EFFECT OF THE ANTIFOLATE PREPARATION PYRIMETHAMINE ON THYMIDYLATE SYNTHETASE ACTIVITY IN THE ANTIMESOMETRIAL AND MESOMETRIAL PARTS OF THE DECIDUAL TISSUE

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The thymidylate synthetase (TS) from the decidual tissue and embryos of rats has a molecular weight of 58,000, a pH-optimum of 6.9, and is rapidly inactivated on heating. In the antimesometrial (A-part) and the mesometrial (M-part) parts of the decidual tissue of rats on the 9th-11th day of pregnancy the profile of specific TS activity and the response of this parameter to injection of the antifolate drug pyrimethamine into the rats were investigated. Cells of the A- and M-parts were found to behave differently. The specific TS activity in the A-part was higher than in the M-part and reached the maximum on the 10th day of pregnancy. Specific TS activity in the M-part fell steadily during the period of investigation. Administration of pyrimethamine on the 9th day of pregnancy caused a wave of increase in TS activity in the A-part, whereas in the M-part the specific TS activity rose slowly.

Key words: thymidylate synthetase of decidual tissue; rat embryos; pyrimethamine as an antifolate agent.

The formation of decidual tissue is an essential factor of implantation and has been well studied morphologically [4, 7]. Meanwhile, however, the molecular mechanisms of decidualization have received little study and the biochemical basis of transformation of the endometrium into decidual cells is not clear.

The test object in this investigation consisted of decidual tissue from rats on the 9th-11th day of pregnancy. Proliferating tissues are characterized by the presence of thymidylate synthetase (TS) activity [5]. This enzyme catalyzes the formation of thymidine monophosphate and at this stage it is able to limit the rate of DNA synthesis [5].

The object of the investigation was to measure the specific TS activity in the antimesometrial (A-part) and mesometrial (M-part) parts of the decidual tissue and also the changes in this parameter on administration of the antifolate agent pyrimethamine [9]. Some physicochemical properties of TS from the decidual tissue and embryos of rats also were studied.

TABLE 1. Specific TS Activity in Decidual Tissue of Rats on 8th-11th Day of Pregnancy (M ± m)

Day of pregnancy	Specific TS activity (in nmoles substrate/mg protein/30 min)		
	whole decidual tissue	A-part	M-part
8th	2,6± 0,2	—	—
9th	2,7± 0,1	2,9± 0,1	2,25± 0,2
10th	2,5± 0,05	4,6± 0,2	1,9± 0,1
11th	1,5± 0,1	2,5± 0,1	1,3± 0,1

EXPERIMENTAL METHOD

Experiments were carried out on albino rats. The first day of pregnancy was taken to be the day on which spermatozoa were found in vaginal smears from the females. In the experiments with pyrimethamine the drug was given in a dose of 25 mg/kg through a gastric tube, once only, in the form of a suspension in a 20% aqueous solution of Tween-20. The A- and M-parts of the decidual tissue were separated in the cold under a binocular loupe; the embryo with the

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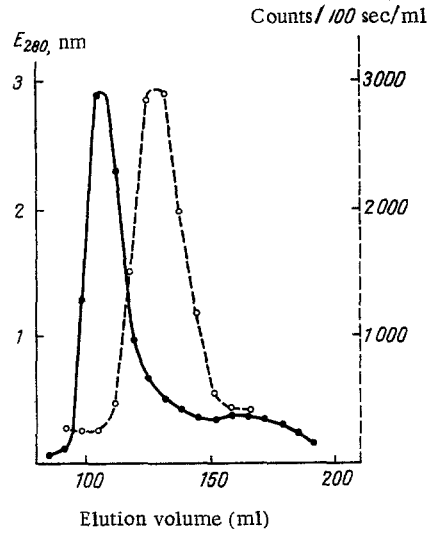

Fig. 1

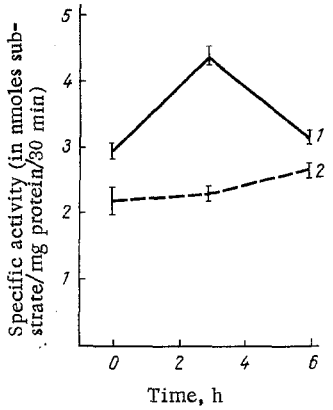

Fig. 2

Fig. 1. Elution profile of TS on gel-filtration.

Fig. 2. Effect of pyrimethamine on specific TS activity in rat decidual tissue: 1) antimesometrial part; 2) mesometrial part.

ectoplacental cone was not used in the work.

Specific TS activity was determined as described earlier [1], but instead of Tris-buffer, phosphate buffer [8] was used in the incubation medium to block the alkaline phosphatase present in the decidual tissue [10]. Formaldehyde-C^{14} with a specific activity of 2.6×10^6 counts/min/μmole and columns of Dowex 1×8 (200-400 mesh) in the formate form, measuring 1×3 cm, were used. The tetrahydrofolate was from Sigma (USA). The results described are mean values from two independent experiments, in each of which 50-100 implantation sites were used.

The molecular weight of the TS was determined by gel-filtration through a column of Sephadex G-100 (2.5×47 cm), calibrated by means of blue dextran and marker proteins. The preparation was applied to the column in a volume of 5 ml and elution carried out with 0.05 M K-phosphate buffer, pH 7.2, containing 0.05 M mercaptoethanol, at the rate of 50 ml/h.

EXPERIMENTAL RESULTS AND DISCUSSION

The study of the dependence of TS activity in the M-part of the decidual tissue on the pH of the medium showed that the pH-optimum was 6.9. The same pH-optimum was found for preparations from the A-part and also for TS from the soluble fraction of rat embryos at the 14th day of development (salting out with ammonium sulfate, TS activity concentrated in the 45-85% saturation fraction). Gel-filtration experiments (Fig. 1) showed that the molecular weight of the TS from the decidual tissue and embryos of the rats was $58{,}000 \pm 2000$. The thermolability of the TS was investigated in preparations of the enzyme from decidual tissue concentrated after gel-filtration with the aid of dry Sephadex G25. After heating for 10 min at 45°C, 39% of the original activity remained.

The results indicate that, with respect to the parameters studied, TS from decidual tissue is indistinguishable from the embryonic type and that rat TS is similar in its characteristics to the enzyme studied from other objects [5, 11].

Measurement of the specific TS activity in the soluble fraction of homogenates from the A- and M-parts of the decidual tissue of rats at the 9th-11th days of pregnancy (Table 1) showed that on the 10th day the TS activity was much higher in the A-part than in the M-part. TS activity in the M-part fell steadily during the period of the investigation. Since the M-part is approximately twice as large as the A-part, when the decidual tissue was used as a whole, no increase in the specific TS activity in the A-part could be detected. The decrease in TS activity observed in the A-part from the 10th to the 11th day and in the M-part earlier still probably reflected the subsequent morphological involution of the decidual tissue.

The M- and A-parts of the decidual tissue also differed in their response to the antifolate drug pyri-

methamine. Like other inhibitors of dihydrofolate reductase [9], pyrimethamine induces a tetrahydrofolate deficiency in proliferating tissues and thereby blocks the reaction in which TS participates [5]. As will be clear from Fig. 2, administration of pyrimethamine to rats on the 9th day of pregnancy caused an increase in TS activity in the decidual tissue. In a dose of 25 mg/kg, a sharp rise of activity was observed initially in the A-part only. TS activity in the A-part did not increase further 3 h after the injection of pyrimethamine and started to return to normal. After 24 h the experimental and control samples (A-part on the 10th day) were indistinguishable from each other. Meanwhile in the M-part administration of pyrimethamine led to a slow rise in TS activity, so that after 24 h it was 63% above normal.

The results of this investigation showed that the A- and M-parts of the decidual tissue have definite biochemical differences. Cells of the A-part, unlike those of the M-part, are polyploid, with many nucleoli [3]. The increased content of TS in the cells of the A-part possibly indicates that DNA synthesis takes place more intensively in these polyploid cells during the period of the investigation than in cells of the M-part, and probably for that reason they were more sensitive to pyrimethamine. The increase in specific TS activity in the A-part observed under these circumstances was probably due to the arrest of DNA synthesis, with a consequent increase in the population of cells in the S-phase of the cell cycle. TS activity is known to rise sharply in cells in the S-phase [6]. The change in the specific TS activity in the A-part coincided in time with another effect of pyrimethamine, which the writers found previously on embryonic tissues: an accumulation of deoxyuridine monophosphate, the substrate of the TS reaction [2]. The disappearance of the action of pyrimethamine on TS after 6 h was most probably due to the accumulation of dihydrofolate which, as the substrate for dihydrofolate reductase, abolishes the action of its inhibitor. Methotrexate, an even stronger dihydrofolate reductase inhibitor, inhibits the reaction of reduction of dihydrofolate in the tissues for a much shorter time than it persists there [12].

The results indicate that in order to understand the molecular mechanisms of decidualization and its functional role and also to analyze the action of inhibitors on decidual tissue, biochemical differences between cells of the A- and M-parts must be taken into account.

LITERATURE CITED

1. B. L. Vaisman and V. S. Repin, Biokhimiya, 38, 375 (1973).
2. B. L. Vaisman, Ontogenez, No. 3, 299 (1974).
3. E. V. Zybina and T. A. Grishchenko, Tsitologiya, 14, 284 (1972).
4. P. G. Svetlov and G. F. Korsakova, Dokl. Akad. Nauk SSSR, 103, 503 (1955).
5. R. L. Blakley, The Biochemistry of Folic Acid and Related Pteridines, Amsterdam (1969).
6. A. H. Conrad, J. Biol. Chem., 246, 1318 (1971).
7. V. J. De Feo, in: R. M. Wynn (editor), Cellular Biology of the Uterus, New York (1967), p. 191.
8. A. Garen and C. Levinthal, Biochim. Biophys. Acta, 38, 470 (1960).
9. G. H. Hitchings, Fed. Proc., 26, 1078 (1967).
10. B. L. Lobel, L. Tic, and M. C. Shelesnyak, Acta Endocrinol. (Copenhagen), 50, 517 (1965).
11. M. Y. Lorenson, G. F. Maley, and Fr. Maley, J. Biol. Chem., 242, 3332 (1967).
12. L. C. Mishra, A. S. Parmar, and J. A. R. Mead, Analyt. Biochem., 48, 515 (1972).