The Effect of Neonatal Administration of Testosterone and Oestradiol on the Liver Nuclear Volume of Male and Female Rats

It is generally accepted that the nuclei of the parenchymal cells of mammalian liver have ploidies (DNA contents) in the ratio of $2N$, $4N$, $8N$[1,2]. These various levels of ploidy correspond in ratio with the cell volume[3,4], nuclear volume[2,5], nucleolar volume[6,7] and nuclear histone levels[8] of hepatocytes. The present investigation is concerned with the neonatal injection of sex hormones on the liver nuclear volume of male and female rats.

Litters of a highly inbred colony of Wistar rats were randomly divided into different groups, each containing males and females. A single s.c. injection of either 500 µg of testosterone propionate or 250 µg of oestradiol benzoate in 0.05 ml of arachis oil was given within 24 h after birth. Controls were given equal volumes of arachis oil or received no treatment. Immediately upon withdrawal of the needle, the injection site was sealed with colourless octaflex (1% w/w octaphonium chloride). Half of the rats were gonadectomized at 4 weeks and the animals were fed a laboratory diet and water ad libitum.

The litters were killed at 6–7 months. Tissue blocks were taken from the left lateral lobe of the excized livers, fixed in Helly's fluid and embedded in paraffin. Sections cut at 10 µm were stained by hematoxylin and eosin for the measurement of the size of nuclei. This method, although it did not provide a direct measurement of the amount of DNA present in the nuclei, such as may be obtained with the Feulgen technique using an integrating microdensitometer, gave a reasonably accurate and quick method for the measurement of a large number of nuclei. The major and minor nuclear diameters were measured using a Leitz 6X ocular µm with calibrated scale and the 100X oil-immersion objective lens. In each animal, 200 nuclei were measured and the nuclear volumes in cubic micra were calculated from the formula of the rotary elliposid: $V = \pi/6\ ab^2$, where a is the major axis and b the minor axis.

The results are summarized in the Table and the distribution of the values obtained were shown graphically in Figures 1–5. The number of nuclei measured falling into each class gave an approximate indication of the various classes of ploidy; presumably A = diploid ($2N$), B = tetraploid ($4N$), and C = octaploid ($8N$). Untreated adult males had a significantly greater nuclear volume than the females. Neonatal injection of testosterone produced no effect in males but significantly increased the nuclear volume in female rats, whereas oestradiol caused a drop both in males and in females. Castration reduced the nuclear volume and the fall could be restored by neonatal treatment with testosterone. The greater nuclear volume of males was apparently due to a higher frequency of tetraploid and octaploid nuclei (Figure 1). In females, although there was a scatter of values approaching the tetraploid and octaploid classes, the liver was predominantly unimodal – presumably diploid. A single s.c. neonatal injection of testosterone, although it produced no significant alternation of polyploid distribution in males (Figure 2), significantly increased the

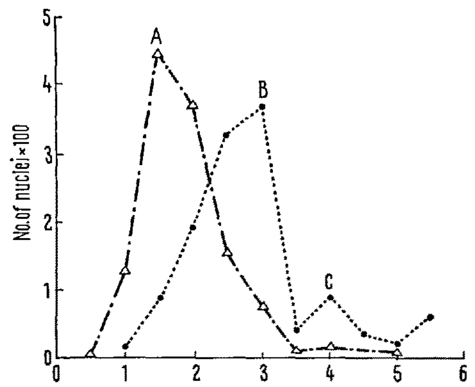

Fig. 1. Distribution of nuclear volume in the liver of male (●···●) and female (△–·–△) rats.

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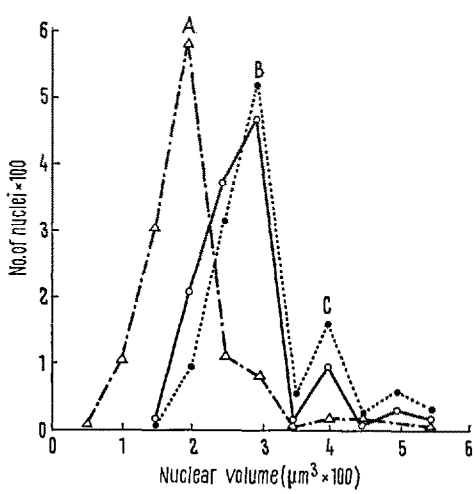

Fig. 2. Comparison of the effect of neonatal administration of testosterone (●···●) and oestradiol (△–·–△) with the control (○—○) on the distribution of nuclear volume in the liver of intact male rats.

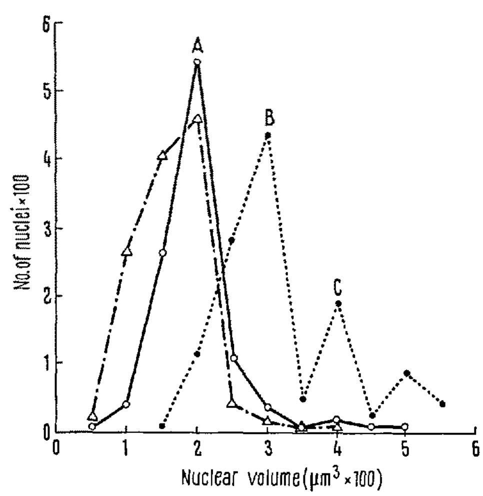

Fig. 3. Comparison of the effect of neonatal administration of testosterone (●···●) and oestradiol (△–·–△) with the control (○—○) on the distribution of nuclear volume in the liver of intact female rats.

The effect of neonatal administration of sex hormones on total liver nuclear volumes of intact and gonadectomized male and female rats

		No. of rats	Intact mean ± S.E.M.		No. of rats	Gonadectomy mean ± S.E.M.	
Normal untreated control	♂	6	265.62 ± 10.35	$P < 0.001$ [a]		–	
	♀	6	164.63 ± 4.23			–	
Oil treated control	♂	6	263.96 ± 6.74	$P < 0.001$ [a]	6	170.79 ± 7.69	$P < 0.1$ [a]
	♀	5	168.41 ± 9.75		6	153.54 ± 7.51	
Testosterone treated	♂	6	287.84 ± 9.77	$P < 0.05$ [b]	4	256.40 ± 10.12	$P < 0.001$ [b]
	♀	6	304.24 ± 8.18	$P < 0.001$ [b]	6	244.18 ± 12.35	$P < 0.001$ [b]
Estradiol treated	♂	6	164.96 ± 7.89	$P < 0.001$ [b]	6	162.20 ± 7.91	$P < 0.4$ [b]
	♀	6	132.87 ± 9.86	$P < 0.025$ [b]	6	154.48 ± 8.21	$P < 0.9$ [b]

[a] Comparison between males and females. [b] Comparison between oil-treated control and hormone-injected groups.

tetraploid and octaploid populations in female rats (Figure 3). Oestradiol, by contrast, reduced the number of big (polyploid) nuclei in the male and in the female rats. Castration of weanling rats consistently decreased the tetraploid and octaploid populations of liver nuclei (comparison of Figures 2 and 4). Testosterone given to new-born rats offset the diminished tetraploid and octaploid nuclear population associated with gonadectomy at the age of 4 weeks (Figures 4 and 5).

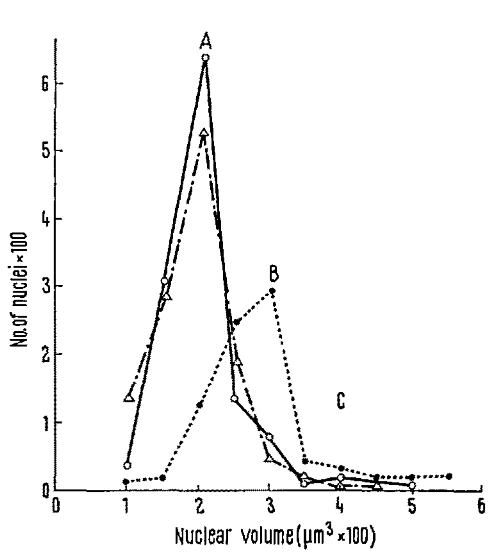

Fig. 4. Comparison of the effect of neonatal administration of testosterone (●···●) and oestradiol (△-·-△) with the control (○—○) on the distribution of nuclear volume in the liver of male rats castrated at the age of 4 weeks.

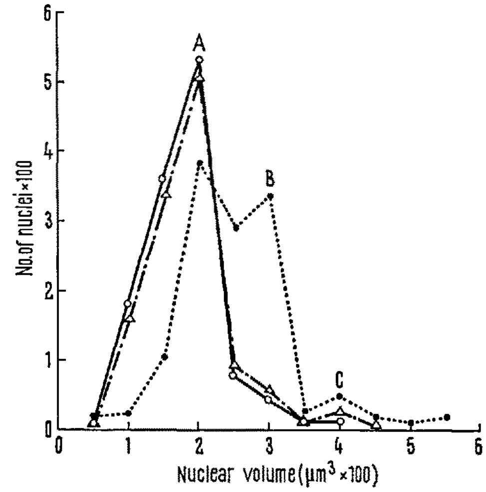

Fig. 5. Comparison of the effect of neonatal administration of testosterone (●···●) and oestradiol (△-·-△) with the control (○—○) on the distribution of nuclear volume in the liver of female rats ovariectomized at the age of 4 weeks.

Swartz et al.[9] also showed a higher percentage of $8N$ nuclei in the liver of males than in female rats. However, Swartz and Sams[10] did not produce any significant effect on liver polyploidization when intact new-born rats were given very large amounts of testosterone (500 μg daily) or estrone (80 μg alternate days) for 80 days. These authors interpreted the failure as a dosage phenomenon, for large quantities of testosterone might actually inhibit body growth[11] while small doses of testosterone produced significant increase of growth rate[12]. Swartz et al.[9,13] demonstrated that neonatally castrated males were more greatly inhibited in growth and polyploidization than males castrated at the time of weaning. If castrations were performed on new-born rats, injection of testosterone (250 μg) or estrone (10 μg) for 22 days beginning at birth, failed to have significant effect on the hepatic ploidy distribution when the rats were killed at the end of the course of injections[14]. Hoffmann and Swartz[14] reported that hypophysectomy interrupted the progress of polyploidization in the liver nuclei and suggested that the effect of sex steroids was mediated via the pituitary rather than by direct action on the liver.

Zusammenfassung. Wirkung von Testosteron oder Östradiol auf die Grösse bzw. das Kernvolumen der Leberzellen von neugeborenen männlichen und weiblichen Ratten. Testosteron verursachte vor allem bei weiblichen Tieren eine erhebliche Volumenzunahme der Leberzellenkerne, während Kastration eine Verminderung der Polyploidie zur Folge hatte.

Yee-Chu Toh

Sub-Department of Endocrine Pathology,
The University of Liverpool, The Liverpool Clinic,
Myrtle Street, Liverpool, L7 7DE (England),
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