl buffer, pH 7.4) were frozen 6 times with solid CO_2 for 4 sec and brought to 0 °C, sonicated for 4 min at maximal speed and stored. β-E levels were determined by radioimmunoassay using the kit from the Immuno Nuclear Corporation (Stillwater, MN, USA). The method consisted of the removal of β-lipotropin by stripping with sepharose-anti-β-lipotropin, extraction of β-E from the acidified stripped seminal plasma or sperm suspension on octadecasilyl silica columns and, after elution with methanol, reconstitution in buffer of the air-dried samples. β-E values were calculated per ml seminal plasma or per 10^8 sperm. In group A values were: volume – 3.5 ± 1.3 (SD) ml; % motility : 45.2 ± 9.8; motility grade : 2.5 ± 0.99; % vitality : 50.5 ± 2; % normal morphology : 39.1 ± 5.3; β-E : 278.6 ± 144 pg/ml; group B: volume : 3.8 ± 1.2 ml; % motility : 39.3 ± 16.9; motility grade : 1.6 ± 0.66; % vitality : 44.0 ± 14.9; normal morphology : 22.7 ± 9.8; β-E : 191.1 ± 127 pg/ml. Values of β-E in sonicates of sperm were 48.4 ± 27.2 and 229.5 ± 225 pg/10^8, respectively. Statistical analysis of the differences between the groups (Student's t-test and Mann Whitney U-test) revealed a p value of 0.05 for the seminal plasma and 0.02–0.01 for the sperm sonicates.

The identification of β-E and related peptides in a variety of tissues and the wide range of their activities constitute an indication of their participation in basic physiological processes[4–15]. Their presence in the male reproductive organs and semen is therefore not unexpected and it appeared to be of importance to clarify: 1) the origin and possible role of the high levels of β-E in the semen; 2) the reason for the differences in concentrations of β-E in normozoospermic specimens and those which were oligozoospermic or azoospermic. In regard to the former, it is worth noting that the values in human blood, as reported by others[16–18] and found in our laboratory as well, for both normozoospermic males and those suffering from fertility impairment, were up to 48 times lower than in the semen. As for the latter, it is stressed that the mean β-E value in group A was 278.6 ± 144 pg/ml, similar to the value reported by Sharp and Pekary[3], whereas that in group B was only 191.1 ± 127 pg/ml, with statistically significant difference ($p = 0.05$).

The high concentration of β-E in the seminal plasma and its abundance in the prostate, seminal vesicle and testes[1,2] do not seem to support passive penetration from the blood. Rather it appears that this substance would be synthesized or actively pooled in the male genital system. This assumption would be in line with the view that β-E and other endogenous peptides have a regulatory function in sexual behavior and are involved in the reproductive process of the male[19]. This is supported by data indicating that β-E, to which the role of both hormone and neurotransmitter have been ascribed[20], participates in the control of pituitary function[21,22], thus modulating the circulatory levels of LH, FSH, PRL, GH[13,23,24] and possibly other hormones. β-E of the male genital system would influence the latter activity by a feed-back mechanism which could obviously be affected by differences in the β-E levels. The significantly higher concentrations of β-E in sperm of pathologic origin as compared to those in the normozoospermic specimens may be associated with membrane abnormalities in the former; these could influence the extractibility or be related to the differences in initial content of β-E, as observed for other constituents of human sperm[25–27]. Should there be a leak of β-E from the sperm into the plasma prior to liquefaction then there should be a more significant difference between groups A and B due to the higher contribution of β-E from the oligozoospermic specimens. On the other hand, the higher sperm count in normozoospermia might act to mask this phenomenon.

On the basis of our present results we are not in a position to explain the differences in β-E content between the plasma of normozoospermic origin and that from semen with a low sperm count. Not to be excluded is the possibility that oligozoospermic and azoospermic specimens are richer than the normozoospermic specimens in peptidases which decompose β-E during the period elapsing between ejaculation and liquefaction.

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Conversion of clionasterol into both fucosterol and isofucosterol by the insect *Tenebrio molitor*

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Summary. [7,7-$^3H_2$]Clionasterol was synthesized and fed, together with [4-^{14}C]sitosterol, to *Tenebrio molitor* larvae; fucosterol and isofucosterol, recovered from the sterol fraction, were found to be doubly labeled, indicating that clionasterol is converted into both the ethylidenic compounds.
Key words. Tenebrio molitor; clionasterol; fucosterol; isofucosterol.

Most phytophagous insects convert C_{29} phytosterols into cholesterol (**3**)[1] through a sequence (scheme 1) involving dehydrogenation, epoxidation and rearrangement with loss of a C_2 fragment followed by reduction of the obtained double bond. In *Tenebrio molitor* both sitosterol (**1a**) and its C-24 epimer clionasterol (**1b**) are metabolized[2] and we have shown[3] that the same insect transforms the former compound into both the E and Z $\Delta^{24(28)}$-ethylidenic intermediates fucosterol (**2a**) and isofucosterol (**2b**). The question whether the dealkylation process of clionasterol (**1b**) shows the same lack of stereospecificity, i.e. whether (**1b**) ist also converted into both the double bond compounds (**2a**) and (**2b**), is still unanswered[4].

In order to clarify this metabolic aspect, we synthesized [7,7-$^3H_2$] clionasterol and tested its conversion into (**2a**) and (**2b**) by *Tenebrio molitor* larvae.

Poriferasterol (**4**), obtained from *Ochromonas malhamensis*[5], was transformed[6] into (22E,24R)-3α,5α-cyclostigmast-22-en-6-one (**5**)[7], from which (24S)-3α,5α-cyclostigmastan-6-one (**6**)[7] was obtained by catalytic hydrogenation on Pd/C. The introduction of tritium at C-7 of (**6**), the reduction of the 6-oxo function, and the treatment with $Zn(OAc)_2$/AcOH were carried out according to the usual procedure[6], and afforded [7,7-$^3H_2$]clionasteryl acetate (7a) which was hydrolyzed to [7,7-$^3H_2$]clionasterol (**7b**) (spec. act. 2.48×10^6 dpm of ^{3}H/mg).

[7,7-$^3H_2$]clionasterol (**7b**) (2.23×10^6 dpm of ^{3}H) was mixed with [4-^{14}C]sitosterol (**8**) (9.50×10^5 dpm of ^{14}C, spec. act. 2.85×10^8 dpm of ^{14}C/mg, the Radiochemical Centre, Amersham) and fed, together with unlabeled fucosterol and isofucosterol as cold traps, to young *Tenebrio molitor* larvae. Two days later the larvae were frozen and from the benzoylated sterol fraction[8] pure fucosteryl (**9a**) and isofucosteryl (**10a**) benzoates were obtained by repeated argentation t.l.c. The 2 benzoates (**9a**) and (**10a**) were diluted with cold material and crystallized to constant specific activity; the free sterols (**9b**) and (**10b**), obtained by alkaline hydrolysis, were also crystallized and counted. The values obtained are summarized in the table.

The data obtained clearly show that clionasterol (**1b**) is converted into fucosterol (**2a**) and isofucosterol (**2b**) as both the compounds were found doubly labeled. Furthermore, from the ^{3}H/^{14}C ratios of the recovered compounds it can be deduced that clionasterol is converted into fucosterol with about the same efficiency as sitosterol, whereas it is transformed into isofucosterol less readily than sitosterol.

Moreover, the different ^{3}H/^{14}C ratios of fucosterol (**2a**) and isofucosterol (**2b**) make it unlikely that the sequential formation of (**2a**) and (**2b**) is the only operative pathway; they seem rather to be in agreement with parallel transformations of the 24-ethyl precursors into the $\Delta^{24(28)}$-ethylidenic intermediates.

Total radioactivities and ^{3}H/^{14}C ratios of the administered precursors and of the recovered products

Compounds	dpm of ^{14}C	dpm of ^{3}H	^{3}H/^{14}C
[7, 7-$^3H_2$] Clionasterol (**7b**) + [4-^{14}C] sitosterol (**8**)	9.50×10^5	2.23×10^6	2.35
[4-^{14}C, 7, 7-$^3H_2$] Fucosteryl benzoate (**9a**)	1.42×10^3	3.58×10^3	2.52
[4-^{14}C, 7, 7-$^3H_2$] Fucosterol (**9b**)	1.48×10^3	3.82×10^3	2.58
[4-^{14}C, 7, 7-$^3H_2$] Isofucosteryl benzoate (**10a**)	0.85×10^3	1.08×10^3	1.27
[4-^{14}C, 7, 7-$^3H_2$] Isofucosterol (**10b**)	0.86×10^3	1.15×10^3	1.34