neutrophilia, and a normal number of immature cells was found in the blood of control and experimental mice. As in C3H mice, myelopoietic cells were predominant in the marrow. The number of femoral marrow cells ranged from 9.51×10^6 to 12.8×10^6 throughout the study.

In conclusion, the administration of a relatively low dose of PHA to mice, for a period of 4 to 6 months, produces a hyperplasia of the lymphoreticular system and minor hematopoietic changes. The number of blast cells in the blood was within the normal range. This study does not provide evidence in support of the development of a leukemoid reaction during prolonged PHA administration[6].

Resumen. La administración de FHA, en dosis de 5 mg/100 g de peso, por períodos de 4 a 6 meses a ratones de la razas C3H y Suiza, causa hiperplasia del sistema limforeticular, que se acompaña de alteraciones mínimas en la sangre periférica. El número de células blásticas en sangre fué bajo y dentro de los límites normales. Estos estudios indican que una dosis relativamente baja de FHA no produce un aumento permanente de células blásticas (reacción leucemoide) en estas cepas de ratones.

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Testosterone Metabolism by Mouse Placenta in vitro

In previous studies we have demonstrated that placental tissue from several strains of mice is capable of converting radioactive pregnenolone and progesterone to deoxycorticosterone and several ring A saturated C_{21} compounds having a 5α-configuration. In addition, pregnenolone and progesterone were shown to be converted to 3α-hydroxy-5α-androstan-17-one and to 4-androstene-3,17-dione. The formation of androgens in small yield following incubation of progesterone has been observed. No estrogens were isolated[1]. The purpose of the present experiment is to demonstrate by incubation with 4-C^{14}-testosterone whether mouse placentae are able to convert androgens to estrogens.

Material and methods. Placentae of 19–20-day-old pregnant mice of the R-III strain were removed immediately after the animal had been sacrificed. The details of the methods used are reported elsewhere[1]. In brief, each placenta was divided and 8 quarters (400 mg/flask) were preincubated for 1 h in Krebs-Ringer bicarbonate medium in an atmosphere of 95% O_2 – 5% CO_2 in a shaking incubator at 37 °C. After preincubation, the medium was discarded and replaced with fresh medium. 4-C^{14}-Testosterone (0.2 μCi, s.a. 29.2 mCi/m*M*) was added to the incubation medium as a small volume of ethanolic solution and the incubation was continued for 3 h. After incubation the placental tissue and media were extracted with chloroform. Following evaporation of the solvent, the residue was partitioned between toluene and N NaOH to obtain phenolic and neutral fraction. Purification and separation of the radioactive metabolites present in neutral fraction was accomplished by paper and thin-layer chromatography. The radioactive metabolites were detected by means of an automatic chromatogram scanner. Radioactivity was quantitatively determined using scintillation spectrometer. The individual labelled compounds were identified by the crystallization to constant specific activity.

Results. The total recovery of radioactivity was 92%. In phenolic fraction less than 1000 cpm remained after the second toluene/N NaOH partition. There was no radioactivity corresponding to estrone or estradiol after paper chromatography of the phenolic fraction in a benzene/propylene glycol system. The neutral fraction was chromatographed in a cyclohexane:benzene (3:1)/propylene glycol system. 6 radioactive zones were detected on the chromatograms. The metabolites of testosterone were oxidized with chromic acid and the oxidation products were chromatographed in Lisboa[2,3] thin-layer system 'O'. From 4 metabolites less polar than testosterone, 2 were oxidized to 5α-androstane-3,17-dione. 2 metabolites were shown unoxidized. Thin-layer chromatography of 4 radioactive metabolites less polar than testosterone revealed that they possessed the same chromatographic mobilities as standards of 3β-hydroxy-5α-androstan-17-one, 3α-hydroxy-5α-androstan-17-one, 4-androstene-3,17-dione and 5α-androstane-3,17-dione. The crystallization data for each of these compounds are presented in Table I. The radioactive material more polar than unconverted testosterone has not been identified. This material had the same mobility as 6β-hydroxy-4-androstene-3,17-dione in Lisboa thin-layer systems 'L' and 'C'. Crystallization studies with this material were not possible due to insufficient amounts

Table I. Identification of 4-C^{14}-testosterone metabolites by recrystallization to constant specific activity (cpm/mg)

Metabolite identified	Crystallization No.[a]			
	1	2	3	4
4-Androstene-3,17-dione	1980 3300[b]	1980 2200[b]	1870 1830[d]	–
3α-Hydroxy-5α-androstan-3,17-dione	7920 9610[d]	7330 7300[b]	7500 7570[e]	7600 7400[d]
3β-Hydroxy-5α-androstan-3,17-dione	5200 7880[c]	4950 5740[d]	4400 4850[d]	4500 4620[b]
5α-Androstane-3,17-dione	4820 6711[b]	4740 5600[c]	4760 4620[c]	–

[a] Upper figure denotes specific activity of the crystals, lower figure denotes specific activity of the corresponding mother liquor. [b] 70% ethanol. [c] *n*-hexane/acetone. [d] 70% methanol. [e] *n*-hexane/ethyl acetate.

[1] R. Rembiesa, M. Marchut and A. Warchol, J. Steroid Biochem. *2*, 111 (1971).
[2] B. Lisboa, Acta endocr. Copenh. *43*, 47 (1963).
[3] B. Lisboa, J. Chromat. *13*, 391 (1964).

of reference 6β-hydroxy-4-androstene-3,17-dione. Percentage formation of steroids from 4-C^{14}-testosterone by placental tissue is summarized in Table II.

Discussion. From these results it is apparent that the only pathway for the metabolism of testosterone by the mouse placenta is via reduction to 5α-androstane derivatives, namely to: 3α-hydroxy-5α-androstan-3,17-dione, 3β-hydroxy-5α-androstan-3,17-dione and 5α-androstane-3,17-dione. The failure of mouse placental tissue to aromatize 4-C^{14}-testosterone suggests that the placenta of mouse is probably not a source of estrogens. These results are in agreement with our previous observations that 4-androstene-3,17-dione formed by mouse placentae from progesterone and pregnenolone was not converted to estrogens[1]. However, VINSON and JONES[4] reported that phenolic compounds appeared when fetal tissue was incubated with progesterone. These observations provide suggestive evidence that the fetus may convert placental androgens to estrogens. This problem is under study in our laboratory.

Table II. Distribution of radioactivity in metabolites isolated following incubation of mouse placental tissue with 4-C^{14}-testosterone

Metabolite identified	Conversion (%)
Testosterone	1.65[a]
I	12.32[b]
4-Androstene-3,17-dione	2.27
3α-Hydroxy-5α-androstan-3,17-dione	43.58
3β-Hydroxy-5α-androstan-3,17-dione	8.78
5α-Androstane-3,17-dione	12.41

The results are expressed as percentage conversion of incubated substrate following recrystallization of the isolated metabolite to constant specific activity. [a] Percentage of substrate remaining following incubation. [b] Percentage conversion was calculated by eluting radioactive peak present on chromatograms.

Zusammenfassung. 4-C^{14}-Testosteron wurde durch Mäuseplazenta in vitro zu Steroiden umgesetzt: 3β-Hydroxy-5α-androstan-3,17-dion, 3α-Hydroxy-5α-androstan-3,17-dion, 4-Androsten-3,17-dion und 5α-Androstan-3,17-dion. Eine Umsetzung von Testosteron zu Östrogenen wurde nicht nachgewiesen.

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Institute of Pharmacology, Polish Academy of Sciences, 52, Ojcowska, Kracow (Poland), 22 February 1971.

[4] G. P. VINSON and J. CHESTER JONES, Gen. comp. Endocr. *4*, 415 (1964).

Varying Degrees of Ventromedial Hypothalamic Destruction in the Weanling Rat and its Effect on Plasma Triglyceride and Cholesterol Levels[1]

Recent studies[2] have shown that bilateral lesions in the area of the ventromedial hypothalamic nucleus (VMN) in weanling rats resulted in hypertriglyceridemia and hypercholesterolemia despite normophagia. The results of these studies suggested that the hypothalamic area involved in the regulation of plasma triglyceride levels is more circumscribed than that involved in the regulation of cholesterol. They also raised the question of whether or not a greater response to destruction required involvement of areas outside the VMN. To examine this question, two experiments were performed, each with three groups of animals. In one group, a small lesion, produced by a single electrolytic focus, was placed bilaterally in the VMN. In a second group, an anteroposterior series of closely-spaced lesions, each of a size comparable to that of the single foci in the previous group, were placed bilaterally in order to destroy a greater longitudinal extent of the VMN while avoiding destruction medial and lateral to these nuclei. A third group of animals served as sham-operated controls.

All methods (operational procedures, maintenance of animals, duration of experiment, and lipid determinations) have been described previously[2]. The lesions were produced in experiment 1 with a current of 1.0 mAmp flowing for 3 sec (3 m-Coulombs), and in experiment 2 with a current of 1.0 mAmp flowing for 4 sec (4 mC). The lesion analysis has been described previously[3] and visualizes the destroyed area common to all rats of each

Experimental data on rats with pairs of single electrolytic lesions ((Group 2) and triple lesions (Group 3) in the ventromedial hypothalamic nuclei compared with their sham-operated controls (Group 1)

Group (N)	Change in body wt. (g)	Change in body length (mm)	Food intake (g/day)	Lee index[b]	Plasma triglyceride (mg/100 ml)	Plasma cholesterol (mg/100 ml)
1 (16)[d] Control	74.0 ± 3.6[a]	60.2 ± 2.7	19.7 ± 0.5	309.4 ± 1.6	49.4 ± 3.4	87.8 ± 2.7
2 (15) Single lesion	57.8 ± 4.4	52.7 ± 3.0	14.0 ± 0.6	313.7 ± 1.2	60.3 ± 5.1	89.0 ± 2.8
3 (30) Triple lesion	79.0 ± 4.5	53.5 ± 2.6	21.8 ± 0.7	318.9 ± 1.9	76.9 ± 5.6	98.1 ± 2.4
	Statistical significance					
1 vs 2	0.01[c]			0.05		
1 vs 3				0.01	0.01	0.02
2 vs 3	0.01		0.02			0.05

[a] Mean ± S.E.M. [b] Lee index, cube root of body weight (g)/naso-anal length (mm) × 10,000. [c] $p <$; no notation when value is not significant. [d] (), refers to the number of animals per group.