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EFFECT OF VULCANIZATION ACCELERATORS ON EMBRYONIC MORTALITY IN RATS

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KEY WORDS: vulcanization accelerators; embryonic mortality; mutagens.

Vulcanization accelerators (VA) are an essential component of any rubber mix. Representatives of the chief classes of VA were chosen for study: from the thiazoles — captax and altax; from the sulfenamides — santocure and santocure-mor; and from the thiurams — thiurams D and E. Data on the effect of VA on fetal development in animals are not available, except for thiuram D [2, 4]. Depending on the aims of their investigations, different workers have used different terminologies and methods of exposure of the animals and have thus strictly speaking examined different aspects of disturbances of reproductive function. It is difficult at present to state what is the primary mechanism (mutagenic action on gametes or blastomeres at the beginning of embryogenesis, toxic action of the chemical on the embryo, or hormonal disturbances in the mother) in the process of injury to or death of the fetus. Depending on the time of exposure, the same factors may be both mutagenic and embryotoxic. There is a definite parallel in the action of these factors [1, 3, 5].

The object of this investigation was to study the level of embryonic mortality (EM) in noninbred albino rats after administration of VA.

EXPERIMENTAL METHOD

VA were administered to noninbred albino rats by two methods: I) to females before the beginning of pregnancy on the 1st and 3rd days of estrus, to males twice at an interval of 3 days (i.e., dominant lethal mutations were induced [5-7]); II) to females on the 4th and

TABLE 1. Characteristics of Groups of Experimental Animals

VA	Number in group			
	females	corpora lutea	resorption sites	living fetuses
Control	25	347	15	309
Administration before pregnancy				
Captax	11	175	14	123
Altax	11	170	33	74
Santocure	10	163	5	120
Santocure-mor	11	167	10	130
Thiuram D	11	181	8	137
Thiuram E	11	173	9	127
Administration during pregnancy				
Captax	12	183	12	146
Altax	15	238	27	148
Santocure	13	181	19	142
Santocure-mor	12	196	11	126
Thiuram D	12	177	10	130
Thiuram E	12	181	12	134

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TABLE 2. Embryotoxic Action of VA (M ± m)

Parameter studied	Control	VA					
		captax	altax	santocure	santocure-mor	thiuram D	thiuram E
Administration before pregnancy							
Total EM, %	10,9±1,6	29,7±3,4	56,4±3,8	26,4±3,4	22,1±3,2	24,3±3,2	26,0±3,3
Postimplantation EM — "index of mutagenicity," %	4,6±1,1	10,2±2,5	30,8±4,4	4,0±1,7	7,1±2,1	5,5±1,8	6,6±2,1
Duration of estrous cycle, days	4,4±0,3	5,7±0,3	6,9±0,7	4,5±0,3	5,4±0,1	5,5±0,4	5,8±0,5
Speed of onset of conception, cycles	1,0	1,6±0,2	1,2±0,2	1,0	2,3±0,3	1,4±0,2	1,7±0,2
Administration during pregnancy							
Total EM, %	10,9±1,6	20,2±2,9	37,8±3,2	21,6±3,3	35,7±3,4	26,6±3,3	25,9±3,2
Postimplantation EM, %	4,6±1,1	7,6±2,1	15,4±2,7	11,8±2,5	8,1±2,3	7,1±2,1	8,2±2,2

11th days of pregnancy; the males were left intact. The VA were injected into the stomach of the rats, dissolved in sunflower oil, in the following doses: captax, altax, and santocure-mor 200 mg/kg each, santocure 2000 mg/kg, thiurams D and E 100 mg/kg (each group consisted of 10 or more females [6]). The same volume of sunflower oil was given to the control animals. Phases of the estrous cycle and the beginning of pregnancy were determined from the ratio of the cells in a vaginal smear and from the presence of spermatozoa. The rats were killed on the 19th day of pregnancy. The number of corpora lutea in the ovaries and the number of dead and living fetuses in the uterine cornua were counted; the fetuses were removed, weighed, and measured (Table 1).

EM for the group of animals (total, pre-, and postimplantation), fertility, and in the case of rats receiving VA before the onset of pregnancy, changes in the duration of the estrous cycle and the rate of onset of conception (estimated from the number of cycles after poisoning during which conception did not take place despite the presence of spermatozoa in the vagina), were calculated for all females [5, 6]. The significance of differences was determined by methods of variance and dispersion analysis (t and F tests).

EXPERIMENTAL RESULTS

Injection of VA into both nonpregnant and pregnant females produced no visible signs of poisoning in the animals. In females receiving the compounds during estrus, changes occurred in the estrous cycle and conception did not always take place in the next cycle. The greatest lengthening of the cycle was produced by altax (up to 6.9 ± 0.7 days), and the greatest delay to conception was produced by santocure-mor (up to 2.3 ± 0.3 cycles). All females showed a significant decrease in the weight of the fetuses: from 3% (thiuram E) to 23% (captax). Fertility (the number of living fetuses per female) was significantly lowered by 46 and 30% after administration of altax before and during pregnancy respectively. The total EM increased significantly after administration of VA to both nonpregnant and pregnant animals. However, the postimplantation EM increased significantly only after administration of altax by both methods, of captax before pregnancy, and of santocure during pregnancy.

On the basis of these experimental results it is impossible to determine precisely which mechanism caused the rise in EM and the decrease in weight of the fetuses. It will be noted that delay in the onset of conception facilitates elimination of affected gametes, and that santocure-mor, which caused the greatest delay of conception, gave rise to the smallest increase in total EM and did not increase the postimplantation EM. By contrast to this, altax did not delay the onset of conception but caused the greatest lengthening of the estrous cycle (possibly with delay of maturation of the gametes), with a sharp increase in all types of EM. Changes in the hormonal balance perhaps took place after administration of altax to the pregnant females. Evidence of this is given by the absence of expulsion of the fetuses in two females after death of all the fetuses.

The clearest manifestation of mutagenic action is an increase in EM after administration of the substances to nonpregnant animals [5-7]. The World Health Organization suggests that the index of postimplantation EM for the group of animals be called the "index of mutagenicity" [7]. Comparison of this index for altax (30.8) with the index of mutagenicity of the carcinogens aflatoxin and benzpyrene (11.0) and of alkylating agents of the ethylenimine and methylmethanesulfonate series (11.0-38.0) [8], shows that altax can be classed among the supermutagens.

Administration of VA during pregnancy caused an increase in total EM, but the postimplantation EM rose only after administration of altax and santocure.

The vulcanization accelerators captax, santocure, santocure-mor, thiurams D and E and, in particular, altax cause sharp depression of reproductive function in albino rats.

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REGENERATION OF SKELETAL MUSCLE AFTER MECHANICAL TRAUMA IN REPTILES

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KEY WORDS: regeneration of muscle; restoration of innervation.

The regenerative properties of muscle tissue have been studied most extensively in mammals [1, 4-10, 12, 14]. The ability of the skeletal muscles of reptiles to recover after injury has not been specially studied. There have been only incidental observations on the regeneration of muscle tissue as part of a study of regeneration after amputation of the tail in lizards [13, 15]. The present writers have shown that the muscles of reptiles can recover when the whole muscle is autografted [2, 11].

It was decided to investigate the regenerative properties of skeletal muscles of reptiles after mechanical trauma in order to compare differences in the regenerative activity of muscle tissue in representatives of different classes of vertebrates, and details of the study are given below.

EXPERIMENTAL METHOD

Turtles (*Testudo horsfieldi*) weighing 200-400 g, kept under animal house conditions with constant illumination and at a constant temperature of 25°C, were used. The gastrocnemius muscle was completely divided transversely. Between 2 and 6 months after the operation the contractile activity of the muscles was tested by electrical stimulation of the tibial nerve, after which the muscles were fixed and sections cut for microscopic and ultrastructural investigation.

EXPERIMENTAL RESULTS

After trauma the muscle stumps were separated by a defect measuring 3-5 mm. The defect 2 weeks after the operation was filled with loose connective tissue, but in the stumps, in the zones adjacent to the region of the defect, a well-marked process of destruction of injured muscle fibers was observed, not only in the immediate vicinity of the site of trauma, but also some distance from it. Destructive changes were more marked in the distal stump at

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