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Hormone residue levels in young bulls after treatment using anabolic agents.(trenbolone acetate and 17 beta estradiol).

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Simultaneous administration of trenbolone acetate(TBA) plus estradiol is a treatment with proven efficiency in improving the growth rate of castrated male farm animals(1,2).
The possible consequences of the treatment,in relation to public health aspect,are of course linked to the residue levels in meat.
Trenbolone(TBOH) is the main metabolite found in meat of treated cattle(3). A specific radioimmunoassay for trenbolone acetate and trenbolone allows sensitive residue determination in meat and in plasma.
The present study investigated the level of trenbolone residues in plasma in the function of time and,after slaughtering,inmuscles of young bulls treated with a mixture of trenbolone acetate and 17 beta estradiol administated as intramuscular injection or subcutaneous implantation.

Materials and methods.

Treatment
TORELOR (200 mg trenbolone acetate plus 40 mg 17 beta estradiol)was administrated as a slow release subcutaneous implant while intramuscular injections were performed using a mixture of FINAJECT(trenbolone acetate) and GYNOESTRYL(17 beta estradiol benzoate).
In the beginning of the experiment,weights of bulls were about 300 kgs.The animals were divided in 3 groups.

Group 1
Four bulls(n°1,2,3,4) were injected twice at 13 days interval as described in table 1.
Table 1:injected doses(mg)

	T1		T2		T3		T4	
	1st	2d	1st	2d	1st	2d	1st	2d
TBA	37	30	75	60	150	30	300	60
17 beta estradiol benzoate	5	4	10	8	20	4	40	8

Plasma samples were taken up 2,4,6 hours and 1,2,3 days after injection.

Group 2
24 young bulls were implanted subcutaneously in the ear basis.After implantation,samples were taken up weekly or every two weeks.Animals were slaugthered in 4 groups;76,110,124 and 138 days after implantation.

Group 3
23 young bulls were first implanted as in group 2.
At day 165, 9 animals received a second implant,while 9 untreated animals received their first implant.
Plasma were drawn monthly.Slaugthering of animals was performed as described in table 2.

Table 2: Delay(days)between last implantation and slaugthering.

Day	66	87	114	211	231	252	280
Number of animals:							
Once treated	4	2	4	5	2	2	4
Twice treated	3	4	2				

In groups 2 and 3,muscles(m.diaphragma) were examined for their trenbolone content.

Measurements of TBA and TBOH in plasma and muscles.

Samples were extracted using diethylether.The extracts were purified by chromatography on dry magnesium oxide.Radioimmunoassay was performed on purified extracts using the procedure described by Hoffman(4). Trenbolone acetate antiserum(batch n°4049) and the radiolabelled 6,7,^{3}H trenbolone(58 Ci/mmole) were kindly provided by Dr Jouquet(Roussel-UCLAF,Paris). Conjugated trenbolone was hydrolysed in the presence of 500 mU/ml of E.Coli beta glucuronidase(E.C.:3.2.1.31) before the assay.

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Results and discussion.

Figure 1 shows the decrease of free TBOH as a function of time in plasma of the 4 bulls of group 1 after first and second injection.The logarithmic plot of figure 2 indicates that the hormone elimination follows an exponential law after injection.For each animal,elimination rate appears constant.The maximum levels of residues in plasma are not proportional to the injected doses as the 4 bull plasmas contained between 2 and 5 ng/ml of free TBOH a few hours after injection while injected doses varied from 37 to 300 mg. Nevertheless,residue levels decrease slower for animal injected with higher doses.
Metabolisation rates after the second treatment does not seem being affected by the doses used in the first injection.
Evaluation of plasma levels in implanted animals of group 2 (figure 3) is clearly different from that observed after injection(group 1,figure 1).Indeed,kinetics of hormone elimination after implantation show irregular profiles involving random peaks.The individual clearance picture is highly variable indicating a discontinuous implant resorption variable with animals.
Levels of conjugated TBOH in plasma are very low and become undetectable after 42 days while,84 days post implantation,free TBOH is still measurable.
In figure 4,plasma levels observed after a second implantation of 9 bulls are higher than that observed for the 9 animals which only received one implant.
As observed in plasma,residue levels in muscles show large individual variations(figure 5).
The elimination rate in muscle is very slow.According to this criteria,animals can be classified in three groups:
a. Slaughtering 2 to 3 months after implantation: 50-100 ppt.
b. Slaughtering 3.5 to 4.5 months after implantation: 0-50 ppt.
c. Slaughtering after 8 months post implantation: no significant difference between treated and untreated animals.
Thus,generally recommended delays between trenbolone implantation and slaugthering(60-70 days) are not long enough to reach residue concentrations under the radioimmunoassay detection limit.This only occurs after about 200 days.
Finally,there is no evidence of correlation between residue levels in blood and in muscle within the same animal.

Fig.1. Kinetics of free TBOH plasma levels (pg/ml) in bulls injected with Finaject + Cypionestryl.Animals were treated at day 0 and 13 days after the 1st injection.

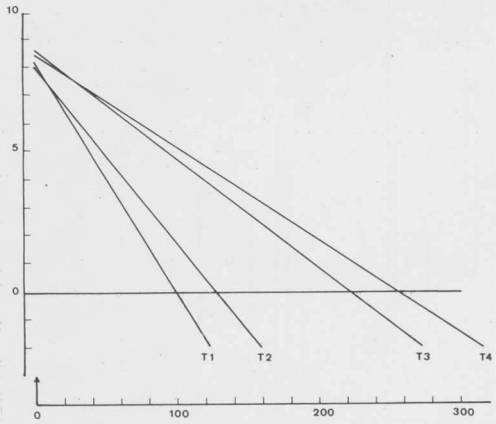


Fig.2. The results of figure 1 are presented as a linear regression of natural logarithms of TBOH plasma levels(pg/ml) versus time(days).

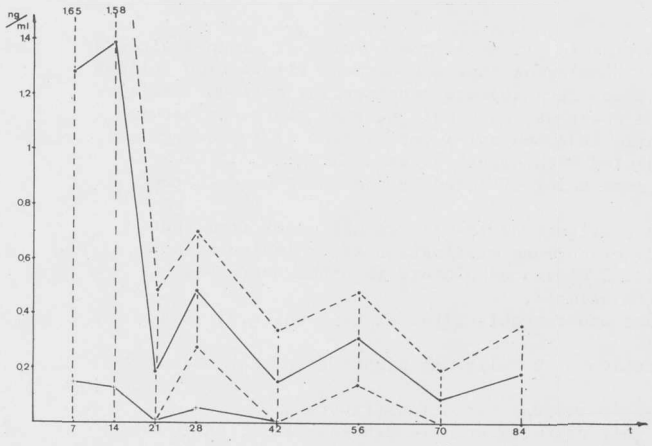
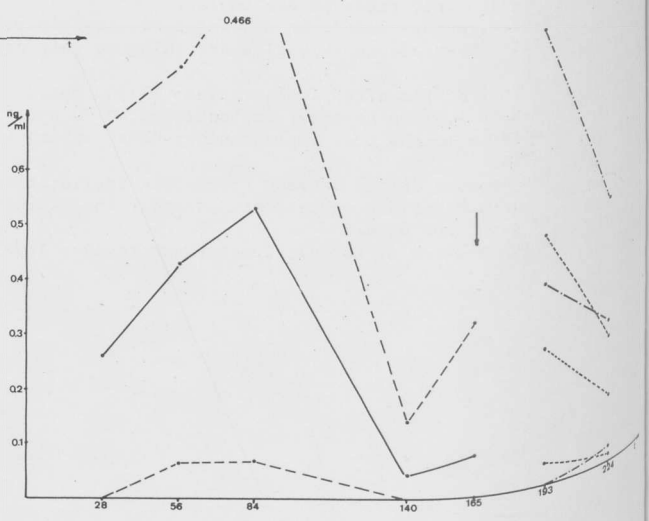


Fig.3. Kinetics of free(o———o)and conjugated (←+——+—)TBOH in plasma of bulls implanted with Torelor.Means of plasma levels(n=24) and limits of standard error(o— — — —o)are show in the function of time (days).

Fig.4. Kinetics of free TBOH in plasma of bulls implanted with Torelor.
The left part of the figure shows means(o———o) of plasma levels(n=23) and limits of standard error(o— — —o) versus time(days).
Comparative data from bulls implanted once or twice are given in the right part of the figure.
o—·—·—o Means of plasma levels in animals(n=9) implanted twice at day 0 and day 165. Limits of standard error.
o— — — —o Means of plasma levels in animals(n=9) implanted once at day 165. Limits of standard error.



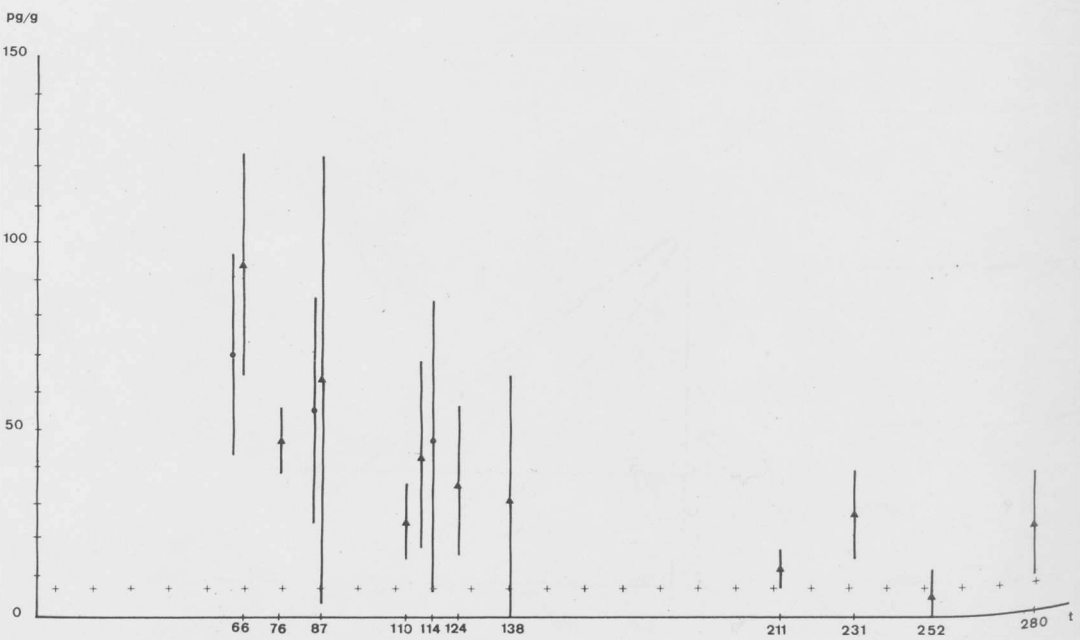


Fig.5. Residue levels of animals implanted with Torelor found in muscles(M.Diaphragma) after slaugthering(days after last implantation).
Means and standard error (—▶—) for animals treated with one implant.
Means and standard error (—●—) for animals treated with two implants at days 165 interval.

Acknowledgment

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