In vitro Metabolism of 4-^{14}C-Testosterone by Thymus of Male Rats of Various Ages

The observations that hypersecretion of androgen causes atrophy of the thymus during puberty and castration prevents this atrophy, point to specific relations between the thymus and the genital organs. Little information is, however, known as to whether the thymus itself is capable of metabolizing androgen and, if it does, whether the metabolism varies with age. In the present study, the uptake of 4-^{14}C-testosterone (T) by the thymus in male rats and the alternation of the in vitro metabolism of 4-^{14}C-T by the thymus of rats of various ages were investigated.

Methods. In the first experiment which dealt with the uptake of 4-^{14}C-T by the thymus, 4 male rats of the Wistar strain, two 21- and two 30-day-old, were used. Each rat was injected i.p. with 0.5 ml of corn oil containing 0.5 μc of 4-^{14}C-T, purchased from the New England Nuclear Corp., USA. 10 or 30 min after injection, the animal was decapitated and the thymus, liver, testes and adrenals were removed. The liver was perfused with saline. Each organ was homogenized with 0.1 *M* Krebs-Ringer phosphate (KRP) buffer (pH 7.4) in a Potter-Elvehjem type homogenizer at 4 °C. The homogenate was shaken 4 times with 10 ml portions of dichloromethane. The combined dichloromethane extract was evaporated and then the residue was redissolved in an adequate volume of ethanol. A suitable aliquot was taken into a planchet and was evaporated. The radioactivities (RA) were counted by a gas flow counter (Aloka TDC-2, Japan).

In the second experiment, which dealt with the in vitro metabolism of 4-^{14}C-T by thymus homogenates from rats of various ages, 2-, 3-, 4-, 5-, 7- and 10-week-old male rats of Wistar strain were used. Immediately after the rat was killed, the thymus was homogenized with 0.1 *M* KRP buffer. To a flask containing the whole homogenates, 0.5 μc of 4-^{14}C-T dissolved in 0.1 ml of ethanol and 500 μg of NADPH dissolved in 1.0 ml of KRP buffer (pH 7.4, 0.1 *M*) were added. The mixture was incubated at 37 °C for 90 min under an atmosphere of 95% O_2 and 5% CO_2, with continuous shaking. The extraction was carried out by OBARA et al.[1] and a suitable aliquot of the extract was chromatographed on a thin layer of silica gel using mostly a benzene:acetone (80:20 by volume) system. After the development of the thin-layer plate, the radioactive spots were detected by an autoradiographic method[1]. As a way to identify the spots, oxidation and acetylation procedures were used[1].

Results. The RA in the thymus 10 and 30 min after the injection of T were 1/1.43 and 1/1.44 of the corresponding accounts in the testes in 21-day-old rats (Table I). The ratio of the RA of the thymus at 10 min after the injection to those at 30 min was 1:3.15. In 30-day-old rats, the RA taken up by the thymus were 1/1.66 and 1/2.75 of the RA in the testes at 10 and 30 min, respectively.

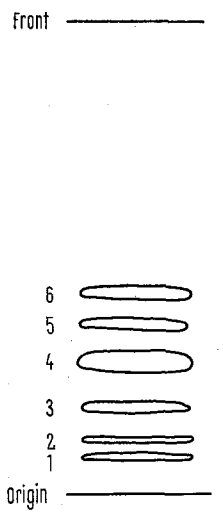
The autoradiogram of the metabolites of 4-^{14}C-T is illustrated in the Figure. Each radiographic pattern of the metabolites obtained from the rats at various periods after birth was essentially similar. Identification or presumption of the substance in each spot was made by the procedures reported previously[1]. The free metabolites of 4-^{14}C-T identified or presumed and the average percentage of the RA of the individual metabolites to the total RA obtained in the rats are shown in Table II. No significant difference was observed in the percentages of RA of T and its metabolites in the rats of various ages.

Discussion. In a similar incubation condition as used in the present experiment, 98.9% of T added was metabolized by homogenate prepared from 0.5 g of adult male rate liver[2]. In this study, only 5–8% of the substrate was metabolized. This finding indicates that the thymus is not an active organ for metabolism of androgen.

The fact that 6β-hydroxy T was found among the metabolites suggests the presence of androgen hydroxylase, which requires NADPH and O_2 for activity[3], in the thymus of rat. Δ^4-androstene-3,17-dione and andro-

Table I. Uptake of injected 4-^{14}C-testosterone by thymus in male rats (%)

Age	21-day-old rats (%)		30-day-old rats (%)	
Minutes after injection	10 min	30 min	10 min	30 min
Organ: Thymus	0.040	0.126	0.059	0.069
Liver	3.350	4.468	3.403	2.985
Testes	0.057	0.181	0.098	0.190
Adrenals	0.019	0.025	0.029	0.026



Autoradiogram of metabolites of 4-^{14}C-testosterone incubated with thymus homogenates.

Table II. Percentage of specific radioactivities of the individual metabolites of 4-^{14}C-testosterone by rat thymic homogenates to the total radioactivity

Age (weeks)	2	3	4	5	7	10
Av. of body weight (g)	23	38	73	112	173	249
Av. of thymus weight (mg)	87	110	206	423	470	546
Testosterone metabolite:						
Unidentified[a]	0.4	0.4	0.5	0.5	0.6	0.6
6β-hydroxy testosterone	0.6	0.4	0.7	0.6	0.7	0.7
5β-androstane-3α,17β-diol	1.1	1.9	0.9	1.7	1.1	0.7
Testosterone	94.8	91.9	95.3	94.3	94.3	95.6
Androsterone	1.5	2.2	1.4	1.8	1.7	1.1
Δ^4-androstene-3,17-dione	1.6	3.2	1.2	1.1	1.6	1.3

[a] Percent-values.

[1] N. SATO, K. OBARA and M. OTA, Tohoku J. exp. Med. *98*, 281 (1969).
[2] N. SATO, M. OTA and K. OBARA, Med. & Biol. *78*, 103 (1969) (in Japanese).
[3] A. H. CONNEY and A. KLUTCH, J. Biol. Chem. *238*, 1611 (1963).

sterone were predominant metabolites of T. This indicates the reduction of the 4–5 double bond and the subsequent reduction of the carbonyl group at C-3. The highest percentage of Δ^4-androstene-3,17-dione and androsterone was observed in the thymus of 3-week-old rats and the lowest was in that of 10-week-old ones. These findings represent that the activities of Δ^4-5α-hydrogenase and 3α-hydroxysteroid dehydrogenase were slightly elevated at 3 weeks of age. The significance of these findings are not known, but it is of interest that a high metabolic rate was observed in a 3-week-old rat which was at the time of prepuberty.

Zusammenfassung. Stoffwechseluntersuchungen von 4-^{14}C-Testosteron wurden in vitro mit Thymushomogenaten, die aus 2, 3, 4, 5, 7 und 10 Wochen alten männlichen Ratten gewonnen wurden, durchgeführt. Die Radiochromatogramme der Metaboliten zeigten ein gleichartiges Bild.

N. Sato, M. Ota and K. Obara

Iwata Medical University,
School of Medicine, Department of Biochemistry,
Morioka, Iwate (Japan), 8 July 1969.

Morphological Counterparts of the Genetically Determined Resistance of Mice to Chloroform Poisoning

Chloroform causes renal tubular necrosis in male mice of the strain C3H/He, resulting in the death of animals within 4–9 days[1]. Necrotic tubules calcify, no signs of tubular regeneration being present. Females of this strain, as well as males and females of the strain C57BL/6JN and BN, survive the chloroform intoxication. The same is true of the males of the generation F1 from the crossing ♀♀ C3H/He × ♂♂ C57BL/6JN[2]. Accidental chloroform poisoning in mice was reported by Christensen et al.[3] and Jacobsen et al.[4].

The purpose of the present experiment was to check the development of renal lesions after chloroform poisoning in males and females of the resistant strains C57BL/6JN, BN, as well as of the generation F1 deriving from the crossing of mice of strain C3H/He and C57BL/6JN.

Material and method. The material consisted of the following groups of sexually mature mice, aged 2–3 months, weighing 18–20 g:

Group I. 16 male and 16 female mice of the strain C57BL/JN.

Group II. 16 male and 16 female mice of the strain BN.

Group III. 16 male and 16 female mice of the generation F1/♀♀ C3H/He × ♂♂ C57BL/6JN.

Each of the mice received s.c. a single dose of 0.1 cm^3 of 0.05 g of chloroform in 1 cm^3 of ethyl laurate.

The animals were destroyed after 6, 12, 24, 48 h, 4, 6, 8, 10 days. After post-mortem examination the kidneys were fixed in 10% formalin. Paraffin sections were stained with hematoxylin and eosin, with PAS, with azan after Heidenhain, and with van Kossa method for calcium.

Results. All animals survived the intoxication, none of them died spontaneously. The kidneys of female mice were normal throughout the course of the entire experiment.

All male mice exhibited morphological signs of renal damage between the 6th and 12th h after chloroform injection (Figure 1). The lesions consisted of the acidophilic necrosis of some segments of proximal convoluted tubules, situated particularly in the outer cortex. Tubular basement membrane was unchanged. Up to the 4th day the lesions gradually became more extensive and were associated with the presence of protein-containing fluid, initially in the lumen of proximal convolutions, later of Henle's loop and collecting tubules.

On about the 4th day, all animals developed tubular regeneration which continued throughout the course of experiment. Necrotic epithelium did not show any calcification, and was undermined by young, flat, basophilic epithelial cells encroaching upon the basement membrane (Figure 2). Between the 4th and 10th day of regeneration basophilic epithelium formed renal tubules, and gradually differentiated into acidophilic form typical of proximal convolutions. As was stated in previous report[2], the kidneys are almost normal, showing only focal regeneration on the 15th day (Figure 3).

Discussion. The present experiment provides a morphological basis of the mechanism of the genetically determined resistance against chloroform poisoning of male mice of the strains C57BL/6JN, BN, and of generation F1 derived from the crossing of a susceptible strain C3H/He and a resistant one C57BL/6JN.

Initially all male mice showed renal tubular necrosis. They were thus susceptible to the harmful action of chloroform. The fact of survival of these mice seems to depend upon two essential 'defensive' phenomena which

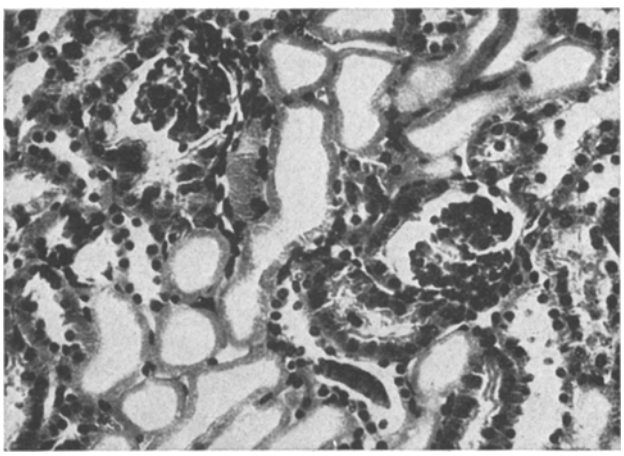


Fig. 1. Male mouse, generation F1 (C3H/He × C57BL/6JN), 24 h after chloroform poisoning. Necrosis of renal tubular epithelium. H.E., magnitude about × 400.

[1] Z. Zaleska-Rutczyńska and S. Kruś, Patol. pol., submitted for publication (in Polish).

[2] Z. Zaleska-Rutczyńska and S. Kruś, Patol. pol., submitted for publication (in Polish).

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[4] L. Jacobsen, E. Krag Andersen and J. V. Thorborg, Acta path. microbiol. scand. *61*, 503 (1964).