Effect of X-Irradiation on Phosphorylase Activity in Tissues of Pregnant Mice

High glycogen content and metabolic activity of placenta make the organ comparable to liver[1]. Our earlier observations[2], however, indicate that placenta and liver respond differentially to X-irradiation with respect to glycogen metabolism. The enzyme phosphorylase, in the active form, is indispensable for the breakdown of glycogen[3-6] and has been shown to be present in rat placenta[7] only in the active form. The present investigation is on the effect of whole body X-irradiation on the levels of total phosphorylase in tissues of pregnant mice.

Materials and methods. Swiss mice, approximately 3 months old, fed ad libitum on a balanced laboratory diet, were used. The presence of vaginal plug the morning after mating, of nulliparous females, was noted and the period of pregnancy was computed on that basis.

Pregnant mice were irradiated on the 15th day of gestation using a Siemens stabilipan X-ray unit operating at 250 kVp and 15 mA tube current with a 2 mm Al filter. The non-irradiated controls were sacrificed every 24 h between 12th and 18th day of pregnancy and irradiated ones between 16th and 18th day. Samples of maternal liver, foetal liver and placenta were assayed for phosphorylase according to the procedure of GRILLO[7]. A 10% (W/V) homogenate was prepared in ice-cold 0.05 *M Tris*-HCl buffer (pH 7.8), centrifuged in the cold at 5000 rpm and an aliquot (0.05 ml) of the supernatant was assayed. The incubation medium (0.5 ml) contained 0.036 *M* glucose-1-phosphate (final concentration, 13.4 mg/ml), 0.1 *M* NaF (final concentration, 4.2 mg/ml), 0.0014 *M* cyclic 3′, 5′-adenosine monophosphate (final concentration, 0.486 mg/ml) and 2% glycogen (final concentration, 4 mg/ml). The pH of the medium was 6.5, optimum for the enzyme activity[7]. Incubation was carried out at 37°C in a constant temperature water bath for 10 min, at the end of which 2 ml 10% trichloroacetic acid was added to stop the reaction. The inorganic phosphate liberated by the action of the enzyme was estimated by the method of FISKE and SUBBAROW[8]. Corrections were made for any residual phosphate in the medium. Protein was estimated by the method of LOWRY et al.[9]. The enzyme activity was expressed as μg inorganic phosphate (Pi) liberated/mg protein/10 min.

Results. The levels of phospohrylase in placenta and maternal liver of non-irradiated mice show diurnal fluctuations (Table I). The foetal liver shows a steady increase in phosphorylase activity from 14th to 18th day of pregnancy. Phosphorylase activity of foetal liver is enhanced more than that of maternal liver and placenta following X-irradiation (Table II).

Discussion. Placenta has less phosphorylase activity than maternal liver but the enzyme levels of both show diurnal changes. There is a parallel rise in the amounts of glycogen[2] and phosphorylase in foetal liver during development. Glycogen synthesis in terms of incorporation of labelled glucose into glycogen is high in foetal liver towards term[10]. The relatively large amounts of phosphorylase suggest that there is high glycolytic activity. It appears that, during the last quarter of gestation, foetal liver is in a state of high metabolic flux, with high rates of synthesis, deposition and breakdown of glycogen. Corroborative evidence for this is available in the reports of BURCH et al.[11] on rats and KORNFELD and BROWN[12] on guinea-pigs.

X-irradiation enhances foetal liver phosphorylase to a greater extent than that of maternal liver while it leaves the enzyme of placenta virtually unaffected. This is different from the effect of X-irradiation on glycogen of maternal and foetal liver and placenta[2].

Résumé. Les niveaux de phosphorylase du foie et du placenta maternels oscillent, mais chez les souris, ceux du foie foetal augmentent constamment pendant la grossesse. L'effet des rayons X sur la phosphorylase du foie foetal est relativement plus élevé.

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Table I. Phosphorylase activity in tissues of pregnant mice (μg Pi/mg protein/10 min)

Tissue	Day of gestation						
	12	13	14	15	16	17	18
Liver-maternal	41.2[a]	44.8	39.1	46.5	35.4	38.8	39.8
Liver-foetal	—	—	27.6	28.3	33.8	38.9	46.1
Placenta	15.5	22.6	18.7	17.4	19.1	18.4	16.6

[a] Each figure is the average of 6–8 experiments.

Table II. Phosphorylase activity in tissues of pregnant mice X-irradiated (500 R) on the 15th day of gestation (μg Pi/mg protein/10 min)

Tissue	Day of gestation		
	16	17	18
Liver-maternal	39.3[a]	39.1	48.7
Liver-foetal	41.5	49.2	60.9
Placenta	22.1	18.4	22.6

[a] Each figure is the average of 6–8 experiments.

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