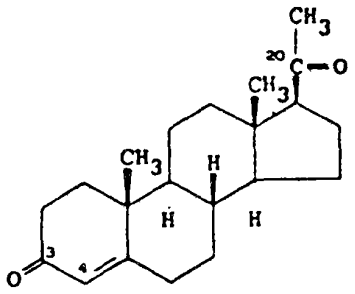
PROGESTERONE

IDENTITY

Chemical name: pregn-4-ene-3,20-dione
Δ4-pregnene-3,20-dione

Synonyms: corpus luteum hormone
luteohormone

Structural formula:



Molecular formula: $C_{21}H_{30}O_2$

Molecular weight: 314.45

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: crystals

Melting point: α-form 127-131°C, β form 121°C

Optical Rotation: $[\alpha]_D^{20}$ = +172 to +182° (c=2 in dioxane)

UV_{max}: 240 nm

(Windholz, 1983)

Technical active ingredients:

USP Grade: >98% purity

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General:

Progesterone is used primarily as a growth promotant in cattle in combination with estradiol or esters of estradiol. Administration is by subcutaneous implant in the ear. The ear, along with any residual drug, is discarded at slaughter.

Dosages:

Synovex-S (200 mg progesterone + 20 mg estradiol benzoate) = steers
Implix BM (200 mg progesterone + 20 mg estradiol) = steers
Steer-oid (200 mg progesterone + 20 mg estradiol benzoate) = steers

RADIOLABELED RESIDUE STUDIES

General:

When administered exogenously, progesterone enters the same metabolic pathways and is indistinguishable from the endogenously produced molecule. (Hoffman and Evers, 1986)

Cattle

Four steers and six non-pregnant cows were given subcutaneous injections of progesterone. Steers were given 50 µg/kg/day twice daily for 15 days. Each of the last three injections contained 0.9 mCi progesterone-4-14C. The cows were given 50 µg/kg/day twice daily for 13-21 days in which radiolabeled progesterone was included in the last 3-5 days.

Animals were slaughtered 2-3 hours after the final administration of radio-labeled progesterone. Samples of fat, muscle, kidney, liver, bile, injection site, urine, and feces were taken. The mean percentage distribution of radio-activity in TLC fractions of muscle, fat, and milk extracts is given in Table I:

Table I. Percentage Distribution of Radioactivity in TLC Fractions of Muscle, Fat, and Milk Extracts

	TLC Fractions			
	1	2	3	4
Muscle				
Steer	7.6 ± 1.3	59.6 ± 1.7	17.7 ± 4.8	7.2 ± 2.7
Cow	10.0 ± 0.9	47.7 ± 2.8	27.3 ± 3.2	9.1 ± 1.5
Fat-Free				
Steer	3.9 ± 1.3	62.7 ± 4.5	21.2 ± 3.6	6.3 ± 1.0
Cow	2.5 ± 0.7	75.9 ± 2.6	15.2 ± 1.8	4.2 ± 0.6
Glucuronide				
Steer	0.9 ± 0.9	70.1 ± 3.7	15.5 ± 2.3	6.6 ± 0.8
Cow	2.0 ± 0.8	76.0 ± 2.9	14.3 ± 1.7	5.1 ± 0.8
Milk	11.5 ± 2.1	57.1 ± 3.0	27.9 ± 4.0	4.5 ± 1.6

The major amount of radioactivity in all the extracts (fraction 2) had the chromatographic mobility of progesterone and is probably the parent compound. Fraction 1 chromatographed like the pregnanediones, fraction 3 like monohydroxylated metabolites including 20α and 20 β hydroxypregn-4-en-3-one; and fraction 4 like the more polar diols or triols. A high percentage of progesterone was found in both the free and glucuronide fractions of the fat extract. (Estergreen, et al., 1977)

Metabolites of progesterone in steers and non-pregnant cows (treated as above) were determined by HPLC analysis. In liver the major metabolites were 3α-hydroxy- 5β-pregnan-20-one and 5β-pregnane-3α, 20β-diol. In kidney tissue the principal metabolites were found to be 20β-hydroxypregn-4-en-3-one, 3 α- and 3β-hydroxy- 5α-pregnan-20-one and 5α-pregnane-3β, 20β-diol, as well as approximately 15% unconverted progesterone. Only traces of compounds other than progesterone were found in kidney fat. (Purdy, et al., 1980)

Metabolites of progesterone in steers and non-pregnant cows (treated as above) were characterized in muscle and adipose tissue by their chromatographic mobility, crystallization to constant specific activity and mass spectra. Progesterone constituted 54% of free radioactivity in muscle and 69 and 73% of radioactivity in free and conjugated portions of extracts, respectively, from fat. Metabolites that were identified are given in Table II: (Lin, et al., 1978)

Four steers and six non-pregnant cows were given subcutaneous injections of progesterone. Steers were given 50 µg/kg/day twice daily for 15 days. Each of the last three injections contained 0.9 mCi progesterone-4-^{14}C. The cows were given 50 µg/kg/day twice daily for 13-21 days in which radiolabeled progesterone was included in the last 3-5 days. Animals were slaughtered 2-3 hours after the final administration of radiolabeled progesterone. Samples of fat, muscle, kidney, liver, bile, injection site, urine, and feces were taken. An estimate of the distribution of total radioactivity in typical cow and steer carcasses is given in Table III: (Estergreen, et al., 1977)

Table II. Metabolites of Progesterone in Muscle and Fat of Cattle treated with 14C-Progesterone (% Extracted Radioactivity)

	Fraction		
	Muscle (Free)	Fat (Free)	Fat (Conj)
Metabolite			
5α-pregnane-3,20-dione	9	0	0
20-β-hydroxy-4-pregnen-3-one	8	11	3
3α-hydroxy-5β-pregnan-20-one	13	2	2
3α-hydroxy-5α-pregnan-3-one	3	3	6
20α-hydroxy-5α-pregnan-3-one	0	2	3
20α-hydroxy-4-pregnen-3-one		1	1
3β-hydroxy-5β-pregnan-20-one		0	2

Table III. Calculated Distribution of Carcass Radioactivity Based on Estimated Carcass Composition

	Radioactivity (% Total)	
Tissue	Cow	Steer
Fat	62.3	54.9
Muscle	15.5	29.6
Liver	21.6	14.9
Kidney	0.6	0.6

RESIDUE STUDIES

General:

Levels of progesterone residues in tissues are generally determined by immunological methods. As with estradiol, the individual methods have different

specificities and recoveries. Therefore, results obtained by different methods may not be directly comparable. For endogeneous compounds, such as progesterone, the important determination is the increase in tissue levels over background levels when the drug is applied exogeneously.

Steers

The concentration of progesterone was measured in edible tissues of control steers and steers implanted with SYNOVEX-S, and untreated pregnant heifers. Residues were measured by using an RIA method with a limit of detection in the low µg/kg range. Mean residue levels of progesterone in tissues at various times after implantation are given in Table IV:

Table IV. Progesterone Mean Tissue Concentrations in Steers Implanted with SYNOVEX-S (µg/kg)

Withholding Period	Tissue			
	Muscle	Liver	Kidney	Fat
Control	0.27 ± 0.33	0.26 ± 0.07	0.17 ± 0.14	2.48 ± 1.61
15 Days	0.23 ± 0.12	0.18 ± 0.03*	0.14 ± 0.05	3.20 ± 1.17
30 Days	0.23 ± 0.53	0.16 ± 0.03*	0.11 ± 0.32	3.48 ± 1.64
61 Days	0.41 ± 0.48	0.35 ± 0.07**	0.20 ± 0.33	3.40 ± 1.32
90 Days	0.44 ± 0.57	0.24 ± 0.12	0.32 ± 0.42	3.67 ± 2.25
120 Days	0.58 ± 0.84	0.27 ± 0.08	0.17 ± 0.17	2.62 ± 1.09

* Significantly lower than controls
** Significantly higher than controls

The concentration of progesterone in fat far exceeds those found in other tissues. The author concludes that, because of the variability of the results, there is no trend towards increased levels of progesterone in animals implanted with SYNOVEX-S. (Kushinsky, 1983)

Pregnant Heifers

Tissue concentrations of progesterone were determined in fat, muscle, liver, and kidney obtained from 3 pregnant heifers at slaughter. The heifers were in mid to late stages of pregnancy and had not been treated with any hormones. The results of this study are summarized in Table V:

Table V. Progesterone in Tissues from Pregnant Heifers (µg/kg)

Tissue	Progesterone
Muscle	10.1 ± 6.65
Fat	239 ± 116
Kidney	6.19 ± 1.86
Liver	3.42 ± 1.37

The author concludes that the concentrations of endogenous progesterone in steers and the tissue concentrations of progesterone in steers treated with SYNOVEX-S are exceedingly small compared to concentrations of progesterone in pregnant animals. (Kushinsky, 1983)

Calves

Nine male, veal calves received implants containing 20 mg estradiol and 200 mg progesterone at three weeks of age. Three each were slaughtered at withholding periods of 15, 30 and 50 days. Two controls were sacrificed at the 30th day withholding period. The tissue concentrations of progesterone, as determined by a RIA procedure, are given in Table VI. (Roberts and Cameron, 1986)

Table VI. Progesterone Mean-Tissue Concentrations in Male Calves Treated with Implix BM (µg/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
Control	0.901	0.749	4.066	1.598
15 Days	0.606	0.599	0.994	5.407
30 Days	0.597	0.924	2.798	6.520
50 Days	0.772	0.771	1.408	8.664

APPRAISAL

The residue data presented in this monograph indicate that residues of exogenously administered progesterone are primarily comprised of the parent compound and its glucuronide conjugate. There is evidence of hydroxylated metabolites, but they form minor metabolite fractions and possess greatly diminished biological activity when compared to parent substance.

As with other endogenous steriod hormones, residue levels of progesterone in tissues are very low. Progesterone levels were measured in tissues from treated steers using a radio-immunoassay technique sensitive at the low ng/kg level, and were found to be about 0.4 µg/kg in muscle, liver and kidney and 3.5 µg/kg in fat; these levels can be compared with normal levels of approximately 0.2 µg/kg in muscle, liver and kidney and approximately 2.5 µg/kg in fat from untreated animals.

Progesterone, like estradiol-17β and testosterone, occurs naturally in mammals, and is normally present in the dairy products and the tissues of untreated animals. In the edible tissues of animals treated with progesterone in combination with estradiol-17β, residue levels are up to twice as high as in the tissues of untreated animals. However, the levels of progesterone found in meat from animals treated with implants according to good animal husbandry practice are extremely low when compared with the amounts of endogenous progesterone produced daily in human beings. The daily production rate of progesterone in humans is given in Table VII. (Farber and Arcos, 1983). Even in prepubertal boys, the 300 ng additional progesterone derived from a 500 g portion of meat from treated animals is considerably less than the amount of endogenous progesterone produced daily. In addition, for those animal classes studied, the progesterone residue levels in treated animals fall well within the normal range of levels founds in untreated bovine animals of different types and ages.

As with estradiol-17β, the methods are primarily RIA procedures. These immunoassay procedures must be rigorously controlled due to the uniqueness of their reagents. Because of the limitations of these procedures, the low levels of progesterone in tissues of treated animals and the wide variations in tissues of untreated animals, it is not possible to monitor tissues of animals for the use or misuse of progesterone implants.

Table VII. Progesterone Production Rate in Humans

Women	mg/day
Follicular phase	0.418
Luteal phase	19.580
Late pregnancy	94.00
Postmenopausal	0.326
Prepubertal girls	0.253
Men	
Adult	0.416
Prepubertal boys	0.150

REFERENCES

Estergreen, V.L., Lin, M.T., Martin, E.L., Moss, G.E., Branen, A.L., Luedecke, L.O., and Shimoda, W. (1977). Distribution of progesterone and its metabolites in cattle tissues following administration of progesterone-4-^{14}C. J. Anim. Sci. 46, 642-651.

Farber, T.M. and Arcos, M. (1983). A regulatory approach to the use of anabolic agents In: Meissonnier, E. (ed.) Anabolics in Animal Production, Office International des Epizooties, Paris, pp. 289-296.

Hoffmann, B. and Evers, P. (1986). Anabolic agents with sex hormone-like activities: Problems of residues. In: Rico, A.G. (ed.), Drug Residues in Animals, Academic Press, New York, pp. 111-146.

Kushinsky, S. (1983) Safety aspects of the use of cattle implants containing natural steriods. Presented at: International Symposium on the Safety Evaluation of Animal Drug Residues, West Berlin. Submitted to FAO by Syntex (U.S.A.) Inc., Palo Alto, CA, USA.

Lin, M.T., Estergreen, V.L., Moss, G.E., Willett, J.D., and Shimoda, W. (1978). The in vivo metabolites of [14C] progesterone in bovine muscle and adipose tissue. Steriods 32, 547-561.

Purdy, R.H., Durocher, C.K., Moor, P.H., Jr., and Rao, P.N. (1980). Analysis of metabolites of progesterone in bovine liver, kidney, kidney fat, and milk by high performance liquid chromatography. J. Steriod Biochem. 13, 1307-1315.

Roberts, N.L. and Cameron, D.M. Steroid levels in tissues of veal calves following implantation with Implix BM/BF and/or Revalor lactose. Unpublished report No. RSL/686 from Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Windholz, M., ed. (1983). The Merck Index 10th Edition. Rahway, N.Y., Merck and Co.

****