THE AUTORADIOGRAPHIC DISTRIBUTION PATTERN
AFTER ADMINISTRATION OF DIETHYLSILBOESTROL COMPARED
WITH THAT OF NATURAL OESTROGENS

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ABSTRACT

The distribution in mice of $^{14}$C- and $^3$H-diethylstilboestrol has been investigated autoradiographically. The results have been compared with those which have been previously reported for natural oestrogens. Many similarities have been demonstrated between the synthetic and natural compounds. Thus a specific accumulation has been observed in the endometrium, the granulosa layer of large ovarian follicles, the adrenal cortex, the interstitial tissue of the testes, and the hypophysis. Natural and synthetic oestrogens differ widely concerning the penetration into and the distribution within the foetus.

The autoradiographic distribution pattern of the natural oestrogens $^{14}$C-oestrone and $^3$H-oestradiol has been reported previously (Ullberg & Bengtsson 1963). Natural oestrogens accumulated specifically in certain sites and the two compounds tested had more or less similar distribution patterns. Natural and synthetic oestrogens differ widely in chemical structure but have similar biological effects. For this reason we wished to ascertain whether the distribution patterns for both these types of oestrogens differ or resemble one another.

This report is concerned with the autoradiographic observations on whole mice injected with monoethyl-1-$^{14}$C-diethylstilboestrol and details of the distribution pattern in particular organs from mice injected with a tritiated diethylstilboestrol compound. The observations will be compared with those obtained earlier for natural oestrogens.

The available literature dealing with the distribution after administration of natural oestrogens has been reviewed in the earlier report. In the case of diethylstilboestrol radioactivity measurements on entire organs and organ ex-
tracts have not led to precise information about specific tissue localization which can be associated with the physiological activity. Thus Twombly & Schoenewaldt (1951) and Twombly (1951) could not detect any accumulation of $^{14}$C in the mammary glands, mammary carcinoma, uterus, adrenals or hypophysis of mice injected with $^{14}$C-diethylstilboestrol. The ovaries of rabbits contained 0.1 per cent of the amount injected and the uterus less than 0.1 per cent. In rats given 2 mg of the same substance there was only slight radioactivity after 6 hours and then mainly in the intestinal tract (Hanahan et al. 1951). At a dose level of 5 micrograms only the liver had a significant content of $^{14}$C (Hanahan et al. 1953).

Steers fed tritium-labelled diethylstilboestrol have shown the greatest accumulation of radioactivity in the liver and kidneys (Mitchell et al. 1956). Dogs injected with $^{85}$P-labelled diethylstilboestrol diphosphate had little or no accumulation of radioactivity in the prostate (Persky et al. 1957).

By using autoradiographic techniques in this investigation it has been possible to detect even small loci of accumulation which can easily be overlooked when using impulse counting methods.

**MATERIAL AND METHODS**

*Whole-animal autoradiography*

Full-grown mice, 9 males and 20 females of which 11 were pregnant, were used for whole-animal autoradiography. Diethylstilboestrol (monoethyl-1-$^{14}$C, obtained from RCC, Amersham, England) was injected subcutaneously into 6 males and 12 females of which 6 were pregnant. Specific activity was 12.6 mc per mmol. The compound was dissolved in peanut oil and 0.2 ml given each mouse. The dose per mouse corresponded to 0.2 mg of the compound or 9 microcuries.

The animals were killed in groups of three (a male, a nonpregnant female, and a pregnant female) at intervals after injection by rapid freezing to about $-70^\circ$C in a mixture of acetone and carbon dioxide. The intervals chosen were 5 min, 20 min, 1 hour, 4 hours, 24 hours and 4 days.

Three male mice and 8 females (5 pregnant) were injected intravenously with the same compound dissolved in glycerol, water and Tween 80. The solution was prepared by dissolving $^{14}$C-diethylstilboestrol in ether. One part Tween 80 was added to 9 parts of a 10 per cent (v/v) solution of glycerol in water and this mixture was added to the ether solution of stilboestrol. The ether was then evaporated at about $50^\circ$C while the mixture was slowly shaken. Each mouse received 0.11 ml in a tail vein corresponding to 0.11 mg or 5 microcuries. The pregnant mice were killed after intervals of 5 min, 20 min, 1 hour, 4 hours and 24 hours. The males and the nonpregnant females were killed after 20 min, 1 hour and 4 hours.

From this point onwards the methods employed correspond to those described previously for natural oestrogens (Ullberg & Bengtsson 1963).

A $^{14}$C-staircase made from known concentration of $^{14}$C in twofold serial dilutions was placed as a reference together with the whole-mouse sections on the films to permit a semiquantitative densitometric evaluation of the autoradiograms.
Detail autoradiography

Particular organs were taken from 3 males and 3 nonpregnant females for more detailed autoradiographic study. The mice were injected subcutaneously and intramuscularly with 0.45 mg diethylstilboestrol-T (Amersham, specific activity 295 mc per mmol) dissolved in peanut oil so that 0.32 ml contained 500 microcuries. The mice were killed 4 hours after injection and the testicles, ovaries, and pieces of the uterus were quickly dissected out and placed in isopentane cooled to -180°C with liquid nitrogen. Five micra thick cryostate sections were dry-mounted on Kodak AR 10 stripping film which was stretched on chrome alum treated glass slides as described previously (Ullberg & Bengtsson 1963).

RESULTS

Stilboestrol given subcutaneously in oil is to a great extent absorbed from the site of injection after 4 hours.

After subcutaneous application a relatively high accumulation of radioactivity was seen in target organs while the blood level was very low. The highest concentration in the tissues appeared 20 minutes to 1 hour after injection. When 14C-diethylstilboestrol was given intravenously the distribution pattern was more diffuse. Four hours after intravenous injection the blood still contained visible amounts of radioactivity. The uptake in the target sites was less distinct after intravenous injection while the accumulation in excretory organs was more pronounced. The differences in the distribution pattern for the two means of administration, however, were not so great that we have considered it necessary to distinguish between them while describing the results. For both ways of administration an accumulation was seen in target tissues such as the endometrium, the granulosa layer of large ovarian follicles, the adrenal cortex, the interstitial tissue of the testes and the hypophysis.

Diethylstilboestrol was excreted fairly rapidly and the autoradiograms representing 24 hours after injection were dominated by the excretory pattern, with most activity in the intestines, gall-bladder, liver, kidneys and salivary glands. Four days after injection the liver was the only site of radioactivity.

The distribution patterns for various organs and tissues at different intervals after injection will be described in more detail.

Skeleton. Cortical bone had a low activity while the activity in the bone marrow was relatively intense.

Muscle tissue. Skeletal musculature had a relatively high level of activity. Activity in the myocardium was much greater than in the heart blood (Fig. 1).

Respiratory tract. The bronchial mucosa and secretions had a high level of activity up to 24 hours after injection.

Digestive tract. There was a relatively strong accumulation of activity in the oral mucosa and tongue as early as 5 minutes after injection. Specific uptake was also observed in the salivary glands. By 24 hours, however, the salivary
Autoradiogram from a male mouse 20 minutes after subcutaneous injection of \(^{14}\text{C}\)-diethylstilboestrol. White areas correspond to high radioactivity. Note low activity in heart blood and high in liver, adrenal cortex, kidney, myocardium, nasal secretion and salivary gland. \(\times 1.25\).

Glands were nearly entirely free from activity. In the gastric mucosa there was a fairly strong accumulation in the basal portions of the glands. Activity in the intestinal lumen gradually increased, presumably as a result of continuous excretion in the bile. There were no signs of excretion through the intestinal mucosa.

Liver. Activity in the liver increased until 4 hours after injection and then slowly subsided but was still notable after 4 days. The bile showed activity by 5 minutes after injection and increased until one hour after injection. After one hour the activity in the bile was greater than that in the liver.

Pancreas. The relatively high content of \(^{14}\text{C}\) was still evident 4 hours after injection.

Kidneys had their greatest activity up to 4 hours. The activity gradually subsided and was quite low after 24 hours.

Central nervous system. There was specific accumulation in the brain and spinal cord. The activity was slightly higher in the grey than in the white matter (Fig. 2).

The eye had a relatively high \(^{14}\text{C}\)-content and of the periorbital tissues the lacrimal gland had a high level of activity.

Lymphatic tissue. There was a low concentration of activity in the lymph nodes and thymus throughout the entire observation period. Activity in the spleen was much greater but was limited to the red pulp.

Adrenals. There was a rapid and intense accumulation of activity in the cortex but there was no visible activity at all in the medulla (Fig. 1). The cortical accumulation was visible by 5 minutes and was still strong after 4 hours but then subsided gradually. The activity seemed to be greatest in the peripheral portion of the cortex. Pregnant females had lower activity than the other mice.
Fig. 2.
Autoradiogram from a pregnant mouse 20 minutes after intravenous injection of $^{14}$C-diethylstilboestrol. High uptake in liver, intestines, nasal secretion, salivary gland, i.e. excretory organs. Note also high uptake in brain, bronchial mucosa and foetal liver.

Pregnant mice have relatively low uptake in the adrenal cortex. $\times 4.5$.

Fig. 3.
Detail from Fig. 2 showing foetal tissues. Strong accumulation in the liver, slight accumulation in the adrenal cortex. $\times 7$. 
Fig. 4.
Detail of whole-body autoradiogram of mouse killed 20 minutes after intravenous injection of $^{14}$C-diethylstilboestrol showing testis and surrounding tissues. High concentration in interstitial cells of testis. $\times 7$.

Fig. 5.
Detail of whole-body autoradiogram of mouse killed 4 hours after subcutaneous injection of $^{14}$C-diethylstilboestrol, showing the ovary. High uptake in corpora lutea. $\times 7$. 
Fig. 6.
Detail of whole-body autoradiogram from a pregnant mouse 4 hours after subcutaneous injection of $^{14}$C-diethylstilboestrol. Note high activity in uterine wall, vagina mucosa and secretion and foetal liver.

The hypophysis had a much higher content of $^{14}$C than the brain. The thyroid had a low level of activity at all intervals studied. There was no specific uptake in the endocrine portions of the pancreas.

In the testicle and epididymis activity was evident from 5 minutes to 4 hours after injection. A specific accumulation was seen in the interstitial tissue of the testicle (Fig. 4).

Ovary. There was distinct accumulation of activity in the ovary with the highest level in corpora lutca and in the walls of large follicles (Fig. 5).

In the uterus there was a high uptake in the endometrium and a fairly low uptake in the myometrium.

The placenta had a somewhat greater degree of activity than the blood. There was a fairly intense accumulation along a narrow zone adjacent to the foetus which probably represents the visceral yolk sac epithelium.

Foetuses. There was a low level of radioactivity in the foetuses 5 minutes after injection. At 20 minutes the activity was still low and distinctly evident only in the liver (Fig. 2). Activity was still evident in the foetal liver and

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gall-bladder after 24 hours. At 4 hours after injection the concentration in
the liver was high and the anterior portion of the intestine also contained
much activity, presumably the result of biliary excretion. Activity levels in the
foetuses never attained those in the dams.

Activity in the foetal brain was less than in the foetal skeletal musculature.
the converse of the relation in the maternal tissues. On the whole, however,
the foetal distribution pattern resembled the maternal distribution fairly close-
ly. Distinct but weak accumulation was evident in the foetal adrenals (Fig. 3).

**DISCUSSION**

**General**

From our studies it is evident that both natural and synthetic oestrogens can
be demonstrated at sites at which local accumulation can be put in relation to
physiological effects. The specific affinity for certain structures is apparently
much the same for both types of substances.

Selective affinity for both types of oestrogens occurs in the endometrium.
the membrana granulosa of the larger ovarian follicles, the adrenal cortex,
the interstitial cells of the testicles, and the hypophysis. Specific localization
was also observed in tissues which cannot be related to endocrine effects, e.g.
bronchial mucosa and red pulp of the spleen. There was a relatively strong
accumulation of both natural and synthetic oestrogens in the brain.

The difference in the distribution patterns for natural and synthetic oestro-
gens were mainly quantitative and of relatively slight degree. Distinct qualita-
tive differences were however found in the placental passage and foetal
distribution.

Specific uptake of diethylstilboestrol and the natural oestrogens is expected
in some sites but more difficult to explain in others. On the whole, the simi-
larities in the distribution patterns for stilboestrol and natural oestrogens sug-
gest the presence of active hormone and not, to any great extent at least, the
accumulation of inactive metabolites in the target sites.

The method of administration has some effect upon the autoradiograms.
After intravenous injection the distribution appears to be more diffuse with
a less pronounced accumulation in the target sites but a more rapid accumu-
lation at the sites of excretion. Subcutaneous injection results in pronounced
accumulation at the target sites but a hardly noticable concentration in the
blood at any of the intervals studied. In studies on a polystilboestrol compound
(Bengtsson & Ullberg, to be published) accumulation was even more intense
in such sites as the adrenal cortex and interstitial tissue of the testicles, pre-
sumably because of slow release of the simple molecules from the polystilbo-
estrol depots.

Below some sites of specific accumulation will be considered in more detail.
Ovary. The observation that not only oestradiol as we showed previously but also diethylstilboestrol accumulates in follicular epithelium cells is a strong evidence that granulosa cells are a target for oestrogens. It is known that mitotic activity increases in the follicular granulosa after the injection of natural (de Wit 1953) and synthetic (Payne & Hellbaum 1955) oestrogens.

Adrenal cortex. Like natural oestrogens (Ullberg & Bengtsson 1963) diethylstilboestrol has a specific affinity for the adrenal cortex. The question then arises whether the adrenal cortex is a true target site for synthetic oestrogens and whether the uptake there is of the same nature as for oestrone and oestradiol. In this context it can be mentioned that the increase in width of the adrenal cortex, which occurs after prolonged administration of natural oestrogens (Korenchevsky & Dennison 1935; Selye et al. 1935; Ellison & Burch 1936) also takes place after treatment with synthetic oestrogens (Allen & Bern 1942; Clegg & Cole 1954; Glasser & Leathem 1955; Vogt 1955, 1957).

Oestrogens are reported to inhibit corticoid synthesis in the adrenals (Vogt 1955, 1957; McKerns 1957; McKerns et al. 1958). According to McKerns (1959) and McKerns & Bell (1960) this effect results from the oestrogens inhibiting the enzyme glucose-6-phosphate dehydrogenase.

Foetus. The greatest difference between the distribution patterns after administration of diethylstilboestrol and natural oestrogens was in maternal-foetal transfer and distribution in the foetus. By 20 minutes after the injection of oestrone and oestradiol the foetuses had a more intense activity than the maternal tissues and maintained their dominance as long as the substance remained visible on autoradiograms. This distribution pattern suggested an active transfer of natural oestrogens across the placenta.

Oestrone and oestradiol differ in this respect not only from diethylstilboestrol but also from a number of other natural steroid hormones (progesterone, testosterone, cortisone) which have recently been studied by whole-animal autoradiography of pregnant mice in our laboratory. The lower content of diethylstilboestrol in foetal tissues during the whole course of distribution and excretion is a sign of a partial placental barrier.

In the foetus 14C-diethylstilboestrol accumulated in excretory organs just as in the dams. There was also some accumulation in the foetal adrenal glands indicating that the foetal just as the maternal adrenals served as a target site.

The placenta seems to have a better capacity than other tissues for distinguishing between natural and synthetic oestrogens since there is active transplacental passage of oestrone and oestradiol but apparently a partial placental barrier to the passage of diethylstilboestrol.

Accumulation of oestrone and oestradiol in the foetus can be a sign of a physiological function in the foetus. If this is the case it may be an explanation for the intense synthesis of oestrogens in the placenta during pregnancy. It seems unlikely that the requirements of oestrogens of the other maternal tissues
increase so much during pregnancy that a manifold increase in synthesis is necessary.

ACKNOWLEDGEMENTS

This investigation was financially supported by grants from Statens Medicinska Forskningsråd, Knut och Alice Wallenbergs stiftelse, Jordbrukets Forskningsråd and Riksforeningen mot cancer.

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Received on February 26th, 1963.