than spleen, lymphoid or bone marrow cells. Thus it may be that the sometimes widely differing observations made by various workers on chimaerism in tolerant mice may be explained on the basis of dissimilar experimental techniques used.

A comparison of the degree of chimaerism produced in the lympho-myeloid complex of adult mice made tolerant by the neonatal injection of allogeneic CBA-T6T6 cells from 4 different tissue sources.

Tissue used to create tolerance	No. of mice examined	Mean % of T6T6 cells in the mitoses of the recipient			
		Bone marrow	Spleen	Thymus	Lymph nodes[a]
Foetal liver	6	8.0	9.7	9.7	10.7
Bone marrow	6	1.0	2.7	4.0	4.0
Spleen	6	0.7	1.7	0.3	7.0
Lymphoid tissue	6	0.7	1.0	1.0	1.0

[a] 50 metaphase plates scored in each tissue.

The explanation for foetal liver cells producing a markedly higher degree of chimaerism than cells from the other three sources, despite the same number of cells from each tissue being injected could reside in the fact the number of cells introduced is less relevant than the relative proportions of stem cells contained within each inoculum, since it is the stem cell component which will implant, proliferate and populate the animal[10].

Résumé. La tolérance immunologique produite chez la souris avec les cellules de foie d'embryon, ou des cellules de rate, de tissus lymphoïdes ou de moelle osseuse d'adulte, a été comparée au degré de chimérisme. Le foie d'embryon a produit le plus haut degré de chimérisme (10,7%).

MARY J. SELLER and A. RONAYNE HARCOURT

Paediatric Research Unit,
Guy's Hospital Medical School,
London, S.E.1 (England), 21 July 1971.

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Studies on Folate Metabolism in Castrated Rats and those Treated with Testosterone

To make the mechanism of action of androgens clear, and chiefly to see how they stimulate RNA and protein syntheses, the effect of testosterone treatment on folate coenzyme storage in the liver and in accessory reproductive glands was studied in a previous paper[1]. It is common knowledge that these coenzymes are directly involved in these biosynthetic processes since they function as actual donors of 1-C groups in the synthesis of purine[2–4] and pyrimidine[5] nucleotides as well as in the formylation of methionine-t RNA_F[6,7]. The data obtained show marked changes in the content and distribution of these coenzymes both in the liver and in 'target' organs of castrated animals. 'Chronic' administration of testosterone normalizes these changes.

To verify the reason for the alterations caused by castration on the tissue level of these coenzymes[8], in the present paper the effect of castration and testosterone treatment on the capacity for conversion of folic acid into its activated forms by the intact animal, has been examined. After injection of folic acid, the liver levels of folate coenzymes and the quantity of folate metabolites excreted in the urine by castrated rats and testosterone-treated castrated rats, were studied.

Materials and methods. 15-week-old male albino rats of Wistar strain, 350–400 g in weight, were used and divided into 4 groups. The animals of groups 3 and 4 were orchiectomized via the scrotal route under ether anesthesia. After 4 weeks the rats of groups 2 and 4 were injected s.c. with 5 doses of testosterone propionate (1 mg in 0.2 ml of sesame oil/100 g body weight) every other day for 10 days. The rats of groups 1 and 3 were injected with the same volume of vehicle. The animals were fed on a stock diet with no restrictions in their food intake throughout the experiment. 36 h after the last injection, 8 rats of each group were injected i.p. with 200 µg of folic acid/100 g of body weight, and received by stomach-tube 5 ml of 0.005 *M* NaCl. The rats were placed in individual metabolism cages and urine samples were collected for 12 h in bottles containing potassium ascorbate (100 mg). The total folate activities in urine were assayed aseptically with *L. casei* ATCC 7469, the

Table I. Effect of castration and of testosterone 'chronic' treatment on the conversion of folic acid to activated forms: urinary excretion of folate derivatives by rats after injection of folic acid

	Folate activity for *L. casei*[a] (µg/12 h/rat)	Folate activity for *P. cerevisiae*[b] (µg/12 h/rat)
Normal rats	357±32	22.6±1.01
Normal rats + testosterone	268±19	18.8±1.37
Castrated rats	294±69	12.7±1.21[c]
Castrated rats + testosterone	307±59	23.1±2.05

[a] Folate activity for *L. casei* is the measure of all folate forms. [b] Folate activity for *P. cerevisiae* is the measure of folate forms reduced to tetrahydro level except the 5-CH_3-H_4 folate All values. represent mean ± S.E.M. of 8 animals; significance of differences from values for normal animals is designated as follows: [c] $P < 0.001$.

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[8] The following abbreviations are used: H_4 folate, tetrahydrofolate; 5 (or 10)-HCO-H_4 folate, 5 (or 10)-formyltetrahydrofolate; 5-CH_3-H_4 folate, 5-methyltetrahydrofolate.

Table II. Effect of castration and of testosterone 'chronic' treatment on the conversion of folic acid to its activated forms: folate coenzymes in the rat liver after injection of folic acid

Compounds	Normal rats	Normal rats + testosterone	Castrated rats	Castrated rats + testosterone
All folate forms	12,725±1,050	11,750±980	11,870±890	12,210±1,100
All non reduced forms	620± 59	510± 70	1,850±203 [c]	1,030±79 [b]
All tetrahydro forms	12,105± 990	11,240±930	10,020±920	11,180±1,025
5-CH_3-H_4 folate	2,065± 280	2,315±195	4,350±358 [b]	3,180± 295
10-HCO-H_4 folate	2,975± 250	2,780±268	1,270±205 [c]	2,885± 307
5-HCO-H_4 folate	2,095± 190	1,850±208	1,340±162 [a]	1,765± 195
H_4 folate	4,430± 395	4,238±345	2,520±290 [b]	3,250± 350 [a]

All values represent mean of 6 determinations on different animals ± S.E.M. and are expressed as ng/g of tissue. Significances of difference from values for normal animals is designated as follows: [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$.

active reduced forms with *P. cerevisiae* ATCC 8081. All rats were killed by cervical fracture 48 h after the last injection of hormone and the liver was quickly removed and placed in ice-cold water.

To determine the folate coenzymes, part of the tissue was homogenized with 9 volumes of 1% potassium ascorbate pH 6.0, another part with 9 volumes of water. Both homogenates were placed in a 95 °C water bath for 5 min, quickly cooled in iced water and centrifuged. To the extracts were added hog kidney conjugase, prepared according to EIGEN and SHOCKMAN[9], 0.1 *M* acetate buffer pH 4.7, 1% ascorbate pH 4.7; the latter was omitted in the enzyme treatment of the water extract.

The samples were incubated 4 h at 37 °C and stored at −20 °C for the determination of folate derivatives. The microbiological 'aseptic' and 'autoclaved' assays were performed by the method described by BIRD et al.[10]. The amount of each component with folate activity was calculated using the procedure recommended by BIRD et al.[10].

Results and discussion. The data of Table I show no significant difference in the amount of folate metabolites active for *L. casei* excreted in the urine by the animals of the 4 groups. On the contrary, a marked fall in the excretion of the compounds active for *P. cerevisiae* has been observed in castrated rats, while castrated and hormone-treated rats eliminate almost the same quantity of these compounds as the control animals.

The data of Table II show no differences in the 4 groups of animals for liver content of total folate activities, while a significant increase of folic acid has been observed in castrated rats, this increase being depressed by testosterone treatment. The total folate reduced forms do not change but, considering the various coenzymes singly, differences have been seen in castrated rats as compared with normal ones. In particular, there is a significant fall of 5-HCO-H_4 folate and 10-HCO-H_4 folate and an increase of 5-CH_3-H_4 folate. Also at this level hormone treatment almost completely restores the liver content of these coenzymes in castrated rats.

The decrease of urinary excretion of folate derivatives active for *P. cerevisiae* found in castrated animals, indicates a low utilization by these animals of administered folic acid. In fact, while *L. casei* measures the total folate activity including folic acid, *P. cerevisiae* only measures reduced derivatives which are the real coenzymatic forms.

The data obtained in the liver content of folate coenzymes, which have pointed out a fall of the active forms of folic acid in castrated rats, would confirm our assumptions.

In conclusion, the whole data make one believe that the lower coenzymatic levels previously observed[1] in castrated animal tissues are due to a block or at least to a slowing down of the processes leading to folic acid conversion into its active forms. It seems possible to affirm that testosterone can somehow influence this metabolic process. A point in favour of this hypothesis is that testosterone treatment of castrated animals, by restoring active form synthesis, normalizes coenzyme levels.

Résumé. Nous avons recherché l'effect du testostérone sur la capacité de l'acide folique de se transformer en ses formes actives chez le rat intact. Les résultats permettent de suggérer l'hypothèse que le testostérone est en quelque manière interessé à ce processus biosynthétique.

B. TOLOMELLI, C. ROVINETTI, C. BOVINA and M. MARCHETTI

Istituto di Chimica Biologica e Biochimica Applicata dell'Università di Bologna I-40126 Bologna (Italy), 28 July 1971.

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