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The Metabolism of the Synthetic Progestational Compound Ro 4-8347

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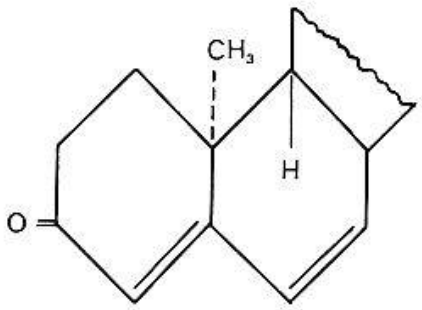
The corpus luteum has been described for 300 years before Fraenkel (1910) demonstrated the dependence of the embryo upon it for survival. In the same year, Ancel and Bouin proved that the secretory changes in the endometrium also depended upon the same ovarian structure. Corner and Allen (1929) isolated a crystalline hormone from the ovaries of sows; the final chapter which proved that this hormone – progesterone – was also synthesised in the human placenta was written almost simultaneously in 1952 by three independent teams led by Salhanick, Diczfalusy and Hoskins.

When progesterone was first available for clinical investigation, the hormone because of its rapid metabolisation was found to be of little value. Today, however, compounds exhibiting some or all of the physiological actions of this steroid challenge antibiotics and aspirin for primacy in universal utilization because of now recognised usefulness.

By 1944, the possibilities of an orally effective progestational compound were beginning to stimulate research endeavour and in that year, a major step towards the development of an ideal progestational agent was realised when ethisterone was synthesised. It took some years to remove the methyl group from the 19th carbon in ethisterone but when it happened, this molecular manipulation opened the way to a new line of highly active compounds of which 19-nor-17-ethinyl-testosterone (norethisterone) was the first and is even today amongst the more widely employed progestagens. It was in 1953 that the flood gates of interest for this kind of synthetic hormone were suddenly burst open by the publication of the findings of Pincus and Chang concerning the effectiveness of oral progestational agents in suppressing ovulation. What has followed since the first field trials in Puerto Rico and Haiti requires no comment.

In 1960, Reerink, Scholer, Westerhof, Wuerido, Kassenarr, Diczfalusy and Tillinger described in a communication to "Nature (Lond.)" a new class of hormonely active steroids – "The Retrosteroids" in which the spatial arrangements at carbons 9 and 10 of progesterone had been



Dydrogesterone

Fig. 1. Dydrogesterone.

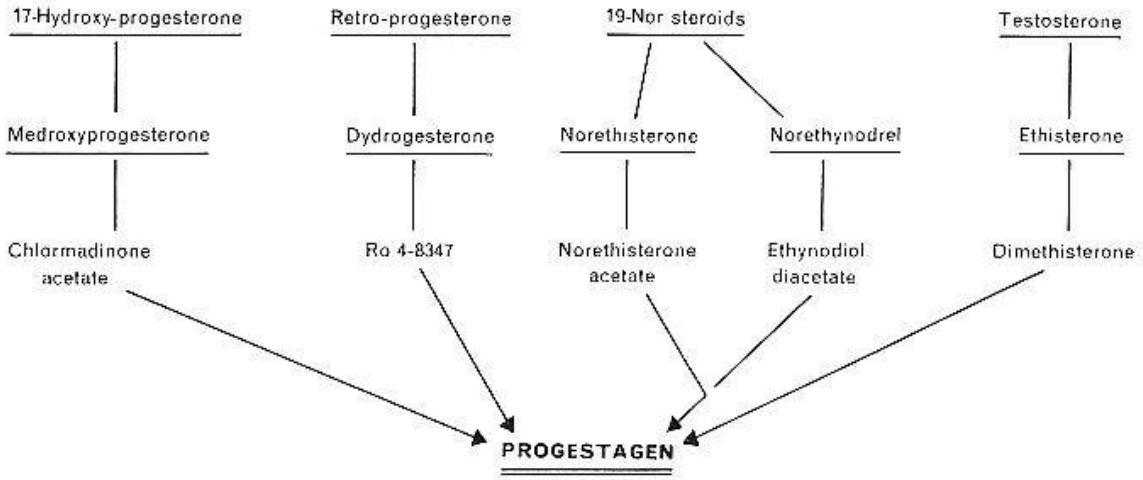


Fig. 2. The four-lane drive to the ideal progestagen.

reversed (Fig. 1). The search for the ideal oral progestational agent was now widened to a four lane drive (Fig. 2).

Elucidation of questions concerning the metabolism of progesterone itself has given criteria by which the excellence of a progestagen may be assessed.

My ideal progestagen would possess these characteristics:

1. Rapid absorption from the gastro-intestinal tract.
2. High activity when administered orally.
3. Effective tissue levels attained and maintained with once-a-day dosage.
4. Possess activity of a single hormone class only.
5. Metabolise to compounds which do not interfere with the activity of the parent substance or its active metabolites, if any.
6. Be non-toxic and non-teratogenic.

Since 1966, my colleagues and I have had the opportunity to study the Hoffmann La-Roche retrosteroid Ro 4-8347.

From the structural formula it may be seen Ro 4-8347 differs from dydrogesterone by having an additional unsaturated bond between carbons 1 and 2 in the A ring and Cl^- at C6 (Fig. 3). Our interest was to study how closely it would conform to the six criteria I have proposed for the ideal progestagen. The results of our investigations will now be presented under these headings:

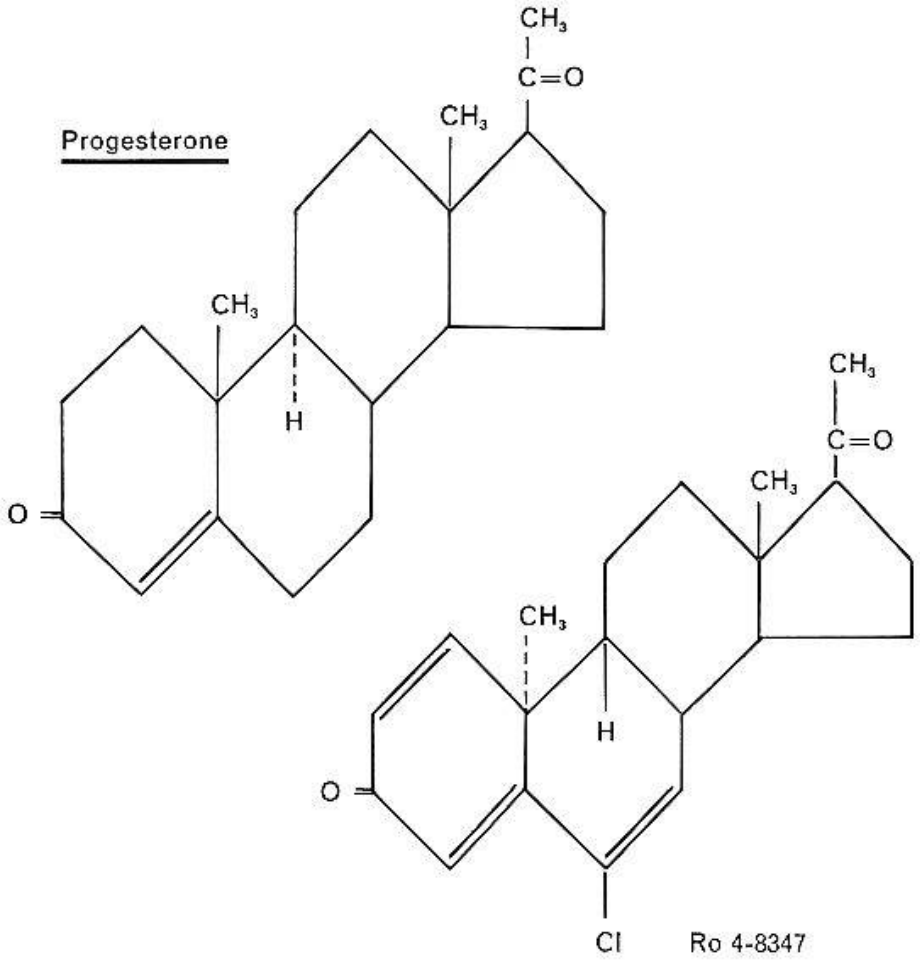


Fig. 3. Progesterone.

1. Absorption

Ro 4-8347 was specifically labelled with tritium and diluted to a specific activity of 5.45 μc/mg. The material was administered in 2 mg tablet form. Purity was ascertained by chromatographic and chemical analysis. Fig. 4 illustrates the uptake in a patient treated with a single dose of 4 mg of Ro 4-8347 labelled with tritium (21 μc ^{3}H). In order to avoid problems arising from unequal dissolution and absorption from the tablets swallowed whole, the patient was instructed to chew the tablets and to rinse her mouth with water and swallow.

In this study, plasma was taken at 15 min intervals during the first hour and the plasma analysed for the presence of free substance and bound substance. It can be seen from curves in Fig. 4 that taken in this way, Ro 4-8347 is rapidly absorbed to give detectable blood levels of the compound in both the free and bound state within 15 min rising to a maximum in 2 h; the concentration of the substance in the plasma being well maintained at 4 h following a single dose. This concentration of Ro 4-8347 in the plasma produced a progestational reaction in the endometrium of the subject (Fig. 5) resulting in a withdrawal bleed while the subject was maintained on continuous estrogen therapy.

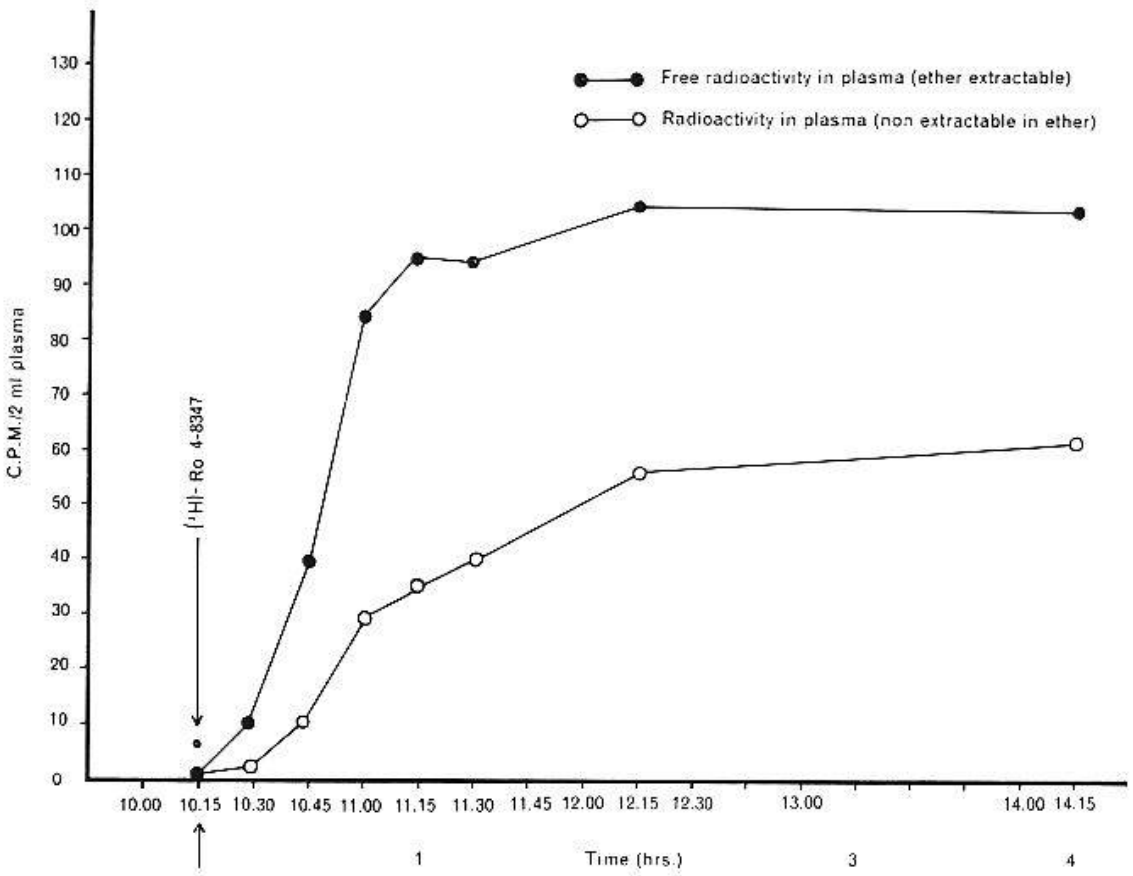


Fig. 4. Radioactivity in plasma in patients treated with tritium-labelled Ro 4-8347.

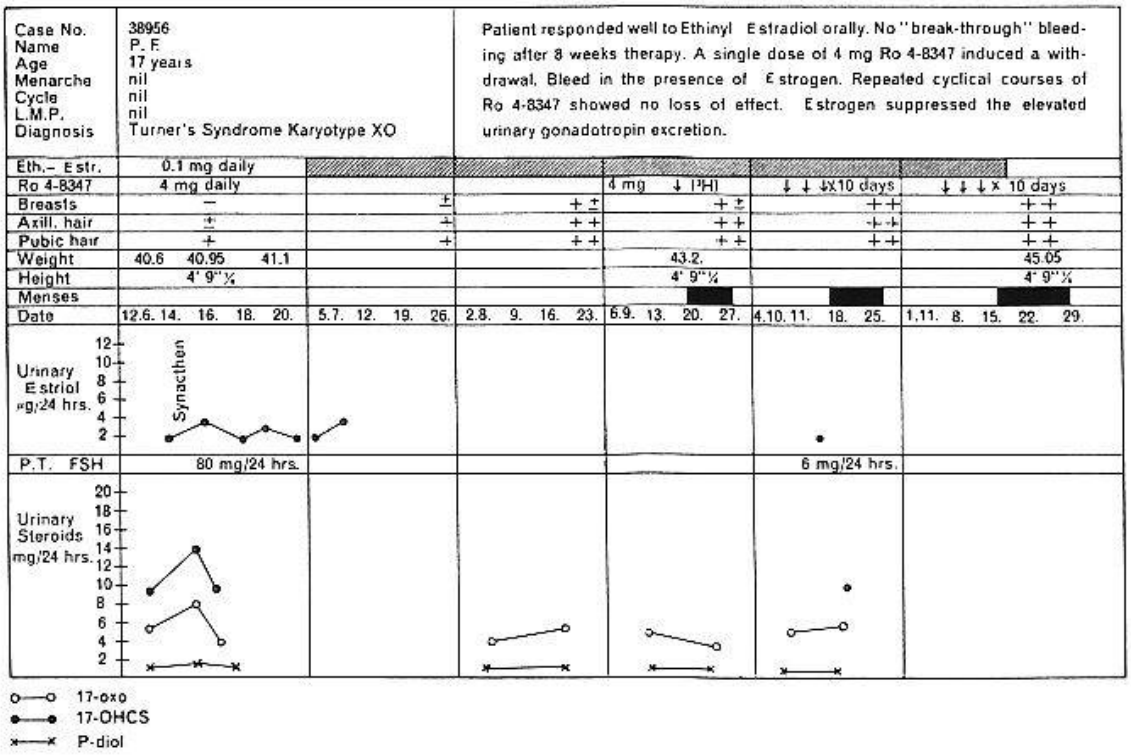


Fig. 5. Progestational reaction in the endometrium produced by concentration of Ro 4-8347 in the plasma.

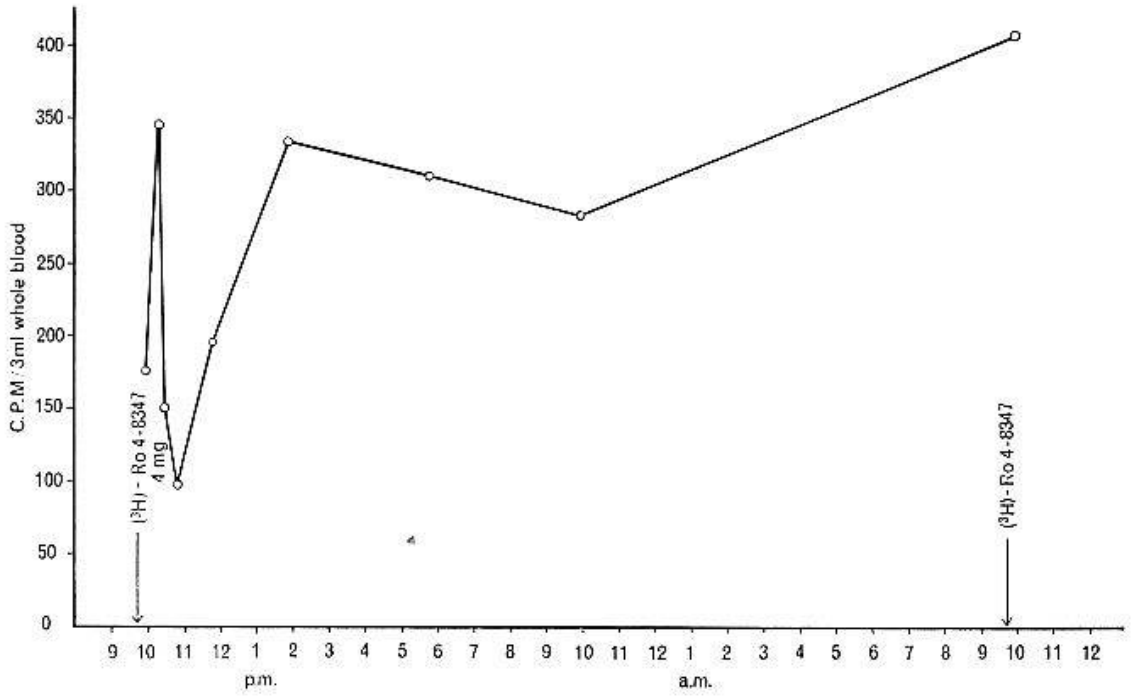


Fig. 6. Timed uptake of H^3 labelled material into blood over the first 24 hours following oral administration of Ro 4-8347 ^3H given as shown.

Fig. 6 shows the uptake curve following the administration of 4 mg tablets swallowed whole. Again, it can be seen that there is a rapid appearance of the substance in the plasma. The concentration falls in the second hour sharply to a low concentration and rises again in the course of the next 2 h to almost the original peak concentration declining thereafter but still being present in a significant concentration 12 h after the administration of the single dose. The administration of a second dose 24 h after the first dose resulted in an increment in the peak concentration attained indicating that a considerable residual activity remained in the plasma after 24 h following the initial dose.

Fig. 7 illustrates the relative distribution between the free and bound forms of Ro 4-8347 in the plasma over a period of 48 h demonstrating that initially the substance is present in plasma in a predominantly free form and as may be expected, the excretion of the free substance via the kidney ultimately reverses the distribution ratio so that at 48 h the residual substance in the plasma is mostly in the bound form and indeed most of the substance has been excreted by that time following the ingestion of a single dose.

For comparison or absorption characteristics tritium-labelled dydrogesterone was administered to a subject in a single dose of 20 mg which had a specific activity of 20 μc ^3H. The curve (Fig. 8) obtained by plotting serial estimations of plasma radioactivity over a period of 24 h is very similar to the pattern demonstrated in the previous studies but there appears to be a more rapid decline in plasma activity than seen in the subjects treated

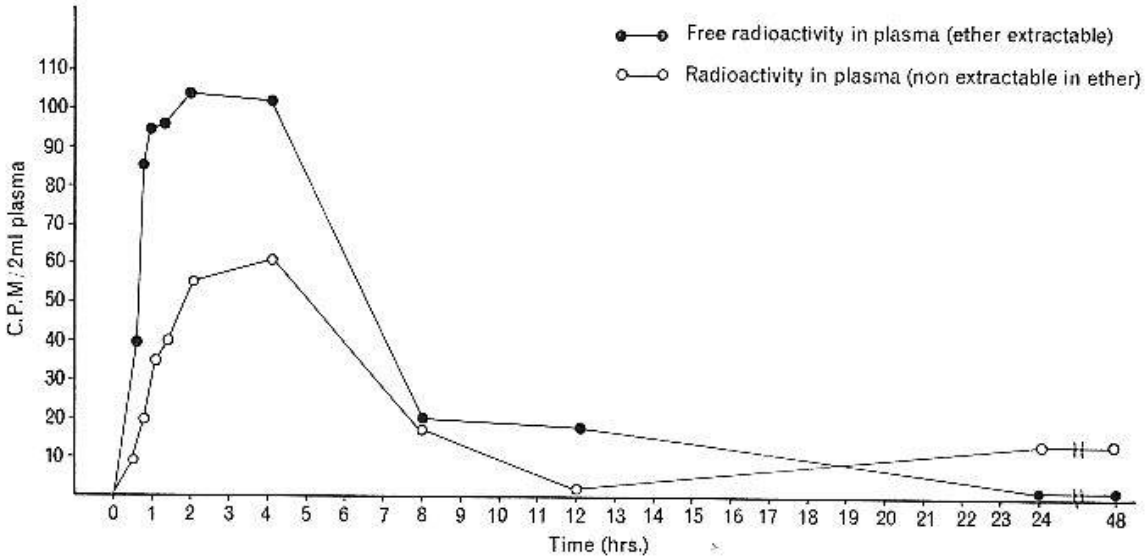


Fig. 7. Relative distribution between free and bound forms of Ro 4-8347 in the plasma.

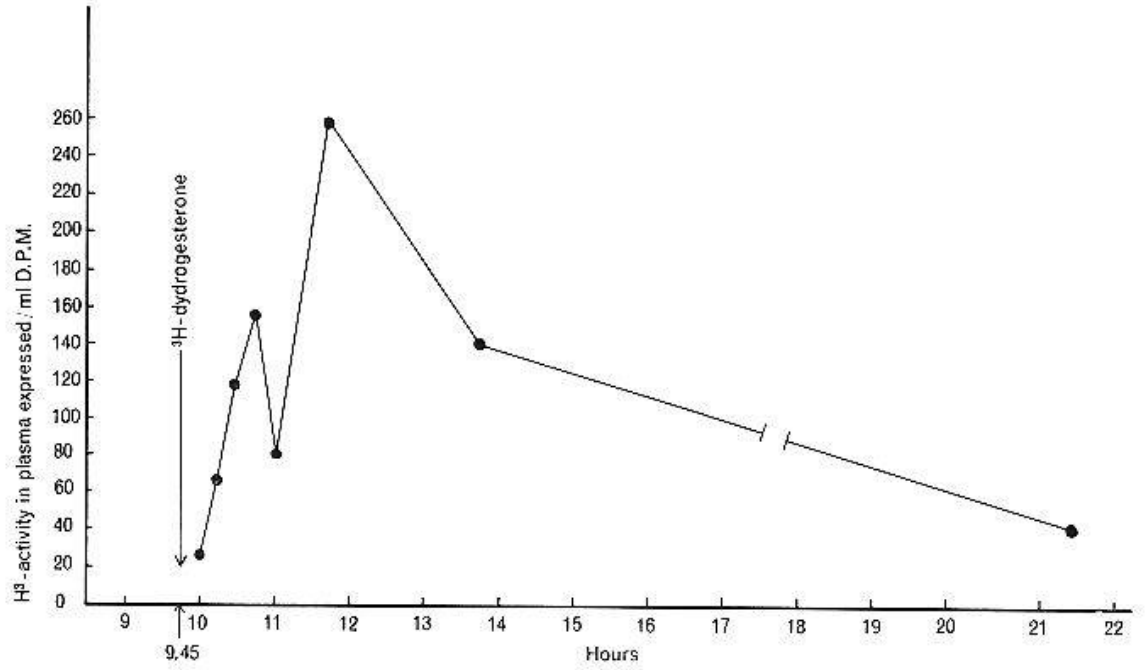


Fig. 8. Plasma levels of ^3H labelled material at timed intervals following oral administration of ^3H labelled Duphaston.

with Ro 4-8347. It would appear that the clearance rate for dydrogesterone through the kidneys could be of the order of 5 times that of Ro 4-8347.

2. Progestational activity when administered orally

In the patients studied and under the conditions of the trial, Ro 4-8347 has proven to be an agent capable of inducing secretory-type changes in the vaginal cytology and mimics progesterone and non-estrogenic synthetic progestagens, e.g. norethisterone in being capable of inducing catamenia in

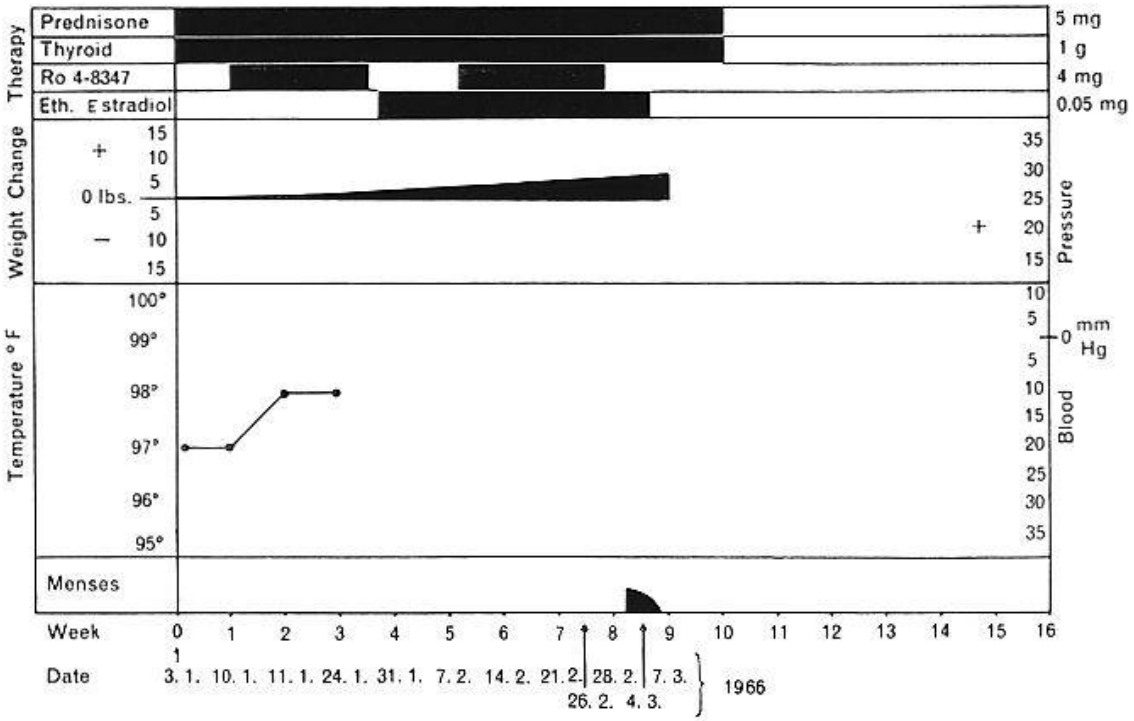


Fig. 9. Progestational activity inducing secretory-type changes and catamenia.

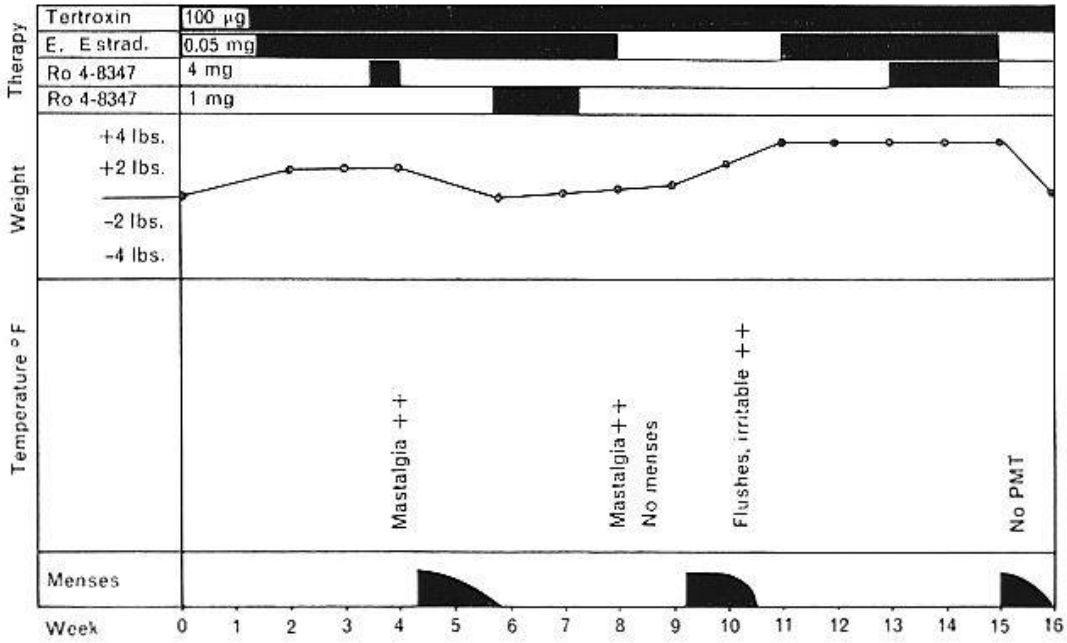


Fig. 10. Withdrawal bleed-production.

estrogen primed women but not in subjects with insufficient estrogen priming (Fig. 9).

Our studies indicated that the compound was consistently and predictably effective at a dosage of 4 mg per day. At a dose of 2 mg per day, the compound appeared to be equally effective; at a still lower dose of 1 mg daily, it was also effective but at this dosage level the preparation was not, in our

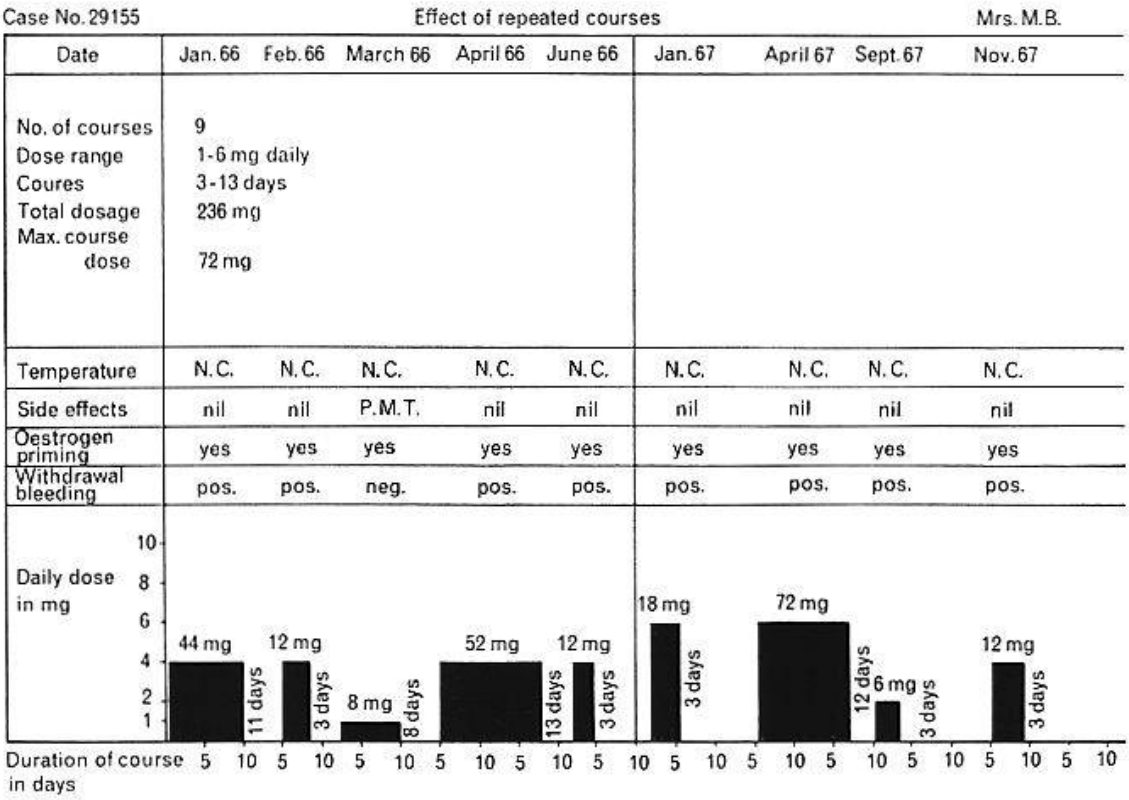


Fig. 11. Effect of repeated courses.

opinion, consistently effective. Fig. 10 illustrated that in the presence of continuing ethinyl estradiol administration at a dose of 4 mg daily, a withdrawal bleed occurred within 4 days of stopping Ro 4-8347 whilst in a subsequent cycle employing a dosage of 1 mg daily of Ro 4-8347 under the same test conditions, the drug failed to produce a withdrawal bleed.

When administered in repeated courses, the compound appears to remain uniformly effective provided the daily dosage level is above 1 mg orally (Fig. 11). This suggests that there is no induction of enzyme systems in the liver or other tissues to accelerate the rate of inactivation of Ro 4-8347.

3. Tissue distribution

Following a single dose of 8 mg of tritium-labelled Ro 4-8347, the labelled material was demonstrated to be present 24 h later in the reproductive organs, skin, fat and muscle, the specimens being obtained from a subject undergoing a hysterectomy because of grossly enlarged fibroids. In its tissue distribution, Ro 4-8347 is very similar to progesterone and appears, like it, to be rather avidly taken up by fat.

Ro 4-8347 is excreted both in the urine and in the faeces, the urinary tract providing the principle route. Fig. 12 illustrates the rate of excretion of labelled material at intervals over a period of 4 days. From this, it can be seen that excretion has well commenced with 4 h and as much as 50% of the total amount ultimately excreted by the urinary tract is in fact ex-

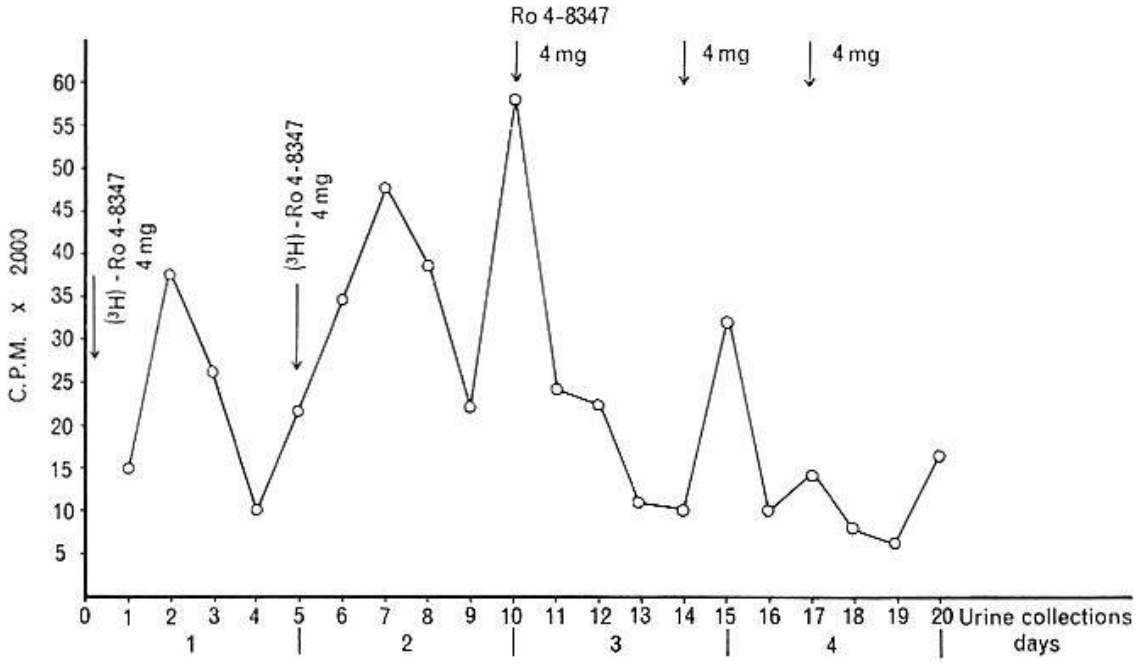


Fig. 12. The excretion of ^{3}H labelled material in urine, collected at timed intervals for 4 days following oral administration of Ro 4-8347 ^{3}H.

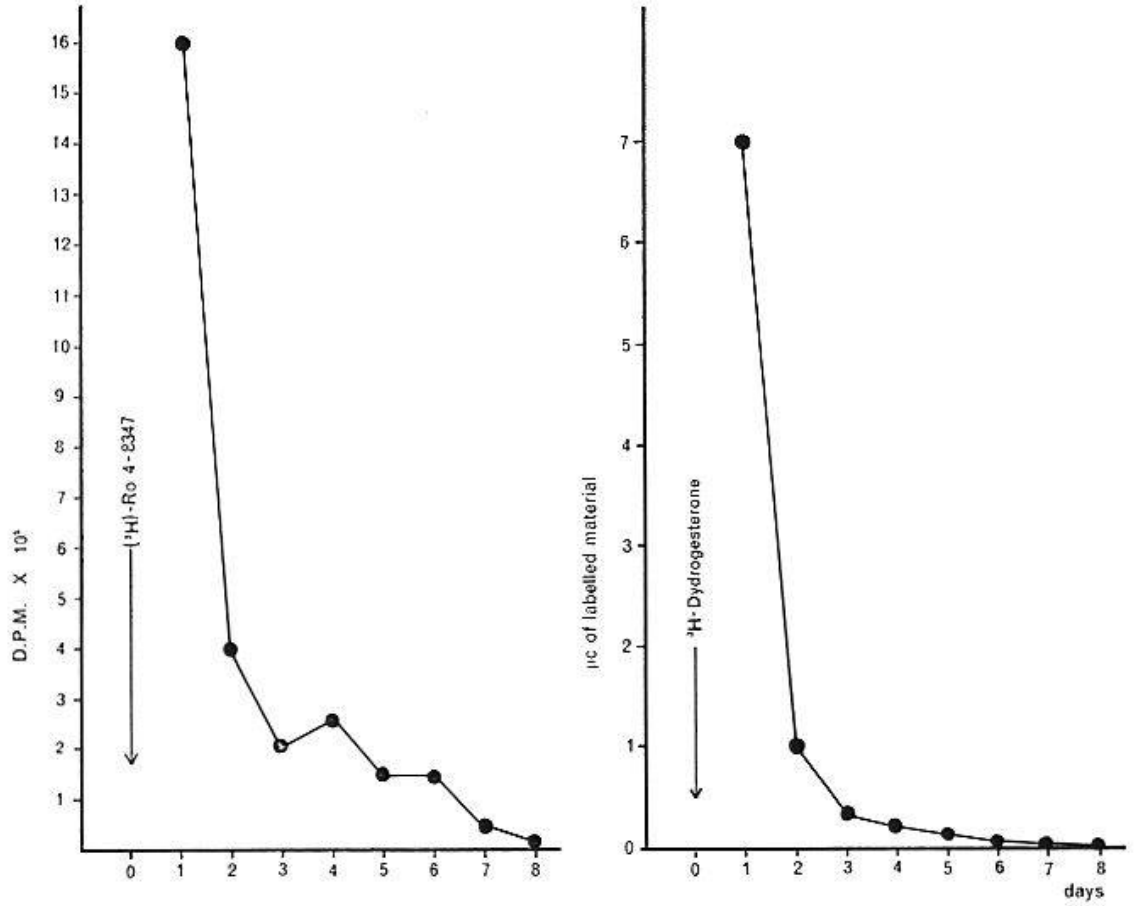


Fig. 13. Excretion of ^{3}H-labelled material by the urinary tract.

creted during the first 24 h. The excretion of dydrogesterone by the urinary tract was also investigated for comparison (Fig. 13). 80.03% of the total urinary excretion of the labelled material was excreted by the test subject in the first 24 h following the administration of a single dose. This higher rate of excretion is consistent with the more rapid clearance of this substance than Ro 4-8347 from the plasma.

4. Hormonal activity

In the experimental animal studies reported and in our clinical experience, Ro 4-8347 demonstrated only progestational activity.

5. Metabolism of Ro 4-8347

Tritium-labelled Ro 4-8347 with a specific activity of 5.45 μc/mg was administered to a group of four female patients whose urine and faeces were then collected for recovery of the labelled material and subsequent analysis. The total recovery of radioactive material in urine was approximately some 25% of the quantity administered. Collection of the faeces during the first 4 days of treatment showed that a major portion of the radioactivity was eliminated by this route. The nature of this material was not investigated. Of the radioactivity recovered in the urine, an average of 19% was in the free form. This metabolite was demonstrated to be identical to Ro 6-9241.

Following extraction with ether, the residual urine was incubated with B-glucuronidase and extracted with ether. The ether was then further extracted with NaOH and both fractions were counted. No radioactivity was found in the alkali phase. The radioactivity in the ether phase was then studied. When the principle metabolite was isolated and subjected to mass spectroscopy, it had a mass and spectrum identical to Ro 6-9241 which is the 20A-hydroxy derivative of Ro 4-8347. The presence of the chlorine atom, the 20-hydroxy configuration and the presence of the 3-keto group in ring A was confirmed, in the metabolite recovered.

Ro 4-8347 is therefore excreted as the 3-keto-20-hydroxy derivative, which there is reason to believe also possesses progestational activity. Unlike the principle excretory product of endogenous progesterone, pregnanediol, which is not only biologically inert but also when given together with progesterone may nullify (perhaps by a competitive inhibition) the activity of certain parameters of progesterone's activity. The non-conversion to a biologically inert metabolite may well explain the longer duration of action of the synthetic progestagen compared with the known short half-life of the endogenous progesterone.

In a number of patients studied, serial observations of the excretion of 17-oxosteroids, 17-OH steroids, estriol and pregnanediol were performed. The results indicated quite clearly that Ro 4-8347 does not contribute to the amounts of these substances appearing in the urine. Therefore, these estimations are valid indices of physiological function in treated cases.

In conclusion, in my opinion considered from the standpoint of these metabolic criteria, synthetic progestagens may exhibit distinct advantages over the naturally occurring hormone, in therapeutics.

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Discussion

B. Lunenfeld: We have just heard an indication that makes me believe that before when Dr. Richter asked whether the 20α compound was biologically active there must have been a misunderstanding in the answer of Dr. Krause. I think that we hear here that there is some activity. Would Dr. Krause maybe confirm or deny the story?

R. Krause: The compound is indeed active.

H. Breuer: You mentioned that in your experiments with tritium-labelled material you found about 25% of the radioactivity in the urine. You stated that a major portion of the radioactivity was excreted in the feces. How big was this major portion?

A. Darragh: In the average it was over 55% in the feces.

H. Breuer: In the experiments where you determined the radioactivity in the blood, you mentioned a free form and a bound form of the material. What do you understand by "bound" form in this particular case?

A. Darragh: Protein bound, i. e. bound to proteins.

H. Breuer: How did you detect this? Could you characterize the protein which binds the steroid?

A. Darragh: We found no evidence of a competitive binding with progesterone. We specifically looked at this point to see if it was being bound to the same protein fraction as progesterone. However, I am informed by the biochemists who carried out the studies that they were able to identify that there was a difference between the free form and the bound form and I believe, though I am subject to corrections by my biochemists on this, that this was by acetone extraction of the plasma.

H. Breuer: It is a very interesting statement that the same protein which binds progesterone also binds the retro-progesterone.

A. Darragh: No. There is no competitive binding between progesterone. Because we have been measuring plasma progesterone by the cortisol-binding globulin technique, we were specifically interested to know whether or not Ro 4-8347 administered to a patient would interfere with the values we were obtaining in our plasma progesterone studies. However, we could find no evidence that retroprogesterones interfere with the binding of natural progesterone to globulins.

H. Breuer: The retroprogesterone is bound by the same globulin as progesterone?

A. Darragh: No. It is bound by a globulin fraction, but not necessarily by the same globulin fraction. The biochemists could find no evidence of competition between progesterone and the retrosteroid.

H. Breuer: You got this increase in radioactivity in the blood after administration of the compound and then one can observe a sudden drop in your slides.

A. Darragh: We found this not only in one case. We found this in all the cases that we investigated. I was at a loss to know what the explanation of that is. Whether it is

a removal of the parent compound and a sudden reappearance of the metabolite? This has to be investigated.

H. BREUER: This could be an extraction effect of course.

A. DARRAGH: Well, we are not certain, maybe.

B. LUNENFELD: To this point you also found the same thing with Duphaston, if I remember correctly.

A. DARRAGH: Yes.

B. LUNENFELD: You mean this is not specific to this retrosteroid?

A. DARRAGH: To Duphaston as well as to the retrosteroid.

J. HAMMERSTEIN: Did I understand you right that you found an excretion of isotopes after administration of labelled retroprogesterone up to eight days? Is there any evidence that retroprogesterone or some of its metabolites is stored in adipose tissue? And, if yes, to which extent?

A. DARRAGH: In the tissues which we studied from patients who had received Ro 4-8347 in a single dose of radioactive material, at 24 hours in fact the richest counts were obtained in adipose tissue. So certainly there is fat storage. My phrase was that it is very avidly taken up by adipose tissue. And this accounts for the protracted excretion.