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Investigations on the Metabolism of the Retrosteroid Ro 4-8347 in Anovulatory Patients

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Tritium-labelled 6-chloro-9β,10α-pregna-1,4,6-tiene-3,20-dione (Ro 4-8347/05; 6μc), together with the non-labelled material (16 mg) were administered orally to an anovulatory patient (W. R.). Samples of the urine collections from the first and second days following administration of the drugs were acid-hydrolysed, extracted with cyclohexane, and the extracts washed with sodium hydroxide then water, dried with anhydrous sodium sulphate and evaporated to dryness. The extracts were dissolved in chloroform and resolved by thin-layer chromatography (stationary phase: silica gel; mobile phase: chloroform:acetone 80:20).

The chromatograms were scanned and a major radioactive peak was found of same mobility as the 20α- and 20β-alcohol of the administered material (Ro 6-9241 and Ro 6-9236, respectively) but different from that of pregnanediol or the administered material. (The substances were visualized after spraying part of the chromatograms with vanillin-ethanol-sulphuric acid and heating.) The radioactive peak area of the first 24-hour urine collection was eluted with dichloromethane and further resolved by different methods as follows. Thin-layer chromatography (stationary phase: silica gel; mobile phase: dichloromethane:acetone 9:1) of a sample from the eluate revealed after spraying with *p*-toluene sulphonic acid a red spot of same mobility as the 20α-alcohol (which also gave a red spot after spraying). No spot similar to the 20β-alcohol was detected. Gas-liquid chromatography resolution on a SE-30 column of another sample from the eluate also gave a peak whose retention time was identical to that of the 20α-alcohol. Again, no peak identical to the 20β-alcohol was detected. The presence of the 20α-alcohol was also confirmed by mass spectroscopy. These results indicated, therefore, that the retrosteroid Ro 4-8347 is metabolized to the 20α-alcohol but not to the 20β-alcohol derivative.

In order to exclude any possibility of artefact formation due to acid hydrolysis of the urine, a further experiment was performed in which enzymatic hydrolysis was carried out.

Tritrium-labelled Ro 4-8347/5 (10.8 μc), together with the non-labelled material (10 mg on day given radioactive Ro 4-8347/05 and the following two days) were administered orally to two anovulatory patients (M. E. and F. K.). The whole volume of urine collections of the two patients from the first and third days following the ad-

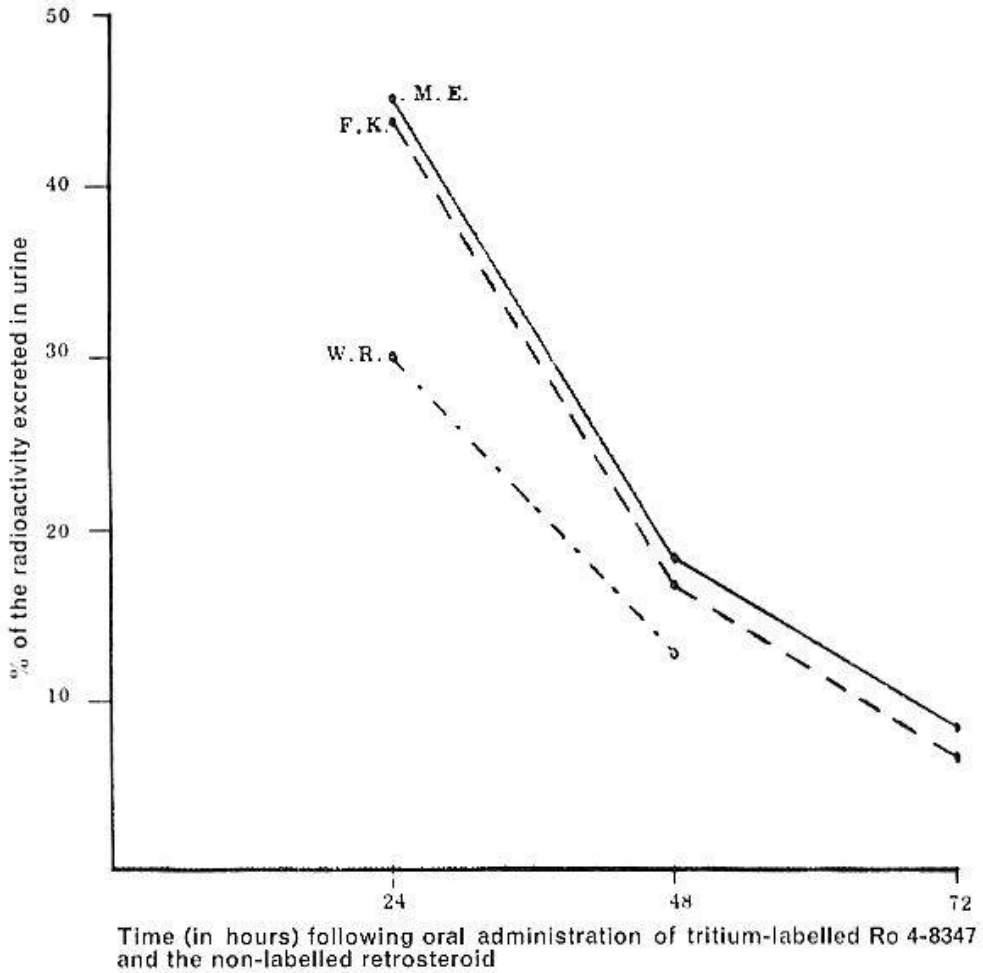

Fig. 1. Rate of radioactivity excreted in urine following oral administration of tritium-labelled Ro 4-8347 and the non-labelled retrosteroid to three anovulatory patients.

ministration of the labelled drug were processed separately in the same manner as for the case discussed above, except that enzymatic hydrolysis (with *Helix pomatia*)[1] was performed this time.

Scanning the chromatograms revealed a major radioactive peak of same mobility as in the previous experiment. The major radioactive peak areas of the different urine collections were eluted with dichloromethane. Gas-liquid chromatography and mass spectroscopical studies revealed that the radioactive spots contained up to 25% of the 20α-alcohol (Ro 6-9241), no 20β-alcohol nor Ro 4-8347 being present.

In order to investigate the urinary excretion rate of radioactivity following administration of the labelled retrosteroid, the amount of radioactivity in the urine collections was estimated (urine samples + ethanol + PPO + POPOP dissolved in toluene measured in Packard Tricarb liquid scintillation spectrometer; corrections were made for quenching). Fig. 1 shows the rate of radioactivity excreted in urine following administration of the labelled retrosteroid to the three patients described above. It is seen that

[1] According to MENINI E. and NORYMBERSKI J. K.: Biochem. J. *95*, 1 (1965).

30–45% of the radioactivity was excreted during the first day, 13–18% during the second day and 6–8% during the following day.

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Discussion

H. BREUER: Did you find any sex difference in the excretion pattern of the 6-chloro compound? Or have you done your studies with females only?

B. LUNENFELD: Unfortunately we have only studied females for the time being and more than this, we have only studied females which we thought belonged to a category which would respond biologically to the compound. But of course in the future we will do it. Or you. One of us!