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A STUDY OF THE EFFECTS OF CONTAMINANTS ON STEROIDOGENESIS IN CANADIAN GRAY AND HARP SEALS

by

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SUMMARY

An *in vitro* study on the effects of the contaminants polychlorinated biphenyl (Aroclor 1254) (PCB), methyl mercury (MeHg), arsenic (As), cadmium (Cd), and selenium (Se) on the biosynthesis of steroid hormones in the gray seal (*Halichoerus grypus*) indicated altered steroid biosyntheses.

Biotransformed Δ^4-androstene-3, 17-dione (Δ^4A), dehydroepiandrosterone (DHA), 11-ketotestosterone (11-KT), and testosterone (T) were detected in all gray seal testicular incubates. Yields of 11-KT were greatly increased in the presence of Aroclor 1254. All contaminants except As and Se stimulated the *in vitro* biosyntheses of T, with the greatest increase in production of T being in the Cd-treated tissue.

Cortisol (F), corticosterone (B), aldosterone (ALDO) but no cortisone (E), were biosynthesized by the gray seal adrenal tissue. Corticosterone (B) was the principal transformation product in all incubations with less B produced by the treated adrenals than by the control. The lowest yield of B was from the Se-treated adrenal. The yield of ALDO was also lower in all contaminant treated incubations, with Se and Cd giving the greatest inhibition. More F was biosynthesized by all the treated adrenals than by the control. The greatest increase of production of F (6-fold) from progesterone was by the As-treated adrenal.

Tissue from a harp seal given methyl mercury at a concentration of 0.25 mg/kg in its diet for 61 days, was highly contaminated with mercury.

Biosynthesized (*in vitro*) F, B, E, 11KT were isolated and identified from the adrenal incubations, and Δ^4-A and T were isolated and identified in ovarian incubations from both untreated and methyl mercury (*in vivo*) treated harp seals.

The ovary from the methyl-mercury treated seal yielded more T and Δ^4-A than that from the untreated seal. The yields of estrogens estrone (E_1), estradiol-17β (E_2) and estriol (E_3) were all low (<1%) from both ovarian incubations but a stimulation of estrogen synthesis in the ovary of the treated seal was suggested. A compound isopolar with ALDO in several chromatographic systems, and thought, but not conclusively identified as aldosterone, was also present in the adrenal incubations.

On histological examination under the light microscope the ovaries and adrenals from both harp seals appeared to be normal with the ovaries being in the follicular phase, yet *in vitro* incubations of tissue from these organs indicated that the methyl mercury treatment caused an altered steroid hormone metabolism in tissue from the treated seal.

The altered steroid hormone metabolism was also demonstrated by autoradiography, and it is suggested that this technique could be used as an indicator of incipient contamination by a pollutant.

INTRODUCTION

Adult Canadian seals have been shown to be highly contaminated with inorganic and organic contaminants (Uthe 1972, Freeman and Horne 1973, Frank *et al.* 1973, Addison *et al.* 1973, and Koeman *et al.* 1973). Seals obtain contaminants from their diet of contaminated fish and shellfish and appear to concentrate them with no apparent ill effect.

It is known that sublethal levels of certain contaminants, even at levels comparable to those found in wild populations, can alter enzyme activities and, in some cases, interfere with steroidogenesis and reproduction (Balazs 1969, Litterst and van Loon 1972, Maugh 1972, Aurlerick *et al.* 1973, Nowicki *et al.* 1972, and Freeman and Idler 1975). The seal is a particularly noteworthy mammal as its amphibious habits subject it to a wide range of stress conditions. In this regard steroids can be expected to play a major role in the seal's physiological requirements.

It was thought important to determine the principal steroids biosynthesized (*in vitro*) in the gray seal, *Halichoerus grypus*, and to study the effects of contaminants, found in the seal's diet, on its enzyme controlled steroidogenesis. The presence of the steroids *in vivo* in the gray seal was confirmed by blood analyses. The *in vivo* exposure of the large gray seals to various contaminants would be difficult to carry out experimentally in our laboratory as we do not have facilities to hold the large animals. Thus we investigated the effects of certain contaminants on the *in vitro* steroidogenic capacities of the adrenals and testes of gray seals shot in the wild. In this study low levels of contaminants were used, for it was thought that high concentrations were not necessary to alter normal steroid hormone metabolism.

In the harp seal the principal adrenocorticosteroids and sex hormones biosynthesized under controlled conditions by untreated and methyl mercury treated (*in vivo*) seals were determined. Any significant change in the fundamental process of steroidogenesis, due to methyl mercury contamination, would affect the physiology of the seal. The concentrations of methyl mercury in tissues from the untreated and treated seals were also determined in order to compare the degree of contamination with the observed effects on steroidogenesis *in vitro*.

METHODS AND RESULTS

Tissue was obtained from two large male gray seals (*Halichoerus grypus*) (weights 410 kg and 225 kg) shot near Amet Island, Nova Scotia, Canada, and two adult female harp seals, one age 16 years, weight 184 kg, the *in vivo* treated seal, and the other age 11 years, weight 172 kg, the untreated seal. Both harp seals were lactating females when captured on the Magdalen Islands, Canada. These seals were transported to the University of Guelph, Guelph, Ontario, Canada where they were held in fresh water and used for experimentation under close surveillance by Dr. Keith Ronald and his group. During the experiment both seals ate well and were fed whole herring (*Clupea harengus harengus*) at 3 to 5% body weight and supplements, beminal with C fortis, thiamine, halibut liver oil, iron tablets, sodium chloride (varied with ionic condition of blood) and vitamin E. For the treated seal 0.25 mg/kg of body weight of methyl mercury in a gelatin capsule was included with the herring. Methyl mercury treatment was continued for 61 days until the animals were killed. Hair and blood were analyzed for mercury every few days during the experiment.

Tissue Steroidogenesis *In Vitro*

On death the adrenals, testes and ovaries were removed immediately. The organs, freed from extraneous tissue, were kept in ice-cold Kreb's Mammalian Ringer buffer 11 (Dawson *et al.*, 1959) until used. The glands were minced and mixed thoroughly to form adrenal, testicular, and ovary tissue pools. One-gram samples of each tissue were used for the incubation experiments which were carried out simultaneously under exactly the same conditions for each species of seal.

In each experiment the tissue was pre-incubated under oxygen in 10 ml of buffer (Kreb's Ringer) containing a NADPH-generating system (Idler and Truscott, 1966) for ½ hr at 37°C to reduce the endogenous pool of steroid substrates. The tissues were then incubated under oxygen in 10 ml of fresh buffer containing enzyme co-factors and equimolar amounts (0.023 µmole) of steroid substrates [^{3}H]-pregnenolone and[4-^{14}C] pregesterone for 1 hr and 4 hrs at 37°C for the harp seal tissue and 3½ hrs for the gray seal tissue. Enzyme co-factors were replenished at ½ time in the 3½ and 4 hr incubations. In the harp seal control experiments, for both testicular and adrenal tissue, incubations were carried out without added contaminants. Separate incubations were also carried out for each contaminant, PCB, methyl mercury, arsenic, cadmium and selenium, where contaminants were added to make a final concentration of 0.45 ppm. Incubations were stopped by freezing in dry ice.

Recoveries were determined by adding [^{14}C] steroid tracers to tissue samples from each pool after incubation without radioactive substrates and analyzing these through the procedures. After the addition of radioinert carrier steroids, the adrenal and testicular incubations were extracted with various solvents according to Freeman *et al.*, 1975. The estrogens and neutral steroids were extracted from the ovary incubations by the ethanol-acetone extraction method of Smith and Zuckerman, 1973, followed by phenolic partitioning according to Brown *et al.*, 1957. One tenth of each extract to which was added 10 µg of the appropriate radioinert carrier steroid was applied to a thin layer chromatoplate silica gel HF254+366. The plates were developed by ascending TLC in solvent system No. 1, Table 1 (Slide 1). The plates were exposed in a light-proof box to a no-screen x-ray film (Kodak Blue M) for 72 hours. The exposed films were developed according to the

manufacturer's instructions and the position of radioactive spots was compared with those of radioinert steroid carriers. The remaining portions of the extracts were also run in system No. 1 and steroids isolated and purified by TLC and PC with derivative formation and recrystallization with radioinert standards to constant $^{3}H/^{14}C$ ratios according to Axelrod *et al.*, 1965.

A Packard liquid scintillation spectrometer, Model 3003, equipped with automatic external standardization was used for all ^{3}H and ^{14}C measurements.

DISCUSSION

Biotransformed double labelled $\Delta^{4}A$, DHA, 11KT, and T were detected in all gray seal testicular incubates. Little [^{3}H] and [^{14}C]-$\Delta^{4}A$ and [^{14}C]-DHA were detected, Table 2 (Slide 2), even in the control incubate suggesting that under the *in vitro* conditions of this experiment, these steroids were further transformed, presumably to the androgens, 11KT and T, which appeared in relatively high yields. In each of the testicular incubates, there were two major radioactive metabolites that were unknown (marked? Figure 1, Slide 3). The considerable radioactivity associated with these products suggests their significance; their identity remains to be investigated.

One of the principal transformation products formed from all testicular tissue was T (Table 2, Slide 2), where the yields were 1.43 and 1.01% for [^{3}H] and [^{14}C]-T respectively for the untreated testes with a $^{3}H/^{14}C$ ratio (7.11:1) greater than the initial precursor ratio (5.53:1) indicating biosyntheses via the normal pathway (i.e. predominantly from pregnenolone and presumably via Δ^{5}-intermediates). All contaminants except As and Se stimulated the *in vitro* biosyntheses of T (Table 2, Slide 2). The greatest increase in T production was in the cadmium treated tissue where the increase was 60% and 85% for [^{3}H]-T and [^{14}C]-T, respectively.

11-Ketotestosterone, an androgen first isolated from salmonids (Idler *et al.* 1961) and to our knowledge never isolated from or biosynthesized from normal mammalian tissue was unexpectedly biosynthesized by the seal testicular tissue. We do not know the significance of the appearance of this steroid; however, *in vitro* incubations only demonstrate the presence of enzyme systems capable of carrying out the steroid transformations and may not indicate the situation *in vivo*. A preliminary search for this androgen in the peripheral blood of the gray and harp seal has indicated no detectable quantities present.

Cadmium was found to cause elevated blood levels of the principal androgens 11-KT and T in the brook trout, *Salvelinus fontinalis* by Sangalang and Freeman, 1974. It is possible that these elevated levels were caused partly by increased biosynthesis as found in the seal. The $^{3}H/^{14}C$ ratios for T in the PCB and As-treated tissue (Table 2, Slide 2) indicated an obvious change in the biosynthetic route to T when compared with the control. These contaminants appear to inhibit 3β-hydroxysteroid dehydrogenase and Δ^{5-4}-3-ketoisomerase activity. This was also indicated in the biosynthesis of $\Delta^{4}A$ and 11KT showing that As and PCB have a significant effect on steroidogeneses *in vitro* in this species. Freeman and Idler (1975) have shown that PCB interfered with *in vitro* androgen biosynthesis in the brook trout.

Biosynthesized F, B, and also ALDO were detected in all gray seal adrenal incubates. No E, T or 11KT were detected (Table 3, Slide 4). Corticosterone (B) was the principal transformation product in all incubations; however, there

was less B produced by the treated adrenals than by the control. Selenium gave the most significant effect, decreasing the yield from the ^{3}H and ^{14}C precursor by 84.0 and 74%, respectively. The $^{3}H/^{14}C$ ratios of B indicated [^{14}C]-progesterone as the principal precursor, approximating the normal pathway in other B producing species. The $^{3}H/^{14}C$ ratios for B from the treated incubations were lower than the control indicating an altered biosynthetic route to B. It is possible that the interference was not at the pregnenolone→progesterone level but at the progesterone→11-deoxycorticosterone (DOC) or DOC→B level indicating some influence on the 21-hydroxylase and 11β-hydroxylase enzyme systems. Evidence from the x-ray autoradiogram of the initial chromatogram of the crude extract (Figure 2, Slide 5) had not indicated significant radioactivity corresponding to the DOC areas. Unfortunately, DOC was not analysed in our experiment although DOC is a principal steroid intermediate in mammalian species.

As for B, the $^{3}H/^{14}C$ ratios for ALDO indicated progesterone as the more efficient precursor. The yields of ALDO from the treated incubates were lower than the control (Table 3, Slide 4). The decrease in yield from the [^{3}H] and [^{14}C] precursors, respectively, ranged from 48% and 46% for the Cd-treated to 78% and 70% for the Se-treated incubations. The decreased productions of B, in the treated samples compared to the control were reflected in the decreased syntheses of ALDO.

More F was biosynthesized from ^{3}H-pregnenolone and ^{14}C-progesterone *in vitro* by treated adrenal incubates than by the control. The isotope ratio of F (9.51) for the untreated adrenal was greater than the initial precursor ratio (5.53) at the end of 3½ hr incubation time, indicating biosyntheses via the normal pathway - i.e. predominantly from pregnenolone and presumably by Δ^{5}-intermediates. The increased production of F by the As-treated adrenal from ^{14}C-progesterone was almost 6-fold (578%).

These contaminants appeared to have altered the biosynthetic route to F, (i.e. ratios were lower than initial precursor ratios suggesting progesterone as the more efficient precursor). This suggests suppression of the 3β-hydroxysteroid dehydrogenase system and/or stimulation of the Δ^{4-5}-3-ketoisomerase and 17α-hydroxylase systems.

The steroidogenesis results given in Table 2 & 3 correlated well with the radioactive profiles of products from the various tissue incubates as seen on the x-ray autoradiographs of the initial chromatograms (Figures 1 & 2, Slides 3 & 5). The autoradiograms carried out on the extracts of the testicular and adrenal incubations from a second male gray seal used in this study confirmed the biosynthesis of the principal steroids as found for the first (largest) male seal. An altered steroid metabolism by the contaminants *in vitro* was also confirmed.

F,B,E, and 11-KT were isolated and identified from the adrenal incubations of the harp seal (Table 4, Slide 6). From the autoradiogram of the first TLC (Figure 3, Slide 7), it was clear that a radioactive metabolite isopolar with ALDO was present. In both adrenal incubates radioactive metabolite remained isopolar with ALDO in later chromatograms but was lost by accident before final isolation and quantification. It appears from the autioradiogram (Figure 3, Slide 7) that a product isopolar with ALDO was biosynthesized in much higher yield by the adrenal of the control than by that of the methyl mercury treated seal. If this compound were ALDO and if the physiological role of ALDO in the seal were as that known in man, then

methyl mercury in this species could have a deleterious effect on its mineral and water balance.

The biosynthesis of F and a compound isopolar with ALDO at 4 hr was greater in the control than in the treated seal (Figure 3, Slide 7), indicating an altered hormone metabolism in the latter. It is suggested that autoradiograms may be used as sensitive detectors to demonstrate that alterations in hormone metabolism caused by sub-lethal levels of a contaminant and to serve as an early warning of pollution.

Biosynthesized T and Δ^4A were identified in the 1 hr and 4 hr incubations of both treated and control ovaries (Table 5, Slide 8). Eleven-ketotestosterone was not detected in any of the harp seal incubations.

The ovary from the methyl mercury treated harp seal yielded more T and Δ^4A than the control tissue (Table 5, Slide 8). There appeared to be a constant turnover of T and Δ^4A in the control ovary for both the ^{3}H and ^{14}C precursors at 1 hr and 4 hr, the yields being approximately the same for each steroid at the given incubation times. By contrast, the 4 hr yields of T and Δ^4A from the ovary of the treated seal were much greater than the 1 hr yield. This indicated that either production of T and Δ^4A was stimulated by the methyl mercury, or conversion of the steroids to other metabolites was inhibited with this endproduct formation. We also note that the biosynthesis of T indicated different patterns in the treated and control ovaries. In the control ovary, T was primarily synthesized from [^{3}H]-pregnenolone, presumably via Δ^5-intermediates. In the treated ovary the Δ^4-pathway predominated in the biosynthesis of T at the end of 1 hr incubation. We can speculate that there may have been an initial inhibition of the Δ^5-Δ^4 isomerase and 3β-hydroxysteroid dehydrogenase systems in the treated ovary.

The radioactive profiles of the steroids and steroid metabolites of the neutral fractions from the ovary incubations showed an altered steroid metabolism in both the 1 hr and 4 hr incubations of the methyl mercury treated seal compared to the controls (Figure 4, Slide 9). The ovaries from the untreated seal biosynthesized two major products slightly more polar than 11KT in this TLC system whereas each control biosynthesized only one minor metabolite at these positions. Again, with the adrenal incubations, an altered steroid hormone metabolism, caused by a contaminant, is indicated by x-ray autoradiography (Slide 7, Figure 3) confirming that found by chemical analysis.

The yields of estrogens biosynthesized by the ovaries from the control and treated seals were less than 1%. These low yields were not unexpected as we used precursors far back in the estrogen biosynthetic pathway. The detectable radioactivity was mostly ^{3}H and the yields were somewhat higher in the ovaries from the methyl mercury treated seal. The autoradiographs of the initial chromatograms confirmed the very low ^{14}C-radioactivity in the estrogen fraction. Radioactive estrogens were not detected in the 1 hr incubations except for possible traces of E_2 from the ovary of the treated animal. E_2 was detectable only in the 4 hr incubation of the ovary from the treated seal but not in the control. Estrone (E_1) was also produced only by the ovary of the treated seal from both ^{3}H and ^{14}C precursors and was detected only from the ^{3}H-labelled precursor of the control (Table 5, Slide 8). Estriol (E_3) was detected in the ^{3}H form in low yield from both control and treated incubations.

If the difference between the low yields of estrogens and in the ovaries from control and methyl mercury-treated were significant (Table 5, Slide 8) it would appear that the methyl mercury treatment stimulated ovarian syntheses of these compounds from both ^{3}H and ^{14}C precursors. This may also be a reflection of the higher yield of T and $\Delta^{4}A$ in the ovary of the untreated seal when compared with the control.

It is significant to note that the levels of total mercury in the adrenals and ovaries from the treated harp seal were high. Mercury levels of various other tissues were higher in the treated seal than in similar tissues from the control seals (Table 6, Slide 10). There appeared to be no other gross differences in these tissues. Histological examination of the ovaries and the adrenals from both seals did not reveal any abnormalities under the light microscope. The ovaries from both seals were in the follicular phase. The adrenals from both seals presented a normal morphological picture. However, results of our tissue incubation studies *in vitro* showed that methyl mercury, at the given dose level *in vivo*, altered the steroidogenic capacities of the adrenals and ovaries of the harp seal, *in vitro*, when compared to the control.

CONCLUSIONS

In conclusion, it is evident that the contaminants PCB, methyl mercury, As, Cd and Se have altered the *in vitro* biosynthesis of steroids in the gray seal. Methyl mercury treatment *in vivo* was also found to alter the biosynthesis of steroid hormones in the harp seal. This is suggestive evidence that contaminants at these levels may have a significant effect on the physiology of the seal. Clearly, much remains to be done in the investigation of the effects of contaminants on steroid metabolism *in vivo* and on steroid hormone controlled physiological functions like mineral and water regulation, carbohydrate metabolism, and reproduction.

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TABLE 1. Chromatographic Separation, Purification and Identification of Steroid Metabolites

STEROIDS	SYSTEMS[a] AND METHODS
4-androstenedione (Δ4A)	1,8,TM-70; reduced to T; 7,TM-70; acetylated; 2,HM-80; recrystallized to constant $^3H/^{14}C$ ratio
dehydroisoandrosterone (DHA)	1[b],2,4,HM-80; acetylated; 9,^{14}C-DHA added; 11,9; recrystallized to constant $^3H/^{14}C$ ratio
testosterone (T)	1;2;6; TM-70; acetylated; 2; HM-80; HAW; 10; recrystallized to constant $^3H/^{14}C$ ratio
11-ketotestosterone (11KT)	1,3,5, TM-70; acetylated; 8; HAW; 10; recrystallized to constant $^3H/^{14}C$
cortisol (F)	1;13;B-50 x 16 hr; acetylated; 2; MMW x 6 hr; recrystallized to constant $^3H/^{14}C$
tisone (E)	1;13;TM-70 x 6 hr; acetylated; MMW; recrystallized to constant $^3H/^{14}C$
corticosterone (B)	1;13; TM-70 x 3 hr; acetylated; 2;10; MWW x 5½ hr; recrystallized to constant $^3H/^{14}C$
aldosterone (ALDO)	1;13; B-50 x 12 hr; E B x 4½ hr; acetylated→18,21-diacetate; 14;15; MMW x 16 hr; (oxidation to 11,18-lactone-21 acetate;16; CMBW x 12 hr; 14; recrystallized to constant $^3H/^{14}C$
progesterone (P)	1;12; HM-80 x 3 hr; (reduced to 20β-hydroxy-P); TM-70 x 3 hr
pregnenolone (PREG)	1;7; HM-80 x 2½ hr; acetylated
DOC	1;17;12; acetylated; 2(sample separated from DOC-acetate)
	18;19;TEG(6 hr); TM-70 (3 hr) (Methyl ether prepared); 1
	18;19;TPG(24 hr); TM-70 (3 hr) (Methyl ether prepared);1
	18;19;TEG(72 hr); B-50(6½hr) (Methyl ether prepared); 1

[a]TLC and PC systems (by vol.).

[b]DHA & T were removed together and separated in 2.

TLC	PC
chloroform:methanol:water (188:12:1)	TM-70, toluene: 70% methanol (1:1)
tertiary butanol:hexane(25:75)	HM-80,heptane:80% methanol (1:)
dichloromethane:acetone (80:20)	HAW, hexane:acetic acid:water (5:4:1)
benzene:ethyl acetate (4:1)	B-50, benzene: 50% methanol (1:1)
chloroform:methanol:water(90:10:1)	MMW, mesitylene:methanol:water (3:2:1)
dichloromethane:ethyl acetate(9:1)	E_2B, isooctane:t-butanol:water (50:25:45)
cyclohexane:ethyl acetate (1:1)	CBMW, cyclohexane:benzene:methanol:water (100:70:100:25)
dichloromethane:butyl acetate (70:30)	TEG toluene:ethylene glycol (1:1)
hexane:ethanol (95:5)	TPG toluene: proyylene glycol (1:1)
ethyl acetate:chloroform:water (90:10:1)	(TEG and TPG are Bush type impregnated paper systems)
dichloromethane:acetone (95:5)	

Table 1 Cont'd

TLC

12. benzene:ethanol (9:1)
13. ethyl acetate: methanol (20:1)
14. chloroform:methanol (96:4)
15. cyclohexane:ethyl acetate (30:70)
16. benzene:methanol (9:1)
17. benzene:ethyl acetate (2:1)
18. chloroform:ethanol (95:5)
19. chloroform:acetone (8:2)
20. benzene:acetone (8:2)

TABLE 2. Effect of Contaminants on *In Vitro* Steroidogenesis in Testicular Tissue

STEROID	CONTAMINANT	$^{3}H/^{14}C$	% Yield[a] ^{3}H	% Change[b]	% Yield ^{14}C	% Change
4A	None (Control)	5:00:1	0.011		0.011	
4A	PCB	3:80:1	0.007	(36. ↓)	0.010	(9.1 ↓)
4A	MeHg	4:69:1	0.002	(82. ↓)	0.002	(82 ↓)
4A	As	3:33:1	0.006	(45. ↓)	0.009	(18 ↓)
4A	Cd	5:00:1	0.011	(0 ↓)	0.011	(0)
4A	Se	4.50:1	0.001	(91 ↓)	0.002	(82 ↓)
HA	None (Control)	645	0.96		0.008	
HA	PCB	182	1.35	(41 ↑)	0.038	(375 ↑)
HA	MeHG	512	1.26	(31 ↑)	0.012	(50 ↑)
HA	As	257	1.18	(23 ↑)	0.023	(188 ↑)
HA	Cd	173	1.29	(34 ↑)	0.038	(375 ↑)
HA	Se	108	0.62	(35 ↓)	0.029	(263 ↑)
1KT	None (Control)	0.33:1	0.007		0.105	
1KT	PCB	0.27:1	0.003	(57 ↓)	0.054	(49 ↓)
1KT	MeHg	0.19:1	0.011	(57 ↑)	0.303	(189 ↑)
1KT	As	0.15:1	0.004	(43 ↓)	0.137	(30 ↑)
1KT	Cd	0.26:1	0.007	(0)	0.146	(39 ↑)
1KT	Se	0.18:1	0.023	(229 ↑)	0.631	(501 ↑)
T	None (Control)	7.11:1	1.43		1.01	
T	PCB	4.81:1	1.54	(8.0 ↑)	1.61	(59 ↑)
T	MeHg	7.52:1	1.62	(13 ↑)	1.09	(8.0 ↑)
T	As	4.54:1	0.88	(39 ↓)	0.98	(3.4 ↓)
T	Cd	6.16:1	2.29	(60 ↑)	1.88	(86 ↑)
T	Se	6.88:1	1.28	(11 ↓)	0.94	(6.9 ↓)

[a] Yields are given as % of precursor and are corrected for recovery.

[b] Values in parentheses indicate increase (↑) or decrease (↓) from the controls valued at 100%.

TABLE 3. Effect of Contaminants on Steroidogeneses in Adrenal Tissue

STEROID	CONTAMINANT	$^{3}H/^{14}C$	% Yield ^{3}H	% Change[a]	% Yield ^{14}C	% Change
F	None (Control)	9.51	0.77		0.41	
F	PCB	3.99	1.40	(82 ↑)	1.76	(329↑)
F	MeHg	4.61	1.68	(118 ↑)	1.82	(344↑)
F	As	3.08	1.70	(121 ↑)	2.78	(578↑)
F	Cd	3.61	1.77	(130 ↑)	2.48	(505↑)
[illegible]	Se	2.15	0.95	(23 ↑)	2.21	(439↑)
B	None (Control)	3.42	15.9		23.5	
B	PCB	2.48	5.58	(65 ↓)	9.12	(61 ↓)
B	MeHg	3.13	5.72	(64 ↓)	9.22	(61 ↓)
B	As	2.15	3.71	(77 ↓)	8.64	(63 ↓)
B	Cd	2.65	5.16	(68 ↓)	9.96	(58 ↓)
B	Se	2.08	2.56	(84 ↓)	6.18	(74 ↓)
[illegible]	None (Control)	3.12	0.29		0.46	
ALDO	PCB	2.78	0.077	(74 ↓)	0.14	(70 ↓)
ALDO	MeHg	3.38	0.073	(75 ↓)	0.11	(76 ↓)
ALDO	As	2.45	0.11	(62 ↓)	0.23	(50 ↓)
ALDO	Cd	2.87	0.15	(48 ↓)	0.25	(46 ↓)
ALDO	Se	2.14	0.065	(78 ↓)	0.14	(70 ↓)

[a] Values in parentheses indicate increase (↑) or decrease (↓) from the controls values at 100%.

4. Biosynthesis (*in vitro*) of adrenocorticosteroids from [^{3}H]-pregnenolone and [^{14}C]-progesterone by adrenals from control and methyl mercury treated harp seals.

ID	Radioactivity dpm (before recrystallization)						% Yield			
	1 Hr			4 Hr			1 Hr		4 Hr	
	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
trol)	132,605	105,129	6.97	1,497,635	205,931	7.19	4.41	3.33	8.91	6.53
ated)	385,095	56,998	6.76	771,487	110,673	6.97	2.32	1.81	4.65	3.51
rol)	130,383	18,138	7.19	124,642	18,025	6.91	0.79	0.58	0.75	0.57
ated)	189,377	25,960	7.29	219,425	27,788	7.62	1.14	0.82	1.32	0.88
rol)	115,404	12,666	0.91	74,360	40,696	1.82	0.69	0.40	0.45	1.29
ted)	46,414	34,579	1.34	28,514	23,485	1.21	0.28	1.09	0.17	0.74
ntrol)	5,773	757	7.60	1,641	794	2.07	0.035	0.024	0.009	0.025
reated)	4,662	1,739	2.68	3,141	1,059	(2.97)	0.028	0.055	0.019	0.033

Biosynthesis (*in vitro*) of estrogens and androgens from [^{3}H]-pregnenolene and [^{14}C]-progesterone by ovaries from control and methyl mercury treated harp seals.

ID	Radioactivity d/m (before recrystallation)						% Yield[1]			
	1 Hr			4 Hr			1 Hr		4 Hr	
	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
trol)	ND[2]	ND		2,000	ND				0.12	
ted)	ND	ND		6,000	1,200	5.0			0.39	0.39
l)	ND	ND		ND	ND					
ted)	700	ND		1,300	ND		≤0.0[3]		0.06	
trol)	ND	ND		1,000	ND				0.07	
ted)	ND	ND		1,300	ND				0.10	
rol)	15,200	2,700	5.63	11,700	1,400	8.35	0.26	0.25	0.20	0.12
d)	14,600	10,400	1.40	68,600	13,400	5.12	0.25	0.92	1.16	1.18
ntrol)	ND	ND		ND	ND					
ated)	ND	ND		ND	ND					
rol)	16,400	4,100	4.0	17,400	4,000	4.35	0.44	0.60	0.47	0.54
ted)	9,700	3,200	3.03	182,300	41,800	4.36	0.26	0.44	4.86	5.82

ected for recovery

detectable

fficient radioactivity for crystallization
if present, yield would be ≤0.03%.

TABLE 6. Mercury concentration (ppm) in seal components

SAMPLE	TIME	CONTROL SEAL		TREATED SEAL	
		Total Hg	Me Hg	Total Hg	Me Hg
blood	0[1]	0.16	0.20	0.14	0.16
blood	death	0.12	0.10	9.93	-
hair	0	10.3	-	11.4	-
hair	death	9.2	-	8.2	-
brain	"	0.45	-	14.8	-
muscle	"	0.78	0.56	27.6	35.2
blubber	"	N.D[3]	0.05	0.28	0.58
liver	"	25.8	0.16	64.0	18.5
spleen	"	9.92	0.63	20.4	-
claws	"	5.10	-	21.9	-
intestine (small)	"	0.44	-	17.2	19.0
heart	"	0.25	-	12.9	16.9[2]
kidney	"	5.98	0.27	69.5	51.6
lung	"	0.12	-	10.6	-
adrenal	"	-	-	14.2	10.8
gonad	"	N.D.	N.D.	13.0	10.1

1. At beginning of experiment.

2. Mean of three sections.

3. N.D. - not detectable.

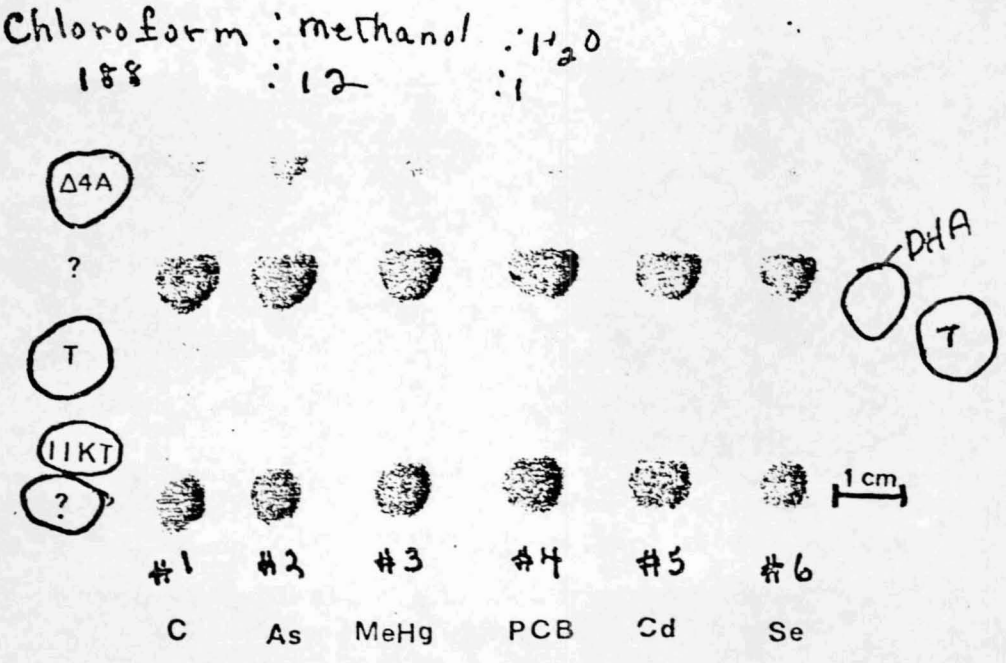

Figure 1. X-ray autoradiogram of 1/10 of the steroid extracts of gray seal testes tissues incubated with radioactive precursors [^{3}H] pregnenolone and [^{14}C]-progesterone with and without contaminants. Spots show the positions of UV fluorescence quenching carrier steroids. ? indicates unknown radioactive products. C, control; MeHg, + methyl mercury; As, + arsenic; Cd, + cadmium; Se, + selenium.

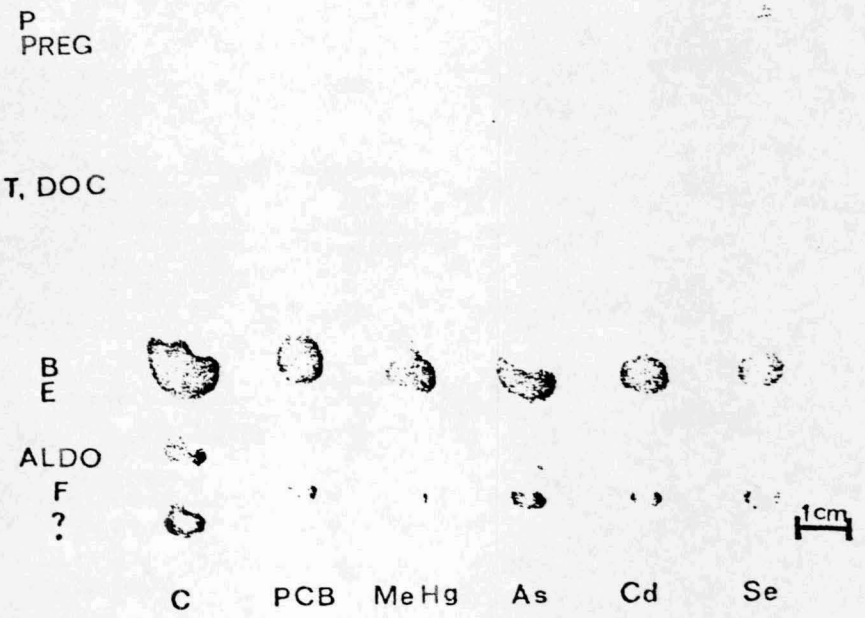

Figure 2. X-ray autoradiogram of 1/10 of the steroid extracts of gray seal adrenal tissues incubated with radioactive precursors [^{3}H] pregnenolone and [^{14}C]-progesterone with and without contaminants. Spots show the positions of radioactive metabolites. Steroid symbols indicate the positions of UV fluorescence quenching carrier steroids. ? indicates unknown radioactive products. C, control; MeHg, + methyl mercury; As, + arsenic; Cd, + cadmium; Se, + selenium.

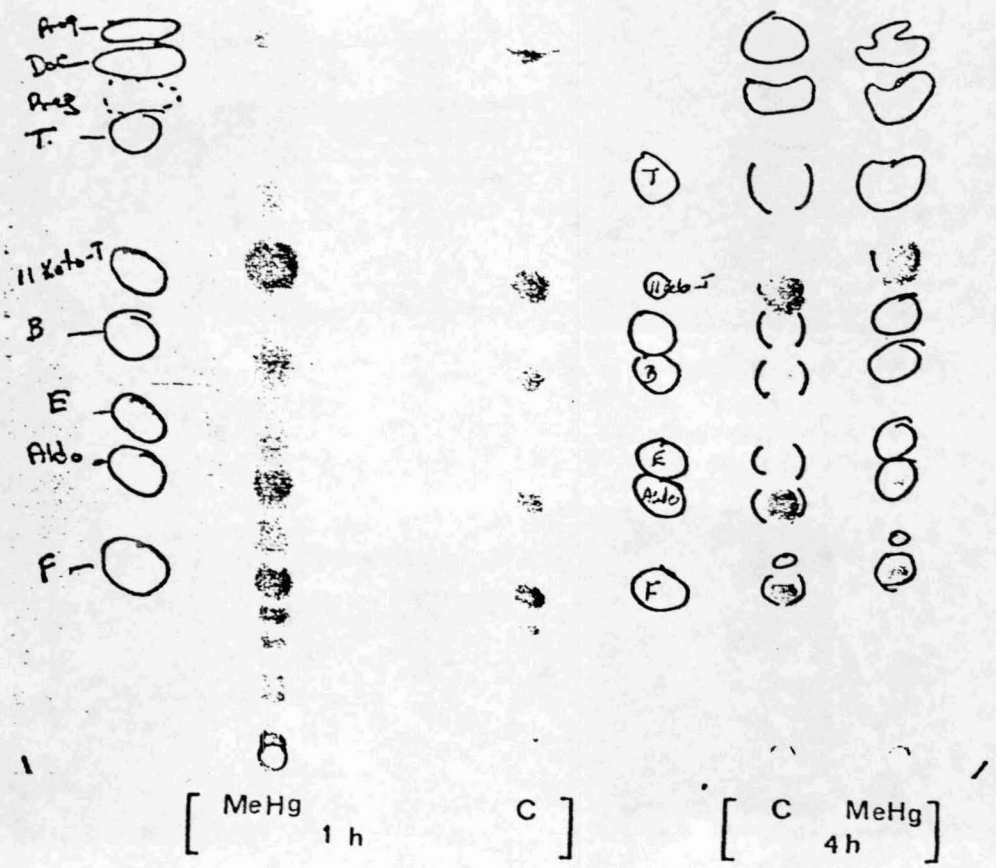

Figure 3. X-ray autoradiogram of the chromatogram of 1/10 of the steroid extracts of adrenal tissues from untreated (C) and methyl mercury treated (MeHg) harp seal. The tissues were each incubated with [^{3}H]-pregnenolone and [^{14}C]-progesterone for 1 hr and 4 hr *in vitro*. Spots show the positions of radioactive metabolites. Steroid symbols indicate the relative positions of UV fluorescence quenching carrier steroids. ? indicates unknown products (x 0.66).

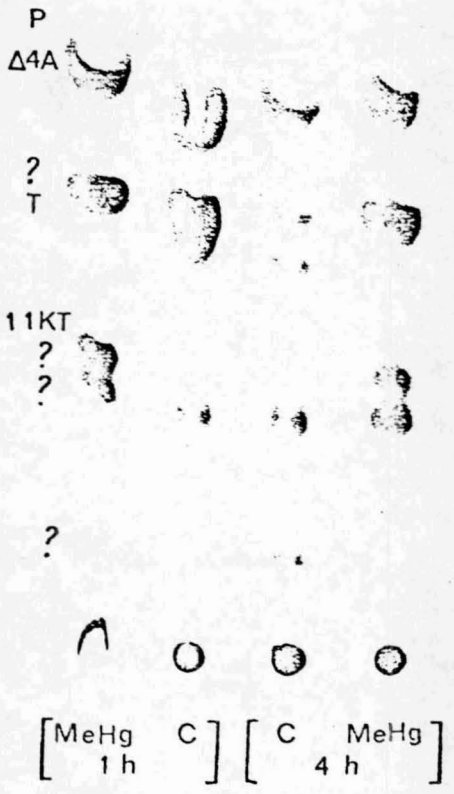

Figure 4. X-ray autoradiogram of the chromatogram of 1/10 of the neutral fractions of steroid extracts of ovaries from untreated (C) and methyl mercury treated (MeHg) harp seal. The tissues were each incubated with [^{3}H]-pregnenolone and [^{14}C]-progesterone for 1 hr and 4 hr *in vitro*. Spots show the positions of radioactive metabolites. Steroid symbols indicate the relative positions of UV fluorescence quenching carrier steroids. ? indicates unknown products (x 0.57).