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DISTRIBUTION OF INJECTED ^{14}C-DIETHYLSTILBESTROL IN THE CHICKEN AND LAYING HEN

WHOLE BODY AUTORADIOGRAPHY AND IMPULSE COUNTING*

By

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BENGTSSON, S. G.: *Distribution of injected ^{14}C-diethylstilbestrol in the chicken and laying hen. Whole body autoradiography and impulse counting.* Acta vet. scand. 1978, *19*, 254—262. — Distribution of intramuscularly administered ^{14}C-diethylstilbestrol (DES) in the chicken and laying hen was studied by whole body autoradiography and impulse counting techniques. In both groups the liver showed the highest concentration of ^{14}C. The ^{14}C-level of the chicken liver and bile appeared greater than the same level in the hen. The ^{14}C-content of the hen kidney was twice that found in the chicken. These findings could mean that the metabolic and excretory function of the kidney is less developed in the young chicken than in the adult bird and that the chicken liver may compensate for insufficient kidney function. The lower ^{14}C-concentration of the chicken adrenal as compared to the hen, could indicate a reduced physiological activity of the young adrenal. The accumulation of radioactivity in the membrane of the follicular yolk should represent excretion. Low ^{14}C-content was found in the skeletal muscle. It is concluded that consumer products based mainly on the liver from DES-treated chickens should not be used for consumption.

diethylstilbestrol; autoradiography; laying hen; chicken.

In several countries diethylstilbestrol (DES) is used as a feed additive in chicken production. Objections have been raised against use of DES, because residual hormone in meat and offal could constitute potential risks to the consumer. Tissue uptake

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after administration of DES to chickens has been studied by biological and chemical methods. In addition, impulse counting techniques after an administration of radioactively labelled hormones have been used. However, with all these methods organs and tissues which are likely to be of interest must be selected without the benefit of prescreening localization.

In the present work the distribution in chickens and laying hens after injection of ^{14}C-DES has been studied using the whole body autoradiographic technique of *Ullberg* (1954, 1958). This method renders it possible to localize radioactivity even in small organs and parts of tissues which can easily be overlooked with other methods. In addition, quantitative measurement of ^{14}C has been performed. The combined techniques of autoradiography and impulse counting have been shown to be suitable in distribution studies in general. Moreover, it should be valuable for the detection of residuals in the edible tissues of chickens supplied feed additives.

MATERIAL AND METHODS

Radioactive material

Diethylstilbestrol (monoethyl-1-^{14}C) was obtained from RCC, Amersham, England. Radiochemical purity was tested by thin-layer chromatography on silica gel in a solvent system of chloroform and acetone (3:2 by volume) and found to be at least 98 %. The specific activity of DES was 39 mCi/mmole. The compound was dissolved in peanut oil to give a concentration of 40 μCi/0.5 ml solution.

Animal material

Eight 2-week old white leghorn chickens weighing about 130 g and 8 10-month old laying hens, live weight about 1.5 kg, were used. The chickens were hatched in a battery and the laying hens were loose housed. They were fed a non-estrogenic Swedish commercial feed mixture containing ground oats, barley, fishmeal and vitamins. All animals had free access to water and the grown up ones to shells. The chickens and the hens were given 40 μCi DES/kg body weight by a single injection in each thigh muscle. Prior to the injection, blood was drawn in heparinized tubes from a superficial wing vein. Further blood samples were taken 30 min., 1 hr., 2, 3 and 4 hrs. later. The last sample was taken immediately before killing.

Whole animal autoradiography

One and 4 hrs. after injection, 2 hens and 2 chickens were sacrificed by anaesthetizing with ether. The feathers were removed and the bodies rapidly frozen by immersion in hexane cooled to about —75 °C with solid carbon dioxide. The subsequent procedure was that described in detail by *Ullberg* (1954, 1958).

Quantitative determinations

Visual reading of the autoradiograms and quantitative determination on sections were made in the way previously reported (*Bengtsson & Hakkarainen* 1975) as was ^{14}C-countings in remaining non-sectioned animals killed 1 hr. and 4 hrs., respectively, after administration of ^{14}C-DES. In addition, a ^{14}C-staircase made from a known concentration of ^{14}C in twofold serial dilutions was placed as a reference together with the whole animal sections on the films to permit a semiquantitative densitometric evaluation of the autoradiograms.

RESULTS

The ^{14}C-concentration in the blood plasma reached its maximum 1 hr. after injection in the chicken and the hen. The distribution picture in the hen and chicken after administration of ^{14}C-DES is shown in Figs. 1 and 2. The relative percentage of the isotope in different organs as compared to the liver is also given in the autoradiograms. In Table 1, absolute amounts of radioactivity in tissues and fluids sampled from animals 4 hrs. after the injection of ^{14}C-DES are given.

In both groups the liver showed the highest concentration of ^{14}C. The ^{14}C-level of the chicken liver, however, appeared greater than that of the hen liver as was the content of the chicken bile and small intestinal ingesta. On the contrary, the accumulation of radioactivity in the hen kidney was twice that found in the chicken. A still larger difference in favor of the hen was obtained for the adrenals. No complete distinction between cortex and medulla could be made, but most of the activity was confined to the outer layers of the adrenal. Large intestinal ingesta demonstrated a very high ^{14}C-activity in hen and chicken.

There was a moderately strong uptake of ^{14}C by the pancreas. The lung and the myocardium showed about the same ^{14}C-content. Very low amounts of radioactivity were found in the brain and skeletal muscles of both age-groups.

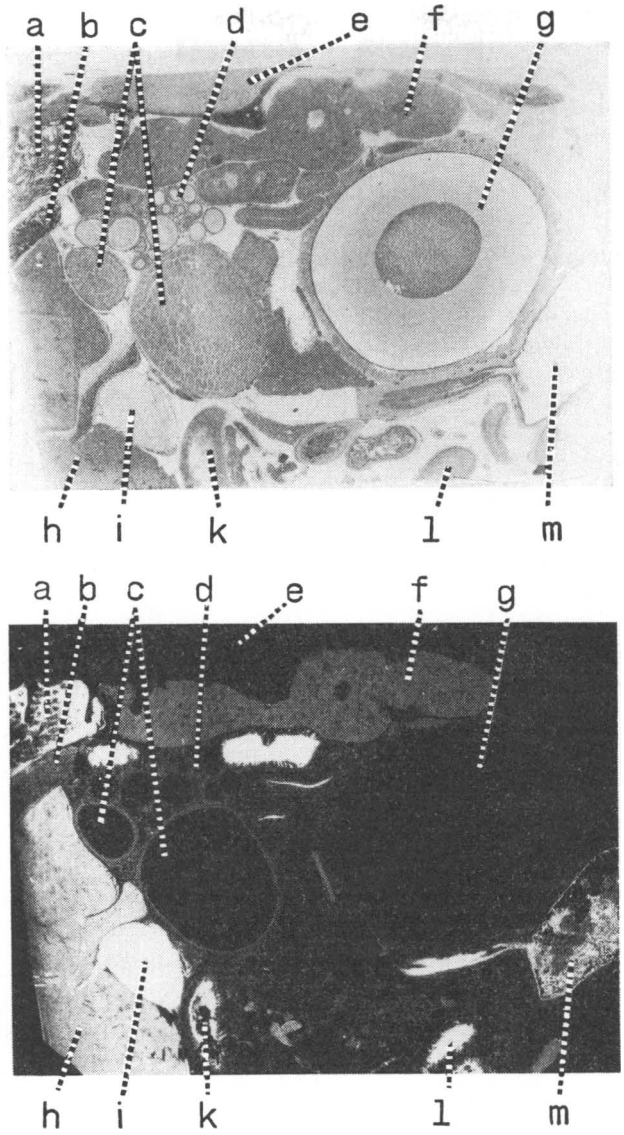

Figure 1. Autoradiogram (lower) and corresponding section (upper) of a laying hen 1 hr. after i.m. injection of ^{14}C-diethylstilbestrol. The letters refer to the following organs and tissues: a lung; b blood 6; c follicular yolk; d ovary; e body muscle 6; f kidney 42; g egg in the oviduct; h liver 100; i bile; k gizzard 2; l intestines 50—250; m cloaca. Figures refer to CPM of corresponding dry section piece relative to the liver. Note high ^{14}C-content of the bile, liver and intestinal ingesta, distinct uptake in the follicular yolk and large yolk membrane. Very low amounts of radioactivity are seen in the mature egg.

Table 1. Radioactivity in tissues of chickens and laying hens 4 hrs. after intramuscular injection of ^{14}C-diethylstilbestrol. Values refer to range given as $10^{-3} \times$ CPM/g wet weight.

Tissue	Chicken	Hen
Liver	162—170	103—138
Kidney	37.2— 37.3	57.1— 98.7
Adrenal	12.1— 13.0	81— 62
Pancreas	18.5— 19.3	20.6— 21.0
Lung	10.6— 13.5	8.1— 13.2
Myocardium	8.8— 11.0	6.8— 9.3
Body muscle	6.3— 8.2	2.4— 2.9
Brain	3.5— 3.8	3.7— 4.0
Gizzard	3.0— 3.6	5.4— 5.9
Bile	6891—9424	2616—3734
Small int. ingesta	525—1217	159—367
Large int. ingesta	1304—1440	1022—2527
Ovary	a	14.4— 17.7
Egg yolk membrane	a	11.9— 12.7
Oviduct	a	8.6— 10.1
Comb	a	5.6— 7.2
Egg albumen	a	0.2
Egg yolk	a	0.01

[a] not counted.

In contrast to small follicles and mature eggs there was a high uptake in the walls of the medium-sized follicles and in the membrane of the follicular yolk in the laying hen. Within the mature egg, the ^{14}C-content was too low to be demonstrated on the autoradiograms. However, the concentration in the albumen exceeded that in the yolk, except in the yolk membrane, as revealed by impulse counting. The ^{14}C-activity of the oviduct was just below that of the follicular yolk membrane and considerably in excess of that of the comb. Within the gizzard there was a quantitative difference in favor of the koilin. A fairly high amount of radioactivity appeared in the ingesta of the glandular and muscular part of the stomach.

DISCUSSION

In the chicken impulse values obtained 1 hr. after administration of ^{14}C-DES varied widely making interpretation of the findings difficult. The results concerning the survival time of 1 hr. therefore have been omitted. As revealed by autoradiography there

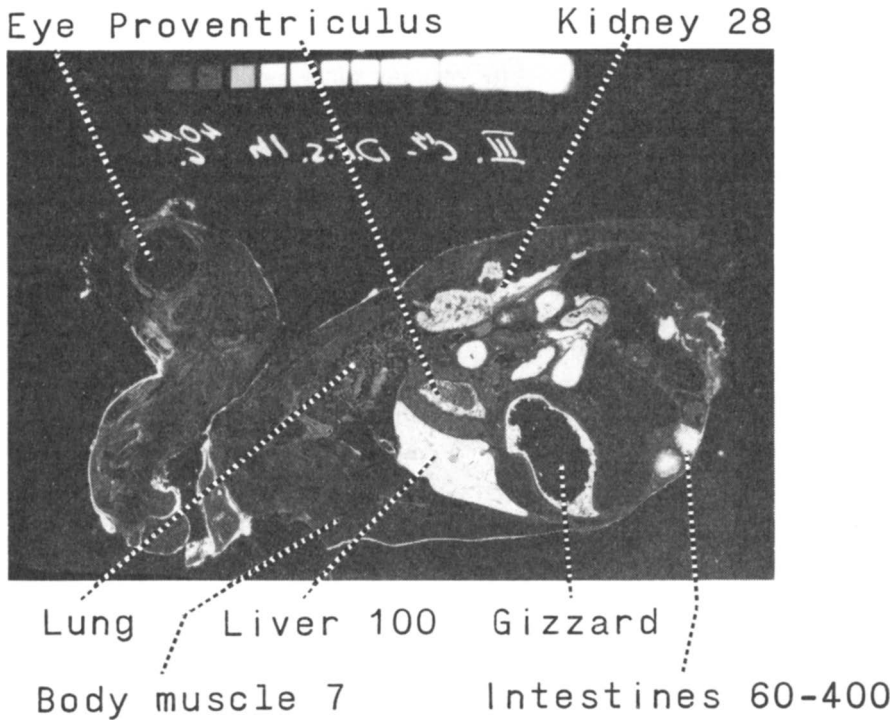

Figure 2. Autoradiogram of a 2-week old chicken 1 hr. after i.m. injection of ^{14}C-diethylstilbestrol. An isotope staircase has been placed above the autoradiogram. For further explanation see legends to Fig. 1. Note high amounts of radioactivity in the liver, intestinal lumen, the koilin of the gizzard and the kidneys. Distinct uptake is seen in the lungs while that of the body muscle is low.

was no qualitative difference in tissue uptake of ^{14}C between animals killed 1 and 4 hrs., respectively, after injection.

The liver and kidney are the main elimination organs of injected DES (*Hopwood & Gassner* 1962 a, b). In the present study the ^{14}C-content of the chicken liver and bile was considerably in excess of that in the liver and bile of the hen. By contrast, the ^{14}C-level in the hen kidney was about twice that in the same organ of the chicken. Under the assumption of equal resorption rate within 4 hrs. after injection of the DES in the hen and the chicken this finding could indicate that the metabolic and excretory function of the kidney is less developed in the young chicken than in the adult bird. Moreover, the liver of the chicken may compensate for insufficient function of the kidney.

DES is to some degree conjugated in the liver (*Hopwood & Gassner* 1962 b). It cannot, however, be established from the present study whether conjugation takes place to an increased extent in the chicken liver, or whether high liver and bile radioactivity represents other metabolites of the hormone injected, or an unchanged DES. The high ^{14}C-level of the chicken liver should scarcely be attributed to an enterohepatic circulation which is reported to occur in rats (*Hanahan et al.* 1953, *Fischer et al.* 1966).

In mice (*Bengtsson & Ullberg* 1963, *Bengtsson* 1963), DES was specifically accumulated in the adrenal cortex. The affinity of both natural and synthetic estrogens for the adrenals was suggested being related to a physiological function at this site (*Ullberg & Bengtsson* 1963). As distinct from the chicken the cortical zone of the hen adrenal showed a very high level of radioactivity indicating low physiological activity of the young adrenal.

The uptake of ^{14}C by the ovary and the oviduct is in agreement with earlier findings in mice (*Bengtsson & Ullberg).* The ^{14}C-content in the egg as a whole was low. Autoradiography, however, revealed considerable accumulation of radioactivity in the membrane of the follicular yolk in later developmental stages. This localization probably represents excretion rather than affinity to the lipid portion of the membrane. In this context, it may be pointed out that residues of DES have been found in eggs of laying hens 7 days after subcutaneous injection of DES in oil (*Umberger et al.* 1963), and that stilbestrol propionate caused lowered egg production in poultry (*Arneja & Arora* 1972). In the present study, with the exception of the membrane, the egg yolk showed a lower ^{14}C-content than the albumen. This difference could be explained by transfer of DES-residuals to the white from the oviduct before shell formation and by the short survival time after injection.

The comb showed a low ^{14}C-uptake. The well known effect on this organ in DES-treated castrated cockerels therefore could be an indirect one, mediated via the endocrine system. The ^{14}C-content of the pancreas probably represents excretion as is apparently the case of the radioactivity in the digestive tract. Part of ^{14}C-DES may be oxidatively metabolized and excreted as $^{14}CO_2$ judging by the comparatively high level of radioactivity found in the lungs. As in mice (*Bengtsson & Ullberg*), the myocardium

showed higher ^{14}C-content than the skeletal muscle in both the laying hen and chicken, indicating increased uptake in the actively working muscle.

The present study deals with a single injection of moderate amounts of DES. However, it may be at least partly comparable with an implantation. Moreover, in the chicken, considerable amounts of ^{14}C-DES-derived radioactivity remain in different tissues more than 3 weeks after implantation (*Hopwood & Gassner* 1962 a). Concerning the question of residuals in the edible tissues and organs of DES-implanted chicken, it seems quite clear that consumer products based mainly on the liver from such chickens should be avoided unless it has been shown that no active DES or DES-residuals occur in this organ. In the case of DES-fed chicken, the same should be true unless the feed additive is withdrawn long enough before slaughter. Both egg albumen and yolk contain DES-residuals as does the body muscle. However, further studies with sensitive methods are required to clarify the contamination level of the carcass residue.

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SAMMANFATTNING

Vävnadsupptag av ^{14}C hos kycklingar och äggläggande hönor efter parenteral tillförsel av ^{14}C-diethylstilbestrol.

På äggläggande hönor och 2 veckor gamla kycklingar har vävnadsupptaget studerats efter intramuskulär injektion av ^{14}C-diethylstilbestrol (DES). Heldjursautoradiografi samt impulsräkning på vävnader, organ och kroppsvätskor har utförts. Hos både kycklingar och hönor visade levern den högsta ^{14}C-koncentrationen. Kycklinglevern innehöll mer radioaktivitet 4 timmar efter tillförseln av ^{14}C-DES än levern från äggläggande hönor. Halten ^{14}C i njurarna från denna sist nämnda grupp var dubbelt så hög som i samma organ från kycklingar. Detta kan indikera att de metaboliska och exkretoriska funktionerna är mindre väl utvecklade hos njuren på unga kycklingar än på vuxna hönor och att levern delvis skulle kunna kompensera den unga njurens bristande funktion i nu nämnt avseende. Jämfört med äggläggande hönor visade binjurarna från kycklingar låg ^{14}C-koncentration antydande reducerad fysiologisk aktivitet hos den unga binjuren. Ansamlingar av radioaktivitet i membranen på folliculära gulor bedömdes vara ett uttryck för elimination av DES eller DES-metaboliter. Relaterat till övriga vävnader innehöll kroppsmuskulaturen små mängder ^{14}C. Det fastslogs att produkter baserade huvudsakligen på lever från DES-behandlade kycklingar inte bör användas för konsumtion.

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