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From the [1]National Centre for Medicinal Products Control, Sven Oftedalsvei 6, Oslo 9, and the [2]Department of Pharmacology and Toxicology, Veterinary College of Norway, Oslo, Norway

Tissue Distribution of ^{14}C-Lynestrenol in Pregnant Rats

By

E. Sannes[1] and I. Nafstad[2]

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Abstract: The tissue distribution of 4-^{14}C-lynestrenol (17 α-ethynyl-oestr-4-en-17β-ol) following oral administration to pregnant rats was studied by whole body autoradiography and liquid scintillation counting. Pregnant females were sacrificed on days 10, 12, 14 and 19 of gestation, in each case 5 hours after oral administration of 43 μCi 4-^{14}C-lynestrenol per animal. The isotopelabelled compound was distributed throughout most tissues, including the fetuses. The highest concentrations were found in the liver, while there was lower activity in the fatty tissues and the activity in the fetuses was comparable with that in the brain. The placental transfer was verified by the results of liquid scintillation counting. The concentration of labelled substance in the fetuses increased with the duration of pregnancy.

Key-words: Lynestrenol – distribution – rat.

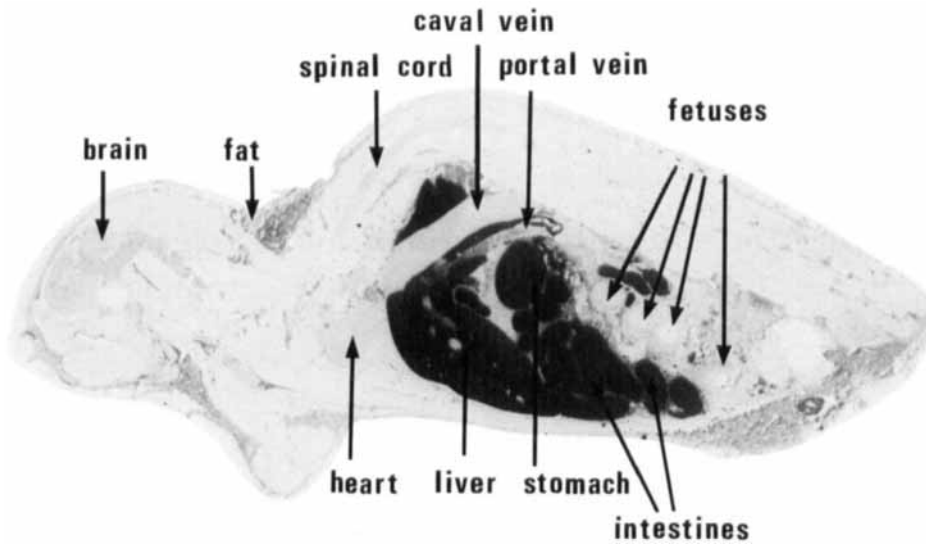
The teratogenic risk involved in the administration of progesterones during pregnancy has been the subject of several reports (M. Halse, personal communication; E. Sannes, A. Lyngset & I. Nafstad, unpublished results). When evaluating teratogenic risk, it is of interest to know whether the compound or its metabolites are able to permeate the placental barrier. Since neither the pattern of distribution nor the metabolism of synthetic progesterones have as yet been fully investigated in different animal species, it was considered to be of interest to study the distribution of isotope-labelled lynestrenol at different stages of pregnancy in an experimental animal model.

Materials and Methods

Isotope-labelled lynestrenol (17α-ethynyl-oestr-4-en-17β-ol) was obtained as 4-^{14}C-lynestrenol. The radiochemical purity was 99% measured by thin-layer chromatography, and the specific activity was 197.4 μCi/mg. The product was stored in benzene until required for use. For oral administration the compound was dissolved in ethanol-A (98.8%) to give a final concentration of 43.1 μCi/50 μl (4.36 mg/ml).

The experiment was performed on young pregnant Wistar rats weighing between 261 g and 395 g. The animals were housed individually in plastic cages with metal bar covers. They were fed a standard laboratory diet *ad libitum* (Norwegian standard for rats BI No. 3155) and they had free access to drinking water. A temperature of 20–23° was maintained with a relative humidity of 40–60%. The day of mating was reckoned as day zero of pregnancy, and the isotope-labelled drug was administered by stomach tube to one animal in each group on day 10, 12, 14 and 19 of pregnancy. Each animal was given 43.1 μCi of 4-^{14}C-lynestrenol dissolved in 50 μl of ethanol A (appoximately 0.7 mg/kg of lynestrenol).

Five hours after administration of the labelled compound the animals were anaesthetized with diethyl ether and immediately frozen in hexane cooled with dry ice to a temperature of approximately −75°. Semiliquid carboxymethylcellulose was used as a mounting medium, and whole-body autoradiography was performed (Ullberg 1954). Sections 30 μm thick were cut on a PMV Cryomicrotome (PMV 450 MP, Sweden) and the autoradiographic exposure was carried out with X-ray film (Kodirex, Kodak Ltd., Great Britain) at −20° for 28 days. The films were developed in D-19 Kodak developer and fixed.



Figs. 1–3 are whole body autoradiograms prepared from sections 5 hours after oral administration of 43 μCi 4-^{14}C-lynestrenol. Dark areas correspond to localization of isotope-labelled substance. Accumulations are seen particularly in the liver and the gastrointestinal tract.

Fig. 1. Whole-body autoradiogram of rat at day 10 of pregnancy. Note levels of radioactivity in placentas comparable with that of the blood in the caval vein.

Tissue samples from liver, brain and pregnant uterus were taken from the frozen blocks and homogenized in water (1:3), incubated with tissue solubilizer Soluene-350 (Packard, Switzerland) for 24 hours at room temperature, and then decolorized with Perdrogen (Riedel-De Haen AG, Seelze-Hannover, Germany). Following the addition of scintillation fluid (Dimilume-30, Packard) and subsequent equilibration at room temperature the samples were counted in a liquid Tricarb scintillation spectrometer (Packard 3310). Counting efficiency was calculated by means of ^{14}C-toluene (Packard) as an internal standard.

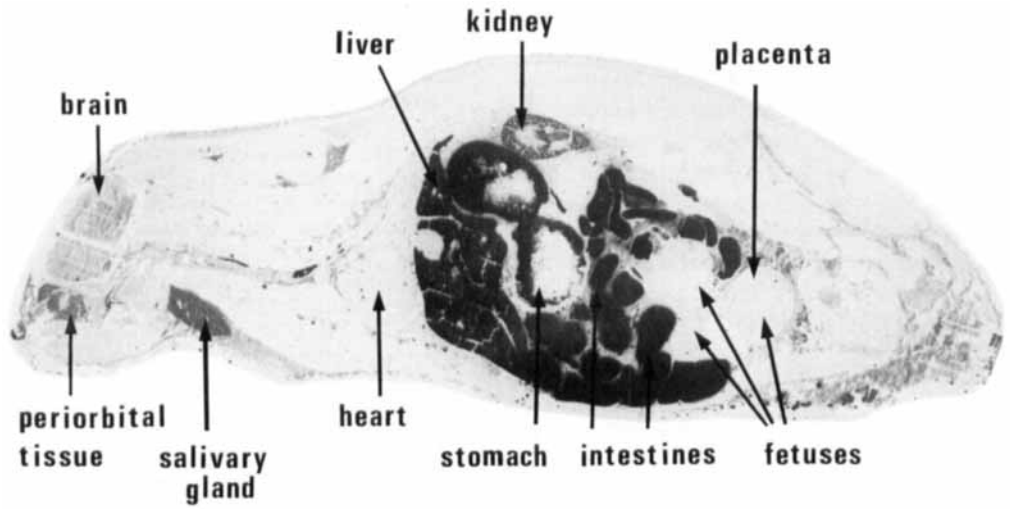


Fig. 2. Whole-body autoradiogram of rat at day 14 of pregnancy. Note high activity in the salivary and periorbital glands and the kidney. The level of radioactivity in placentas is similar to that observed at day 10.

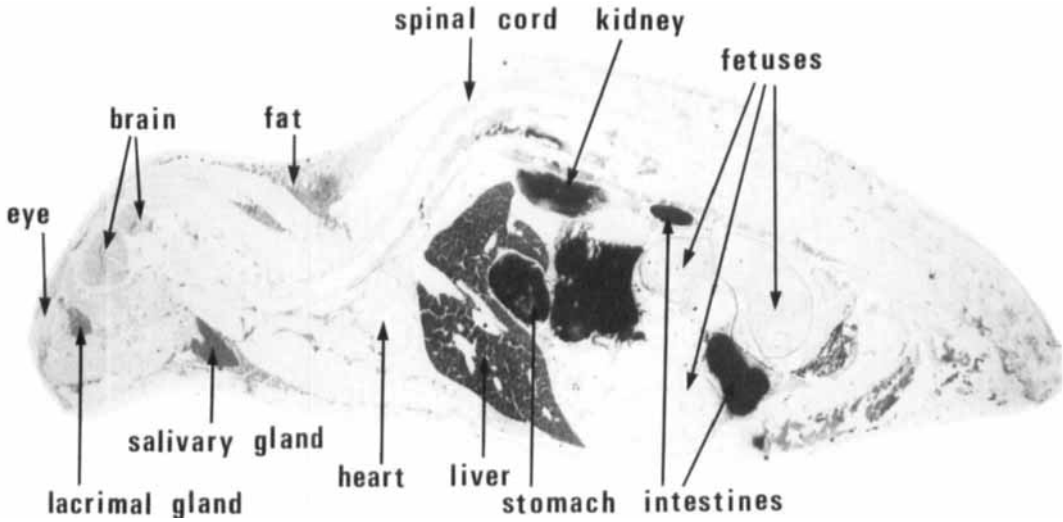


Fig. 3. Whole-body autoradiogram of rat at day 19 of pregnancy. Note the general distribution of radioactivity in the foetuses. Note increased level of radioactivity in foetuses as compared to foetuses at days 10 and 14.

Results

Whole body autoradiography.

Autoradiograms prepared 5 hours after oral administration of the labelled compound showed radioactivity in most of the tissues, with a large accumulation in the gastrointestinal tract, the liver and the renal cortex (figs. 1–3). Substantial amounts of radioactivity were also present in the adipose tissue, the salivary glands and the periorbital glands (fig. 2). Smaller amounts of radioactivity were found in the brain and the spinal cord, the blood, the heart muscle, the adrenals and the placenta (figs. 1 and 2). The level of radioactivity in the placenta was comparable with that of the blood, while the amount in the foetuses was obviously lower than in the placenta of rats sacrificed at days 10, 12 and 14 of gestation (figs. 1 and 2). At day 19 of pregnancy, however, no difference could be observed between the radioactivity in foetuses and placenta respectively, and the radioactivity now appeared evenly distributed throughout the foetus without any tendency to particular tissue accumulation (fig. 3).

Liquid scintillation counting.

The results of scintillation counting in table 1 show that 5.2 to 7.5% of the ingested dose was present in the liver. Foetus concentrations were comparable with those in the brain, and represented between 0.05 and 4% of the amount in the liver. The amount of radioactivity in the foetuses showed a tendency to increase proportionally to the duration of pregnancy.

Table 1.

Results of scintillation counting performed on organ samples and foetuses from pregnant rats (approximately 300 g) administered 43 μCi (approximately 0.73 mg/kg) ^{14}C-lynestrenol orally and killed 5 hours later.

Rat No.	Day of pregnancy	Foetus Radioactivity d.p.m./g	Foetus % of dose/g	Liver Radioactivity d.p.m./g	Liver % dose/g	Brain Radioactivity d.p.m./g	Brain % of dose/g
1	10	34362	0,03	518333	0,5	47583	0.05
2	12	23236	0,03	n.i.	n.i.	25833	0,03
3	14	56817	0,06	553446	0,6	25706	0,03
4	19	46884	0,05	305443	0,3	38371	0,04

Discussion

The finding that the isotope-labelled compound was distributed throughout most tissues 5 hours after oral administration corresponds with the knowledge of a rapid absorption and distribution of synthetic progesterones of the 17α-ethynyl-group in rabbit and in man (Kamyab *et al.* 1967; Fotherby *et al.* 1968; Fotherby 1974). In man, the peak concentration of lynestrenol in plasma was observed 4–6 hours after oral administration (van der Molen *et al.* 1969).

The high radioactivity in the liver demonstrated by whole-body autoradiography in the present study is in agreement with previous investigations which have reported the liver as being the organ chiefly responsible for the metabolism of synthetic progesterones (Kamyab *et al.* 1967; Mazaheri *et al.* 1970). The large amounts of labelled compound detected in the kidney can be explained by the role of the urinal pathway as a major excretion route in man and in rabbit for lynestrenol and its metabolites (van der Molen *et al.* 1969; Kamyab *et al.* 1968). The relatively high radioactivity in the salivary and lacrimal glands indicates some excretion through these organs. The concentrations of labelled substance in the placenta and the foetuses have not, to our knowledge, been reported earlier. The high radioactivity in foetuses at day 19 of gestation compared with earlier stages of pregnancy, revealed by whole-body autoradiography, were also demonstrable by scintillation counting. The percentual increase of radioactivity in the foetuses in relation to duration of pregnancy indicated an increased placental transfer in the final stage of gestation.

The observation that lynestrenol permeates the placental barrier may be of importance in the discussion of the possible teratogenic effect of synthetic progesterones.

Acknowledgement

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