upper part of the Figure. Accordingly, the PHA stimulated lymphocytes may be considered to show a behavior similar to that which has been described by KLEVECZ[4] for a body of diploid cells obtained from Chinese hamsters. In a body of murine fibroblasts (L cells), a different rate of DNA synthesis in the S phase was also found; here, however, the delay was found to be at the very start of the S phase[5-8]. The authors believe that eu- and heterochromatin show a different behavior here. It should also be considered that the synthesis of DNA, on the one hand, and the synthesis of RNA and protein, on the other hand, show a reciprocal relation[4,9]; this may necessitate a transient slowing down of DNA synthesis in the S phase, in order to make increased synthesis of both RNA and protein possible. Anyway, our studies have shown that it is necessary for different bodies of cells to be examined for their different behavior in the S phase[10].

Zusammenfassung. Karyologische und zytophotometrische Untersuchungen an PHA-stimulierten menschlichen Lymphozyten ergaben nach 76 h im DNS-Karyogramm einen weiteren Gipfel zwischen diploiden und tetraploiden Werten. Daraus wird geschlossen, dass bei Lymphozyten in der Kultur die DNS-Synthese in der S-Phase diskontinuierlich verläuft.

M. ZANK, BRIGITTE KRÖBER, P.-F. MAHNKE and H. KRUG

Pathological Institute, Karl Marx University, Liebigstrasse 26, DDR-701 Leipzig (DDR), 26 March 1971.

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Hematopoietic Alterations Produced by Long-Term Treatment with Phytohemagglutinin

It is known that the lymphoreticular system of the mouse undergoes profound cellular modifications in response to a single dose of phytohemagglutinin (PHA)[1-5]. The characteristic changes are depletion of mature lymphocytes and the appearance of numerous large undifferentiated cells in the spleen, lymph nodes, and thymus. Lymphopenia, neutrophilia and an abnormal number of lymphoblastoid cells are seen in peripheral blood[3-4].

It is not known, however, if repeated administration of PHA for a long period of time would produce permanent cell changes similar to those described earlier. This study was undertaken to find out whether the long-term treatment with PHA produces a leukemoid reaction in C3H and Swiss inbred strains of mice.

The administration of PHA (Bactophytohemagglutinin-P, Difco, Detroit, Mich.) began when the mice were 2 months old, and continued twice weekly for 16 (Swiss) and 24 (C3H) weeks at the dose of 5 mg/100 g of body w. i.p. Control animals were injected with heat-inactivated PHA[2] at identical intervals and doses. Groups of 10 mice were sacrificed on the 1st week of treatment and every 2 weeks thereafter to the end of the experiments. 50 mice of each strain served as untreated controls. A total of 320 mice were studied. Peripheral blood values, marrow (one femur), and splenic cell counts were assessed by standard techniques. Histologic sections were stained with hematoxylin-eosine. Cytologic examinations were also performed in smears stained with Giemsa.

Mice (C3H) given PHA or inactivated PHA developed splenomegaly (+40%) and hypertrophy of lymph nodes. The splenomegaly was mainly caused by a hyperplasia of splenic follicles and proliferation of hematopoietic foci, among which myelopoietic cells were predominant. The number of nucleated splenic cells was between 185×10^6 and 252×10^6. Untreated control mice had 150 ± 20.6 (S.D.) $\times 10^6$ splenic cells. Thymic atrophy was only observed in C3H mice throughout the study (Control thymus $0.10 \pm 0.01\%$ body wt.; Experimental 0.044 to 0.067% body wt.). Treatment with heat inactivated PHA did not change the thymus weight. No alterations of erythrocyte values and platelet counts were found in C3H mice. Control, given inactivated PHA, and experimental animals of this strain had leucocytosis (14,800 to 24,600/mm³; Normal = $9,200 \pm 950$/mm³) due to absolute lymphocytosis and neutrophilia. The number of immature cells in peripheral blood was within normal limits (0.2% of the total leucocyte count). Myelopoietic cells were predominant in the marrow. The number of nucleated marrow cells in the right femur was similar to that of untreated control and ranged from 7.23×10^6 to 9.40×10^6 regardless of the treatment.

Similar alterations were observed in the spleen of Swiss mice under chronic treatment with PHA. The spleen cell number was 201×10^6 in untreated control animals. Following PHA or inactivated PHA their number rose to a maximum of 332.1×10^6 cells on the 6th week of the treatment. Normal erythrocyte ($9.61 \pm 0.16 \times 10^6$/mm³) and platelet ($1.11 \pm 0.01 \times 10^6$/mm³) counts were found from the 1st to the 10th week of PHA administration. Anemia as indicated by erythrocyte counts of 6.71 to 7.14×10^6/mm³, with normal reticulocyte counts (4.8 to 5.8%) was observed from the 12th to the 16th week of the treatment with PHA. The administration of inactivated PHA did not cause anemia in Swiss mice. The normal leucocyte count was $24,800 \pm 1,230$/mm³. Following either PHA or inactivated PHA the total leucocyte counts were between 24,700/mm³ and 33,800/mm³. Lymphocytosis,

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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.

B. B. Lozzio

The University of Tennessee, Memorial Research Center and Hospital, 1924 Alcoa Highway, Knoxville (Tennessee 37920, USA), 23 March 1971.

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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.

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