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Lack of Influence of Ro 4-8347 on the Steroidogenesis of Human Corpus Luteum Slices in vitro in Contrast to Clomiphene and Cyclofenil

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Since some time ago we have been investigating the problem of whether ovulation inducing agents such as clomiphene may exert direct effects on the ovarian steroidogenesis. For this purpose slices of human corpora lutea were incubated from 2 to 6 hours' duration in the presence of various drugs using acetate-1-^{14}C as precursor. The work-up of the incubates followed the principle of the reverse isotope dilution technique with crystallization of the isolated steroid fractions to constant specific activity whenever possible.

As the starting point it was found that the incorporation of acetate-1-^{14}C into progesterone was reproducibly reduced in this model when clomiphene citrate was added to the medium in concentrations even as low as 0.034 μmol (Hammerstein, 1969 a). This inhibitory effect of clomiphene was not confined to the formation of progesterone but was demonstrable for all of the steroids in the C_{21}-, C_{19}-, and C_{18}-series studied so far. Cyclofenil, another ovulation inducing drug, also known as Sexovid, F 6066 or F 6060 (free compound), has similar effects, but – on a weight basis – to a somewhat lesser extent. Diethylstilbestrol, on the other hand, though close related in structure to clomiphene and cyclofenil, failed to show any influence on the ovarian steroidogenesis in vitro. Notably, this synthetical, non-steroidal estrogen does not possess ovulation inducing properties in the treatment of human anovulation. It is, therefore, tempting to assume that the inhibitory action on steroid formation of clomiphene and similar agents as shown in our in vitro studies has also some meaning in respect to the in vivo mode of action of these drugs (Hammerstein, 1969 b).

Consequently, it was of interest to study other ovulation inducing compounds in the same way. Among them 6-chloro-9β,10α-pregna-1,4,6-triene-3,20-dione (Ro 4-8347) deserves special attention, since it is neither a stilbene derivative nor a steroidal estrogen. Preliminary results of such an experiment are given in Fig. 1. The numbers in the upper part of the graph represent the absolute amount of radioactivity in decompositions per

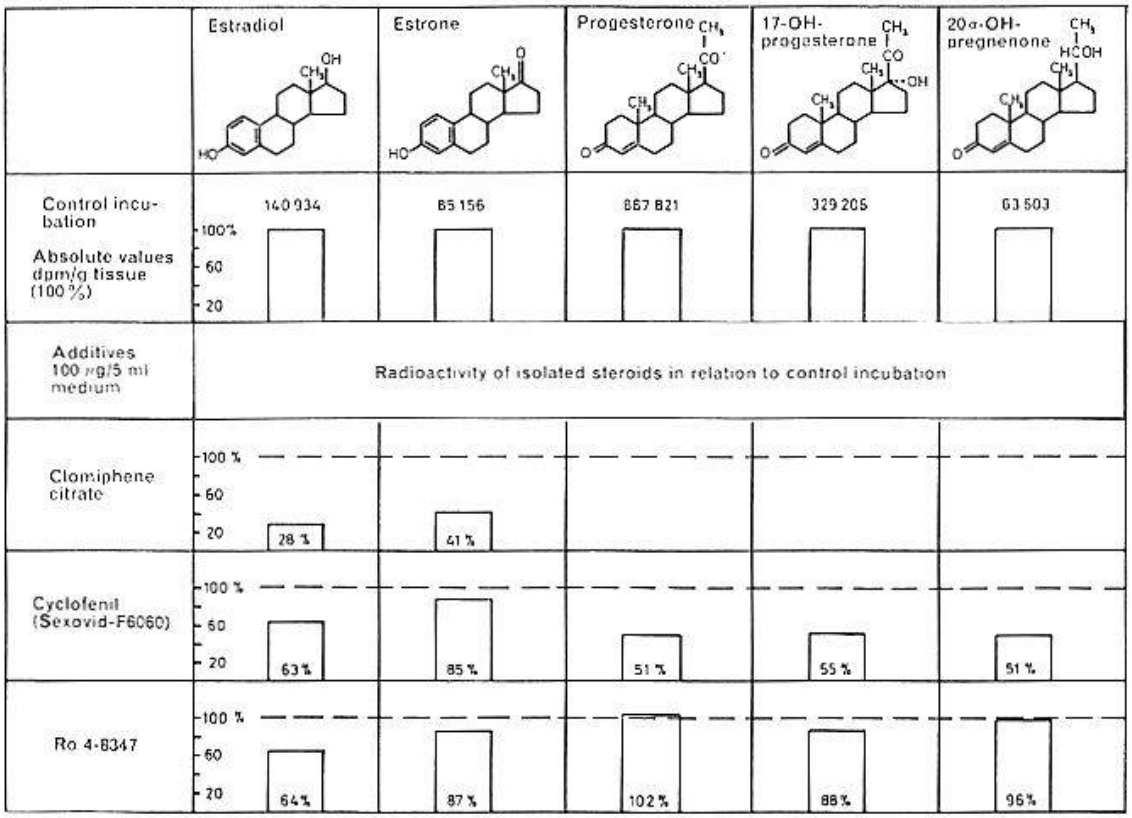

Fig. 1. In vitro incorporation of 50 μc acetate-4-^{14}C into 5 steroids by slices of a human corpus luteum of pregnancy (mens II) as influenced by various ovulation inducing drugs.

minute (d.p.m.) of the five steroids so far isolated from the control incubate. All values are corrected for analytical losses and adjusted to 1 g of luteal tissue. The high yield of radioactive steroids after 6 h of incubation with 50 μc acetate-1-^{14}C indicates a very viable luteal tissue which is a prerequisite for a valid interpretation of the experimental results. With clomiphene present in the incubation medium the amount of radioactive estradiol and estrone was only 28% and 41% that of the control. These findings compare well with the results of our previous experiments. In this incubation C_{21}- and C_{19}-steroids are not yet analyzed. A similar reduction by clomiphene, however, may reasonably be assumed on the basis of our former studies. When cyclofenil was added to the medium the biosynthesis of steroids from acetate-1-^{14}C was also reduced, but to a lesser extent. This finding meets our expectations, too. In the presence of Ro 4-8347 on the other hand, no major change with regard to the quantitative yield of radioactive steroids was to be found. Four values were well within the $\pm 15\%$ variance of the controls and, therefore, within the limits of failure due to analytical plus biological causes. Only estradiol contained one third radioactivity less. Future experiments will show whether these results are as reproducible as those observed with clomiphene and cyclofenil.

On the basis of these findings it would appear that Ro 4-8347 differs in action on the ovarian steroidogenesis from those ovulation inducing drugs related to stilbene. As a consequence, also a different mode of action in vivo

would be reasonable to assume. It may well be that the retroprogesterone derivative acts analogously to the ovulation inducing effect of the short-term application of progestogens. This subject will be reviewed later in this meeting by Professor BETTENDORF.

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Discussion

H. BREUER: Do you think that the concentrations used in your experiments were within the physiological range? You added between 10 and 100 μg of steroid to an incubation volume of 5 ml. Significant inhibition was observed only with concentrations between 30 and 100 μg.

I noted from your slide that at very low concentrations you got an increase in the formation of Δ_4-androstenedione and at higher concentrations a decrease. Now, have you got any explanation for this observation? Do you think that this reflects the true situation as it is in vivo?

J. HAMMERSTEIN: Honestly, I do not know, Professor Breuer. Some of the work we are now planning to do is to go still further down with the concentrations not only of clomiphene but also of the other substances. I could think of an increase in acetate incorporation into all steroids, e.g. at very low drug concentration in the medium. Then we would have to change our hypothesis, of course.

B. LUNENFELD: I wonder whether for the drugs you were studying the corpus luteum would be the ideal tissue.

J. HAMMERSTEIN: This question is always brought up in discussions like this, and principally you are, of course, all right. We chose the corpus luteum slices because they are suited best for a biosynthetic working model. If you work for example with stromal tissue, the incorporation of acetate is usually so low that the significance of the results is becoming doubtful. In addition, I think we should not forget that the corpus luteum consists of two cell types which are already present in the follicle prior to ovulation. These are the granulosa and the theca lutein cells. It appears to me very probable that the basic steroidogenetic processes are the same prior to and after ovulation and consequently that the use of luteal tissue for this purpose is justified.

H. BREUER: I would agree that these experiments are very important, but as far as the mechanism of action is concerned, I think one must be very careful to draw any final conclusions from these in vitro studies. I wonder whether you have studied various concentrations of steroids added to your incubation medium, because from these experiments you could find whether this is a competitive or a non-competitive inhibition. I would suspect that in these experiments one gets a non-competitive inhibition. However, this would not mean very much about the physiological significance as to the

mechanism of action. You may get an inhibition of the incorporation of acetate into steroid molecules by many compounds.

J. HAMMERSTEIN: But not with retroprogesterone which is also used in ovulation induction.

H. BREUER: I think it would be very nice to have these measurements at various concentrations of the steroids, so that you can say this is a competitive inhibition and may have a physiological significance.

J. HAMMERSTEIN: Professor Breuer, we discussed this before. It is very laborious, as you know, to perform such studies and it takes us not less than 3 months to carry out one single experiment. Being therefore still in the beginning of our investigations, we focuse our main interest on the more general effects of these drugs on the over-all steroidogenesis from early precursors such as acetate. Consequently, we are far from having a detailed insight into the biochemical mode of action of these compounds. At the moment we do not even know as yet which enzymatic steps are affected by these drugs. Only very recently, experiments using pregnenolone and progesterone as precursors were started to study this point. Once the locus of action is clarified, experiments of the sort you just mentioned will surely be done. It might then become useful to switch from slices over to subcellular fractions.