ACTION OF SOME PYRIMIDINE DERIVATIVES ON EARLY STAGES OF CHICK EMBRYOGENESIS

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Pyrimidine derivatives differ in their teratogenic and harmful action on chick embryos. In order of diminishing activity these substances may be arranged in the following order (by groups): triazines (5-azauracil, potassium salt of 2,6-dihydroxytriazinecarboxylic acid) and 6-thiouracil; 4-methyluracil and orotic acid; uracil, isouracil, and pentoxyuracil; citrazinic acid.

* * * *

Methacil (4-methyluracil), pentoxil (pentoxyuracil), and certain other pyrimidine derivatives are widely used in clinical practice to stimulate regeneration, to regulate the course of inflammatory processes and, finally, as antileukemic and antitumor drugs [2-6].

However, the mechanism of action of pyrimidine derivatives on growth, multiplication, and differentiation of cells has received little study. Animal embryos, including chick embryos, are a suitable biological model for investigating the action of various pharmacological substances.

Few studies of the effect of pyrimidine derivatives on animal embryogenesis have been published and there is no information on the effect of uracil and its analogs on embryogenesis of the chick. It has been concluded from investigations of the action of physiological pyrimidine nucleotides on development of the chick that the sensitivity of the embryo to these substances is due to its inability to cause breakdown of these compounds [9]. Nucleotides not occurring in nature have also been shown to inhibit embryogenesis of the chick [7, 11].

In the present investigation the effect of uracil and some of its analogs on embryogenesis of the chick was studied.

EXPERIMENTAL METHOD

Fertilized hens' eggs (Russian White breed) were used in the experiments and were incubated at 38° and 60% relative humidity. The substances were injected into the yolk in doses of between 0.25 and 4 mg on the 1st day of incubation. In control tests the solvent was injected in the same volume as in the experimental series (0.1 ml). The results were read on the 7th day, because by this time all the principal processes of organogenesis are complete.

EXPERIMENTAL RESULTS

The experiments showed that the agents tested differ in their action on embryogenesis of the chick (Table 1). The teratogenic action of uracil, isouracil, and pentoxyuracil was approximately identical and weak, increasing with an increase in dose of the agent. Few embryos died as a result of the action of these agents, and with an increase in dose the number varied within narrow limits, close to the control level (Table 1). These compounds produced various developmental anomalies, of which the most frequent were malformations of the eyes (anophthalmia, microphthalmia), reduction of the tail, deformation of the trunk axis, and retardation of development. Following the action of small doses of uracil and isouracil (0.25-

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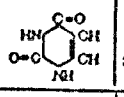
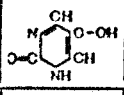
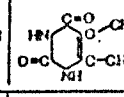
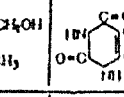
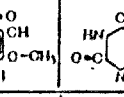
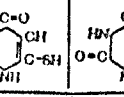
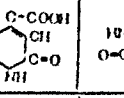
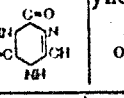
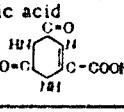
TABLE 1. Teratogenic and Lethal Action of Uracil and Its Analogs on Chick Embryogenesis

Dose (in mg)		Uracil 	Isouracil 	Pentoxyuracil 	4-Methyl-uracil 	6-Thio-uracil 	Orotic acid 	5-Azauracil 	Potassium salt of triazinecarboxylic acid 	Citrazinic acid 	Control (0,1 ml solvent)
0,25	I	0	—	—	4,2±2,8	—	—	8,0±5,3	—	—	5,9±2,4
	II	10,6±5,5	—	—	17,7±5,0	—	—	16,6±6,7			19,2±3,7
0,5	I	0	2,8±2,8	11,6±4,8	15,2±5,3	6,2±4,1	3,0±3,0	25,8±7,8	7,1±4,7	0	
	II	19,2±5,6	16,0±5,0	18,8±5,3	15,6±4,2	27,2±7,0	17,5±6,0	32,0±7,0	24,3±2,8	7,1±4,6	
1	I	3,7±2,6	10,0±5,4	15,0±5,5	29,5±8,6	19,2±7,6	8,0±5,6	31,0±8,5	18,5±5,0	0	
	II	10,0±3,8	19,0±5,6	15,1±4,6	49,0±6,2	41,0±7,4	32,0±7,7	38,0±7,0	28,7±7,3	12,0±6,6	
2	I	10,0±4,2	26,1±9,1	18,3±6,1	29,5±8,6	44,0±9,8	21,0±10,4	75,5±6,4	35,4±9,1	—	
	II	16,6±4,8	25,0±6,7	22,4±4,6	56,0±5,2	45,0±7,1	42,0±8,6	53,6±5,0	46,0±7,5	—	
4	I	23,8±6,5	11,8±5,0	24,1±8,0	25,0±8,8	66,6±7,6	28,0±10,5	85,7±9,6	46,1±9,7	12,5±5,2	
	II	22,2±5,6	25,6±6,7	23,6±6,7	61,3±6,1	50,0±7,3	50,0±7,0	63,1±8,1	51,8±2,6	20,0±7,2	

Legend: I) teratogenic action (number of malformed embryos in percent of number of living); II) lethal action (number of dead embryos as percent of total number of embryos).

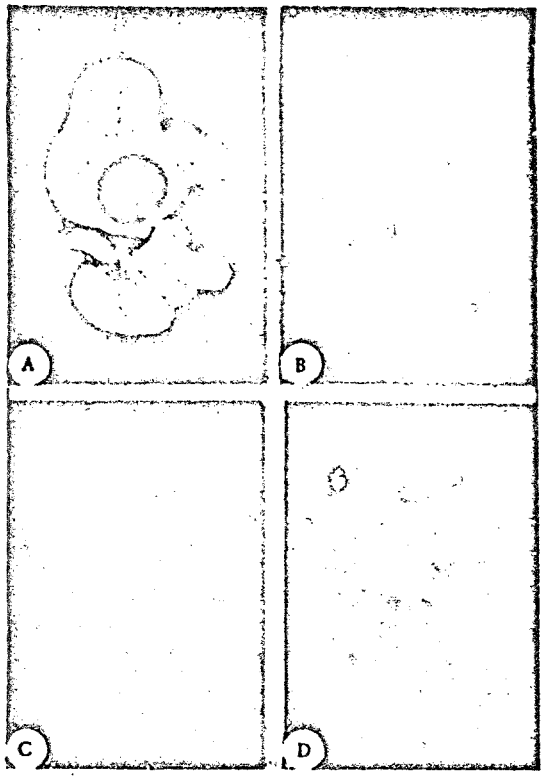


Fig. 1. Teratogenic action of pyrimidine derivatives. A) Control 7-day embryo; B) 7-day embryo after injection of 2 mg 6-thiouracil: obliteration of cerebral vesicles, deformation of beak, ectocardia, eventration of abdominal viscera; C) 7-day embryo after injection of 2 mg 5-azauracil: microphthalmia, deformation of beak, under development and deformation of wings, fusion of the limbs ("mermaid"); D) 7-day embryo after injection of 4 mg 5-azauracil: microphthalmia, under development and deformation of beak and wings, reduction of tail, micromelia with deformation, ectrodactyly.

0.5 mg), some embryos showed characteristic signs of an older age – well-developed digits of the limbs, well marked optic papillae – which are not normally observed in 7-day embryos.

6-Thiouracil and, in particular, 5-azauracil possess much greater teratogenic and lethal activity than uracil, isouracil, and pentoxyuracil. These substances caused severe and multiple malformations. The commonest of these were delay in development, failure of fusion of the neural tube, malformations of the eyes and cerebral vesicles (cerebral hernias, obliteration of the cerebral vesicles), reduction of the tail, various developmental defects of the limbs, eventration of the abdominal viscera (Figs. 1A-D). Malformations caused by the action of the potassium salt of triazinecarboxylic acid in most cases were similar to those observed by the action of azauracil.

Citrazinic acid exhibited only slight teratogenic and lethal activity (Table 1).

Analysis of the results of the 7th day of development showed that following the action of orotic acid and 4-methyluracil malformations were found mainly among the dying embryos (these were not considered when the experimental results were recorded). It was therefore interesting to study the action of 4-methyluracil and orotic acid on embryos incubated by the open method. At certain time intervals (6, 12, 24 h, and so on) after treatment with 4-methylthiouracil and orotic acid the embryos were examined and the number of somites counted under a binocular loupe with a point source of light. The embryos continued to develop normally during the first 4-6 h after injection. Delay in development was found after 6-12 h, followed by complete arrest of development in some embryos, a decrease in the number of somites and in all dimensions of the body compared with the control, and a poorly developed vascular system; development of the cerebral vesicles and neural tube was disturbed. In most such embryos the somites were irregularly rhomboid in shape, and in some cases several somites were separated by areas of undifferentiated mesoderm. The highest percentage of surviving malformed embryos was noted on the 2nd day after treatment. Nearly all the embryos retarded in their development died at the end of the 4th day or on the 5th day.

The results of these investigations demonstrate a definite link between chemical structure and character of action of the pharmacological agents used in the experiment. Whereas citrazinic acid (the pyridines) exhibits only slight activity on the chick embryo, the pyrimidines, especially orotic acid, give a much higher mortality and exhibit higher teratogenic activity. The triazines possessed the most harmful action on the developing embryo. The potassium salt of triazinecarboxylic acid exhibited higher teratogenic activity than orotic and citriazinic acids and caused death of a higher proportion of embryos (Fig. 2).

The differences in harmful activity of these substances may evidently be attributed to differences in their ability to influence nucleic acid synthesis in the embryonic cells, thereby inhibiting these cells. Thiouracil, for instance, which in our experiments showed high teratogenic activity, is capable of taking part in RNA synthesis and replacing up to 20% of the normal uracil. A second highly active preparation, 5-azauracil, by blocking the decarboxylase of orotidylic acid, prevents synthesis of orotic acid. The harm-

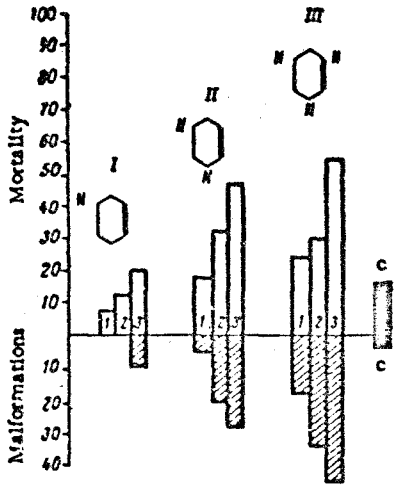


Fig. 2. Relationship between teratogenic and lethal action of citrazinic (I) and orotic (II) acids and the potassium salt of 2,6-dihydroxytriazinecarboxylic acid (III). Ordinate: percentage of malformed and dying embryos. Abscissa, dose of substance injected: 1) 0.5 mg; 2) 1 mg; 3) 4 mg; c) control.

ful activity of orotic acid and 4-methyluracil, as of all the other agents used in these experiments, may be connected with the ability of these substances to act as antimetabolites during the synthesis of nucleic acids [1, 8, 10].

The results of investigations on the chick embryo can thus be used for the further analysis of the mechanism of action of pyrimidine derivatives.

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