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Incorporation of (^{3}H) Thymidine into the Nuclear DNA in the Brain and Liver of Chicken Embryos

Inkorporacja (^{3}H) tymidyny do DNA jądrowego w mózgu i wątrobie embrionów kurzych

Включение (^{3}H) тимидина в DNA ядер мозга и печени куриных эмбрионов

The evolution of organs is accompanied by changes in the size of nucleic acids synthesis. Therefore investigations were undertaken to determine the specific activity of RNA and DNA from embryonic organs. The data obtained so far concern mainly the different kind of RNA (1, 8). The present study was designed to compare the labelling patterns of nuclear DNA of the brain and liver during embriogenesis.

MATERIALS AND METHODS

Radioactive compounds

(^{3}H) thymidine (9 Ci/mmole) was purchased from UVVVR — Prague, Czechoslovakia. The purity of this material was tested chromatographically.

Preparation of tissues

9 and 12-day-old chick embroys were used for the experiments. (^{3}H) thymidine was amniontically injected in a dose of 40, 50, 60 or 80 uCi in 0.1 ml of 0.9 per cent NaCl. Brains and livers were prepared 5 or 13 hrs after the injection. The tissues (50 brains or 50 livers) were weighed and then homogenized in 100 ml of ice-cold 0.44 M sucrose solution in 0.01 M Tris-HCl buffer, *pH* 7.2 containing $5 \cdot 10^{-4}$M EDTA.

Preparation of DNA from nuclei and mitochondria

The nuclei and the mitochondrial fractions were isolated by differential centrifugation in 0.44 M sucrose solution (2, 9). The nuclei were purified by centrifu-

gation in 1.5 sucrose in 0.01 M Tris-HCl, *pH* 7.2 containing $5 \cdot 10^{-4}$ M EDTA. The mitochondrial fractions were incubated with DNase (12) to reveal any contamination by fragments of nuclei. Nucleic acids were then extracted by the phenol technique described previously (4). RNA was removed by the alkaline hydrolysis (13) and DNA was hydrolized according to the method described by Borkowski et al. (3). In some experiments after RNase treatment (11) DNA was separated by chromatography on a methylated albumine kieselguhr column (10).

DNA determination

DNA was assayed spectrophotometrically (7) and by the diphenylamine reaction (6).

Radioactivity counting

100 µl samples of hydrolized DNA solution were neutralized and transfered to scintillation vials. The amount of radioactivity was measured by the method of Bray (5) by a Intertechnique scintillation counter.

RESULTS AND CONCLUSIONS

Comparative investigations showed that the wet weight of both 9-day old embryos and that of 12-day old ones was 4-times bigger than the weight of 1 liver. During those 3-days of embriogenesis the organs doubled their weight. However the DNA content did not increase in proportion to the organ weight. A 12-day old chicken brain contained only 2.3 µg of nuclear P-DNA more than that of a 9-day old embryo. On the other hand a fourfold increase of nuclear DNA content was observed in the liver during the same period (Tab. 1).

Table 1. Amount of nuclear and mitochondrial DNA in chick embryo tissues

Embryo's age (days)	Weight of fresh tissue		Nuclear fraction		Mitochondrial fraction	
	brain (mg)	liver (mg)	P-DNA/brain (µg)	P-DNA/liver (µg)	P-DNA/brain (µg)	P-DNA/liver (µg)
9	100 (97—101)	24 (23—25)	9.7 (8—10.7)	3.0 (2.8—3.2)	0.04 (0.024—0.072)	0.038 (0.033—0.045)
12	210 (194—228)	46 (42—49)	12.0 (11—13)	12.4 (11.3—15.6)	0.056 (0.04—0.082)	0.035 (0.032—0.036)

The results point to a very intensive liver DNA synthesis between 9-th and 12-days of embryogenesis whereas the slight increase of DNA content in the brain may indicate an earlier genom formation the tissue. No significant quantitative differences were found in the mitochondrial fraction. In experiments with isotopes it was found that the amount of radioactive thymidine incorporated into nuclear DNA was small as compared to the injected quantity (Tab. 2).

After 5 hours incubation irrespective of dose, only 0.1% radioactivity

Table 2. Radioactivity of nuclear DNA from chick embryo tissues after (^{3}H) thymidine injection

Embryo's age (days)	Dose (μCi/1 egg)	Time of incubation (hrs)	Tissue	Radioactivity of DNA (μCi)	Per cent of the total radioactivity (μCi)	Specific activity (imp/min/ /μg DNA)
9	50	13	brain	0.14	0.28	144.0
			liver	0.12	0.24	400.0
9	60	13	brain	0.19	0.31	196.0
			liver	0.19	0.31	633.0
12	40	5	brain	0.03	0.07	25.0
			liver	0.07	0.17	56.0
12	80	5	brain	0.08	0.10	66.0
			liver	0.14	0.18	113.0

was found in brain nuclei DNA and 0.18% in liver nuclei DNA but after 13 hours the values became equalized to about 0.3%. However taking into consideration the fact that the 13 hours incubation dealt with 9-day old embryos whose DNA content in liver is three times lower than in the brain the values obtained from radioactivity measurements clearly indicate that liver DNA metabolism in this period of embryogenesis is more dynamic than that the metabolism of brain DNA. Higher specific activities of liver nuclear DNA confirm this conclusion.

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STRESZCZENIE

Badania przeprowadzone na embrionach kurzych wykazały, że między 9 a 12 dniem embriogenezy synteza DNA wątrobowego przebiega o wiele intensywniej od syntezy DNA tkanki mózgu.

Niezależnie od czasu inkubacji ilość zainkorporowanej (^3H) tymidyny do DNA jądrowego nie przekracza 0,3% wstrzykniętej dawki, przy czym aktywność właściwa DNA wątrobowego przewyższa 2—3 razy aktywność DNA mózgowego.

РЕЗЮМЕ

На основе опытов, проведенных на куриных эмбрионах определено, что синтез DNA печени происходит между 9—12 днем эмбриогенеза и является более интенсивным, чем синтез DNA в мозговой ткани.

Независимо от времени инкубации количество включенного (^3H) тимидина не превышает 0,3% исходной радиоактивности; при чём удельная активность DNA печени превышает 2—3 раза активность DNA мозга.