TERATOGENIC ACTION OF AMINOPTERIN AND 5-FLUOROURACIL ON CHICK EMBRYOS OF 4-23 SOMITES WHEN APPLIED IN OVO

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An important method of investigation of teratogenesis is by modifying morphogenesis, which may be done by disturbing the specific biochemical processes in early embryogenesis with the aid of antagonists of metabolism, i.e., of chemical teratogens [1, 2, 4].

The object of the present investigation was to study the teratogenic reactions of the chick embryo by using aminopterin and 5-fluorouracil, the mechanism of whose teratogenic action has been adequately explained as by depressing the synthesis of nucleic acids [5-10].

EXPERIMENTAL METHOD

Experiments were carried out on 1- or 2-day chick embryos (4-23 pairs of somites). Pieces of ash-free filter paper measuring 1 × 1 mm were soaked in solutions of aminopterin (0.032%) or 5-fluorouracil (0.1%) and applied for 10 min to the postsomite areas of the embryos. After this treatment the eggs were again incubated until the end of 7 days. All the developmental anomalies observable visually in the surviving embryos and also in those dying on the 5th-7th days of incubation were recorded.

EXPERIMENTAL RESULTS

Aminopterin and 5-fluorouracil produced various developmental anomalies in the embryos, the principal of which were microphthalmia and malformations of the trunk and limbs (Table 1).

In some cases the developmental anomalies of the eyes took the form of a moderately severe microphthalmia, i.e., the eyes were simply reduced in size but were correctly subdivided into their component parts. In other cases, besides a decrease in the size of the eyes, their structure was grossly disturbed. On visual inspection these eyes had the appearance of a small formation bounded by rudimentary eyelids, in the depth of which could be seen a pigmented lump (Fig. 1a). On section the rudimentary structure of the eye could be seen (Fig. 1b). The optic vesicle ceased to develop, evidently, soon after application of the substance, and its anterior segment invaginated only partially or not at all. The cavity of the optic vesicle communicated with the cavity of the brain; in the 7-day embryos the cells of the optic vesicle contained pigment. The lens, most frequently appearing as an irregular vesicle, lay in a small cavity separately from the optic vesicle. The gross disturbances of development of the optic vesicle and lens did not, however, prevent the formation of the eyelids and the convexity of the anterior part of the rudimentary eye. Such eyes may be classes on the whole as "rudimentary". Following the application of aminopterin mainly a moderately severe microphthalmia developed, while fluorouracil caused the formation mainly of "rudimentary" eyes.

TABLE 1. Teratogenic Action of Aminopterin (AM) and 5-fluorouracil (FU) on Chick Embryos of 4-23 Somites (results obtained at end of 7th day of incubation)

	Embryos			
	4-13 somites		14-23 somites	
Substance	AM	FU	AM	FU
Total	21	26	51	94
With malformations (in %)	67±10,2	81±7,7	43±7,0	56±5,2
of the head (microphthalmia)	33±10,2	38±9,5	10±4,2	7±2,6
ot the eyes (micro-	57±10,8	64±9,5	27±6,2	25±4,4
(microphthalmia)	52±10,9	50±9,8	30±6,4	56±5,2
of the trunk and tail	38±10,6	46±9,8	30±6,4	15±3,6
of wings, lower limb	43±10,8	32±9,1	27±6,2	31±4,8

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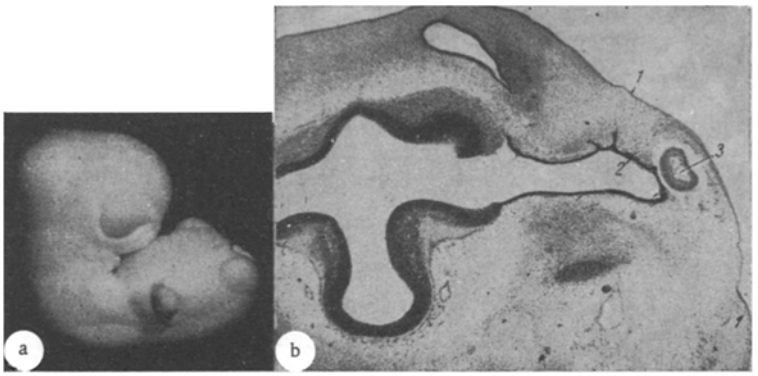

Fig. 1. A 7-day chick embryo after treatment with aminopterin. a — General appearance. 4.5×; b — section at the level of the "rudimentary" eyes: 1) rudiments of lids; 2) pigmented wall of the optic vesicle; 3) lens. Fixation in Bouin's fluid. Hematoxylin-eosin. 35×.

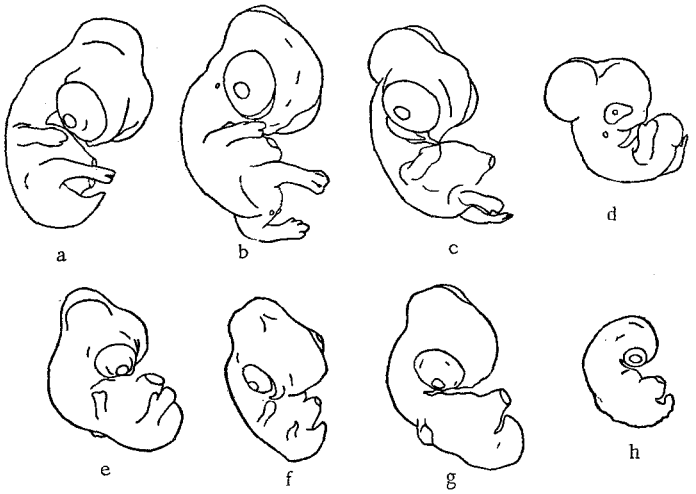

Fig. 2. Comparison of malformations arising by the 7th day of incubation following application of 5-fluorouracil (b-d) and aminopterin (e-h) to chick embryos at the stage of 4-23 somites. a — control. Drawn from photographs. Explanation in text.

In the experiments with aminopterin the trunk was nearly always completely formed as far as the tip of the tail (Fig. 2e-h), but often it was shortened, curved, covered with tubercles or swellings; occasionally developmental defects of the tail were present. After treatment with 5-fluorouracil the malformations of the trunk were more severe than in the experiments with aminopterin and were characterized reduction of its caudal part: in relatively mild cases the tip of the tail was absent, but in more severe cases the tail was rudimentary up to the level of the lower limbs (Fig. 2b, c), and in the severest cases the trunk failed completely to develop below a level a little behind the alar region (Fig. 2d).

Following treatment with aminopterin and malformations of the limbs were the most severe and obvious defects. The wings and legs consisted of short, pointed projections or very thin threads, and often they were absent altogether (Fig. 1a and Fig. 2e-h). In contrast to the changes observed after application of aminopterin, the developmental disturbances of the limbs caused by 5-fluorouracil, although frequently seen, were much milder and were always combined with severe developmental disturbances of the trunk (which failed to develop from a level corresponding to the rostral border of the rudimentary hind limbs).

In these cases the hind limbs were usually absent, but if they did develop they differed very little in size and shape from the control, and their only developmental defects were minor anomalies in their distal parts: imperfect formation of the digits, irregularly indented edges, and other defects. The developmental disturbances of the wings were confined entirely to defects of their distal parts.

This investigation of the action of aminopterin and 5-fluorouracil on the chick embryo also showed that the sensitivity of the embryos to these substances depends, as is reported in the literature in the case of other harmful agents [3], on the stage of development at the moment of application: the younger embryos are always more sensitive (see Table 1). The teratogenic action of the agents used is certainly connected with the nature of their biochemical action: aminopterin, by disturbing the conversion of folic acid into its reduced forms essential for nucleic acid synthesis indirectly depresses the synthesis of DNA [9], while 5-fluorouracil, which blocks thymidylate synthetase, depresses DNA synthesis directly [6]. However, these properties alone cannot explain the different reactions of the embryos. Why, it may be asked, when embryos are treated at the same periods of development, should the limbs be mainly affected in one case (aminopterin) and the caudal segments of the trunk in the other (5-fluorouracil)? This selective sensitivity is evidently connected not only with the biochemical action of these substances, but also with the m morphological and physiological features distinguishing the development of these analgen in the different periods of embryogenesis.

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