of various age groups, have observed a high incidence (85%) of positive antibody in the cord serum. The frequency of positive serum, according to their study, decreased to 30–40% between 1 and 24 months old but again increased to about 80% by 3 years of age. It remains to be seen whether such a sharp rise of antibody prevalence is due to de novo infection by EBV during the early infancy or immunological reactivity of the infants to latent EBV transmitted vertically in utero.

Zusammenfassung. Antikörpertiter gegen EBV wurden in 40 gepaarten Seren von pränatalen Müttern und Neugeborenen gleichzeitig mit indirekter Immunofluoreszenz bestimmt. In 95% hatten sowohl Mütter als auch Neugeborene den Antikörper. Die Titer der Mütter waren mehrheitlich gleich oder höher jenen der Neugeborenen, woraus geschlossen wird, dass der Antikörper gegen EBV von der Mutter diaplacentar auf den Fetus übertragen werden kann.

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A Comparison of the Degree of Chimaerism Produced when Allogeneic Cells from Four Different Tissues are used to Create Tolerance in the Mouse

Immunological tolerance, the specific non-reactivity to a foreign antigen initiated by contact with that antigen, was first described by BILLINGHAM, BRENT and MEDAWAR[1]. For tolerance to persist, the antigen must remain[2], and when the antigen is in the form of whole cells, these should be detectable in the tolerant individual[3]. In the mouse, using a chromosome marker, quantitative estimates of the degree of chimaerism have been made. However, conflicting findings have resulted. Some workers have found 80–90% of allogeneic i.e. donor cells in the organs of the recipient mice[4], whilst others have been unable to detect any[5]. An explanation for these differences may lie in the fact that there has been no uniformity in the various experiments for such factors as the source of the tolerogenic tissue, the route of entry of the cells or the histocompatible relationship between host and donor.

The present work examines the possibility that the source of the tolerogenic tissue may influence the degree of chimaerism achieved.

The donor mice were a pure line CBA/H-T6T6 strain, the T6 chromosomes were used as cell markers since they were not possessed by the recipients. The recipients were +/+ mice of the W-series, phenotypically completely normal.

For the preparation of the tolerogenic cells, either one spleen, or the thymus together with the subcutaneous and mesenteric lymph nodes (referred to as lymphoid tissue) from one adult CBA mouse, or the livers from a litter of 16–18-day-old CBA foetuses, were removed and placed in Hanks' B.S.S. A suspension of single cells was produced as described previously[6]. In the case of bone marrow, 2 adult femurs and tibiae with the ends removed were in turn attached to a 14G hypodermic needle on a 1 ml syringe, and the marrow flushed out with Hanks' B.S.S. Cell clumps were broken up by aspiration through a 27G hypodermic needle. Following centrifugation at 1500 *g* and resuspension in Hanks' B.S.S., the cells were counted, and the dilution of the cell sample adjusted so that 5×10^6 cells were present in 0.02–0.04 ml of solution.

Neonatal +/+ mice were injected i.v. with 5 million cells of one of the tissues. At 10–12 weeks of age each was challenged with a skin graft from a female CBA mouse, using the technique of BILLINGHAM and MEDAWAR[7]. In untreated mice such grafts were rejected in a mean time of 11.2 days[8]. Those mice which did not reject the skin grafts were accepted as having been rendered tolerant. At least 6 weeks after grafting, when the graft had a good growth of hair, the mice were subjected to mitotic chromosome studies after an injection of Colcemid (Ciba), using the method of FORD[9]. Chimaerism was sought in the bone marrow, spleen, thymus and lymph nodes of each animal.

The results were compared statistically using the Student's *t*-test, and differences were regarded as significant if $p < 0.05$.

The Table shows the results obtained. Overall, the degree of chimaerism was very low, the acme for all tissues being reached by the foetal liver cells, but even then, the highest value, found in the lymph nodes, was only 10.7%. Statistically, this level of chimaerism produced by the foetal liver cells was significantly higher than that produced by lymphoid cells in each of the 4 recipient tissues examined. When foetal liver was compared with spleen cells as the donor tissue, there was a significantly greater degree of chimaerism in the bone marrow, spleen and thymus, and when bone marrow was used as the source of donor cells, the chimaerism produced by the foetal liver was only significantly higher in the recipient bone marrow and spleen. Foetal liver cells apart, bone marrow, and spleen and lymphoid cells all produced the same very low level of chimaerism in the recipient tissues.

The chimaeric mice were quite healthy at the time of sacrifice, occasionally, individuals were found to have slightly enlarged spleens, which is regarded as one of the signs of graft versus host disease.

The present experiments have shown that when a mouse is made tolerant to allogeneic cells, their descendents will be found in all of the lympho-myeloid tissues of the recipients, albeit at a low level of chimaerism. If the tolerogenic tissue source is varied, foetal liver cells produce a higher degree of chimaerism in the recipients

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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%).

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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.