EFFECT OF 2,4,5-TRICHLOROPHENOXYACETIC ACID AND ITS BUTYL ESTER ON EMBRYOGENESIS OF RATS

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The teratogenic action of 2,4,5-trichlorophenoxyacetic acid and its butyl ester was studied. Preparations of the acid itself and its butyl ester were shown to be highly specific teratogens inducing anomalies of uniform type: cleft palate, hydronephrosis, brachydactylia, and gastrointestinal hemorrhages. In the case of the butyl ester of 2,4,5-trichlorophenoxyacetic acid these changes were more marked. It is postulated that the teratogenic action of the compounds is due to the presence of dioxide.

The teratogenic action of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been described in experiments on two strains of mice, rats, and hamsters [3, 4]. The compound 2,3,7,8-tetrachlorodibenzo-p-dioxine, present as an impurity in the preparations of 2,4,5-T, also had a teratogenic action on rat embryos [2, 6]. It is not yet clear whether pure 2,4,5-T possesses teratogenic properties or its teratogenic action is due to the presence of the dioxide.

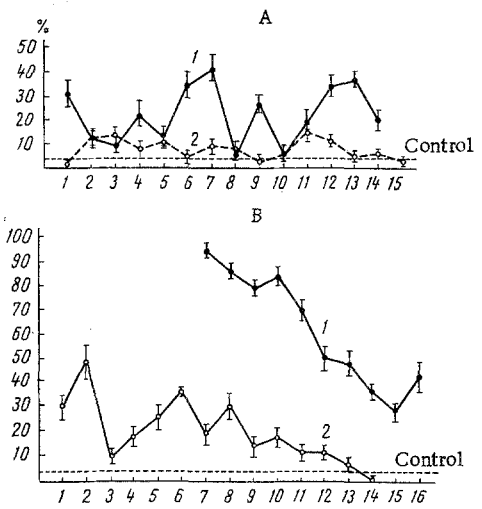

Fig. 1. Relationship between embryotoxic action of the compound and time of its administration. Abscissa, days of pregnancy; ordinate, dosed implantation mortality of embryos (in %); A) compound 2,4,5-T; 1) dose 400 mg kg; 2) dose 100 mg/kg; B) butyl ester of 2,4,5-T; 1) dose 200 mg/kg; 2) dose 50 mg/kg.

Experiments were carried out to study the teratogenicity of 2,4,5-T and its butyl ester.

EXPERIMENTAL METHOD

Experiments were carried out on 513 experimental and 73 control rats obtained from the Rappolovo Nursery. Two preparations of 2,4,5-T were tested: one was synthesized at the Ufa Chemical Factory, the other at the Institute of Toxicology, Ministry of Health of the USSR.* The butyl ester of 2,4,5-T consisted of a 42% emulsion with a filler. The 2,4,5-T was tested as an aqueous emulsion with the addition of Tween-20 and Tween-40 emulsifiers in doses of 100 and 400 mg/kg, and the butyl ester of 2,4,5-T was given in doses of 50 and 200 mg/kg (calculated as butyl ester). The preparations were given as a single dose from the first to the fourteenth or the first to the sixteenth days of pregnancy through a gastric tube.

The first day of pregnancy was determined from the presence of spermatozoa in vaginal smears. The pregnant females of the experimental and control groups were examined on the nineteenth-twentieth day of pregnancy. The number of living and dead

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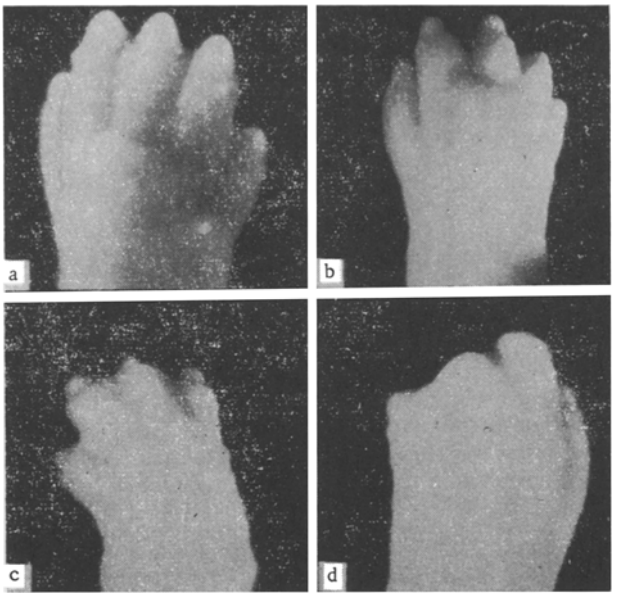

Fig. 2. Types of brachydactylia arising after administration of the butyl ester of 2,4,5-T in a dose of 200 mg/kg: a) normal limb, b, c, d) examples of brachydactylia.

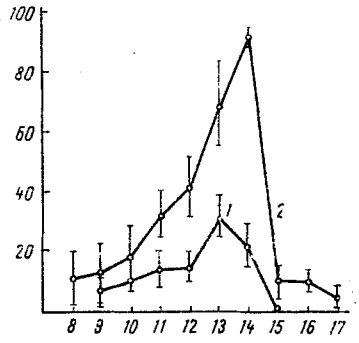

Fig. 3. Teratogenic activity of butyl ester of 2,4,5-T in a dose of 200 mg/kg as a function of time of administration. Abscissa, days of pregnancy; ordinate, percentage of deformed embryos; 1) incidence of brachydactylia; 2) incidence of cleft palate.

fetuses, of implantation sites with resorbed embryos, and the number of corpora lutea in the ovaries were counted. The embryos were freed from fetal membranes, examined with the unaided eye to determine any external anomalies, and then fixed in Bouin's fluid for a study of the state of the internal organs by Wilson's method as modified in the Department of Embryology, Institute of Experimental Medicine [1].

EXPERIMENTAL RESULTS

After administration of 2,4,5-T in a dose of 100 mg/kg the embryonic mortality in the early period of pregnancy was low. An increase in the dose to 400 mg/kg increased the embryotoxic effect (Fig. 1A).

In this series of experiments solitary fetuses with external deformities were found. In a dose of 100 mg/kg, 2,4,5-T produced embryos with a combination of deformities: absence of the lower jaw, changes in the hind limbs, and exophthalmos. After administration of 2,4,5-T in a dose of 400 mg/kg one embryo was found with tridactyly of the upper limb combined with syndactyly; and another embryo had brachydactylia of the upper limb.

Investigation of the state of the internal organs after treatment with 2,4,5-T revealed single cases of cleft palate, hydrocephalus, and hydronephrosis. The butyl ester of 2,4,5-T proved to be much more toxic for the embryos (Fig. 1B). After its administration in a dose of 200 mg/kg from the first to the seventh days of pregnancy most of the embryos died, so that only single embryos were obtained at autopsy. In some animals all the embryos had died, while in others pregnancy had evidently been interrupted in the early stages of development, so that at the ordinary times of autopsy no corpora lutea of pregnancy or implantation sites could be found. For these reasons it was impossible to establish the dynamics of the postimplantation mortality of the embryos in this series of experiments before the seventh day of development. However, the embryonic mortality exceeded 30% at the end of organogenesis. The butyl ester of 2,4,5-T caused high mortality among the embryos in a dose of 50 mg/kg also.

External deformities induced by the butyl ester of 2,4,5-T in a dose of 200 mg/kg from the ninth to the fourteenth day of pregnancy consisted of a uniform anomaly of the limb – brachydactylia.

It is striking that only the hind limbs were affected in all embryos with brachydactylia except one.

The degree of involvement of the digital phalanges varied considerably in different embryos: from shortening to total absence (Fig. 2). The butyl ester of 2,4,5-T in a dose of 50 mg/kg caused no deformities of the limbs.

Furthermore, the butyl ester of 2,4,5-T induced cleft palate, hydronephrosis, hydrocephalus, and extensive gastrointestinal hemorrhages. Most embryos with brachydactylia had cleft palate. When the dose of the compound was reduced to 50 mg/kg, cleft palate was found only occasionally.

The relationship between the frequency of cleft palate and brachydactylia induced by the butyl ester of 2,4,5-T in a dose of 200 mg/kg and the time of administration of the compound is illustrated in Fig. 3.

It can be concluded from the results of these experiments that the butyl ester of 2,4,5-T has a highly specific teratogenic action. Comparison of the embryotoxic and teratogenic effects of the two preparations studied reveal only quantitative differences. The teratogenic and embryotoxic activity of the preparation of the butyl ester of 2,4,5-T was stronger than that of the preparation of 2,4,5-T.

The identical teratogenic action of the two preparations is probably attributable to the presence of dioxine, while the quantitative differences between the effects are attributable to differences in the content of the dioxine. This hypothesis is supported by the fact that in experiments on rats of the Sprague-Dawley strain and on New Zealand rabbits a preparation of 2,4,5-T with a reduced dioxine content had only an embryotoxic action and the teratogenic effect was absent [5]. In addition, identical effects have been described for preparations of 2,4,5-T with a high content of dioxine and the pure dioxine [4, 7].

The results of the present experiments, like those described in the literature, suggest a high teratogenic activity of preparations of the 2,4,5-T group.

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