THE ACTION OF ASCORBIC ACID ON THE REPRODUCTIVE SYSTEM OF LABORATORY ANIMALS

E. P. Samborskaya

Laboratory for the Trial and Study of Contraceptives (Scientific Director, Professor E. F. Shamrai), Ukrainian Research Institute for Care of Mother and Child (Director, Docent A. G. Pap), Kiev
(Presented by Active Member AMN SSSR A. P. Nikolaev)
Translated from Byulleten, Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 10, pp. 110-114, October, 1962
Original article submitted January 15, 1962

During recent years ascorbic acid (AA), like other vitamins, has become widely used as a nonspecific agent for the treatment of many different diseases, and has been given in large doses [4, 5, 6].

It is generally accepted that AA is nontoxic in any dose, for it is readily excreted by the kidney. Mourican and Edel gave AA to guinea pigs in doses of 500 and 1000 mg daily for 150-170 days without observing any toxic manifestations. When studying the toxic properties of AA, these and other workers used the usual tests but paid no attention to the state of the reproductive system. This accounts for the absence of information in the literature concerning the action of large doses of AA on the reproductive system.

Preliminary investigations in sexually immature female guinea pigs showed that the subcutaneous injection of large doses of AA, and also of doses close to the daily requirement of vitamin C of these animals, led to a decrease in the weight of the reproductive organs.

These investigations have been continued. Three series of experiments were carried out: 1) Measurement of the weight of the reproductive organs (uterus, ovaries, testes) of sexually immature male and female guinea pigs and female rats after parenteral (subcutaneous and intravaginal) administration of AA; 2) the study of the sexual cycles of mice receiving AA subcutaneously; 3) the study of the histological structure of the uterus, ovaries, and testes of guinea pigs receiving subcutaneous injections of AA.

EXPERIMENTAL METHOD

The AA solution for subcutaneous injection was prepared in such a concentration that the necessary dose was contained in 0.1-0.2 ml of physiological saline. The test doses of AA were 50 and 15 mg/kg body weight.

AA was given daily for 30 days, and in the series of experiments to study the reproductive cycles for 60 days every other day. The animals of the control group received physiological saline in the same volume as the AA solution and at the same times. AA was introduced intravaginally as a solution of suitable concentration (50 mg/kg), with which a cotton-wool tampon was soaked. The tampons with AA solution were wedged into the vagina with a dry tampon. The control group of animals received tampons without AA daily. Vaginal smears were taken from the mice by L. P. Grigoliya's method [2].

Organs for histological investigation were fixed in 5% formalin solution and embedded in paraffin wax. Sections were stained with hematoxylin-eosin and by van Gieson's method. The summer diet for the guinea pigs consisted of fresh hay, beetroot, carrot, and oats (in winter the fresh hay was replaced by dried). The rats received meat, milk, bread, gruel, oats, and hay; the mice the same without meat.

Experiments were conducted on 144 guinea pigs, 76 rats, and 22 mice.

EXPERIMENTAL RESULTS

The weight of the reproductive organs of sexually immature laboratory animals may be used to determine the functional state of the sex glands of these animals. We therefore used for our experiments sexually immature female and male guinea pigs with a mean weight before the experiment of 150-170 g. The observations were made in August and September, 1960, on 144 guinea pigs (104 females, 40 males) and 76 female rats (see table).

Mean Weight of Uterus of Guinea Pigs after Administration of Various Doses of Ascorbic Acid by Different Methods (daily for 30 days)

Group of animals	Substance administered	Dose (in mg/kg)	Mode of administration	N	M	m	t
Experimental	AA	50	Subcutaneously	24	139.5	±0.7	7.8
Control	Physiological saline		"	24	454	±0.5	
Experimental	AA	15	"	20	167	±0.6	7.1
Control	Physiological saline		"	23	454	±0.5	
Experimental	AA	50	Intravaginally	13	222	±0.6	3.6
Control	Tampons		"	10	371	±0.5	

Note. N—No. of animals; M—arithmetical mean weight of uterus (in milligrams); m—mean error of arithmetical mean; t—coefficient of reliability.

The table shows that AA, when administered daily to sexually immature guinea pigs by the parenteral route, caused a decrease in the weight of the uterus by comparison with that of the control group of animals (there was an identical change in the weight of the ovaries).

In order to ascertain the effect of the AA, when administered parenterally, on the weight of the reproductive organs of animals synthesizing the vitalin, experiments were carried out on 76 sexually immature female rats. AA was given in the same doses daily to two groups of animals subcutaneously, and to one group intravaginally. The results of the investigations confirmed a decrease in the weight of the reproductive organs of the female rats after parenteral administration of AA.

To discover the effect of AA on the weight of the male sex glands, experiments were carried out on 40 sexually immature male guinea pigs. For 30 days, 20 experimental animals received daily subcutaneous injections of AA in a dose of 50 mg/kg; 20 guinea pigs were controls. The results showed that the weight of the testes in the animals of the experimental and control groups was nearly indistinguishable (mean weight in the experimental group 645 mg, in the control group 648 mg).

Observations on the sexual cycles of the animals were conducted from July to October, 1961. The experiments, which lasted 83 days, were performed on 22 sexually mature nulliparous mice with a mean weight of 18-20 g. AA was injected subcutaneously into the (11) mice of the experimental group on alternate days in a dose of 50 mg/kg, starting on the 18th day of observation (after establishment of the normal duration of the sexual cycles). The control group of (11) animals received subcutaneous injections of physiological saline on alternate days.

The sexual cycles of the mice of the experimental and control groups followed a normal and generally similar course before administration of AA (Fig. 1). The mice in which sexual activity began later were three in number (Nos. 6, 12, and 13) in the experimental group, and four (Nos. 4, 9, 10, and 13) in the control group. The remaining animals of both groups had already passed through several sexual cycles at the time of administration of AA. Injections of AA and physiological saline began on July 30, but a difference in the course of the sexual cycles began to be noticeable only after the middle of August. Counts of the number of estrous cycles in ten animals of the experimental and ten of the control group showed that during the period of observation there were 31 cycles in the first group and 64 in the controls (mouse No. 2 of the experimental group and mouse No. 1 of the control group died at the beginning of the experiment and were excluded from the counts). The predominance of the resting phase (diestrus) in the animals of the experimental group was also obvious.

The changes in the mice in which the beginning of sexual activity was delayed are interesting. The onset of sexual activity in the animals of the control group was shown by the regular sequence of the sexual cycles. In the

mice of the experimental group estrus developed, but either only once during the whole period of observation, or at long and irregular intervals thereafter.

At the end of September (70th day of observation) both groups of animals were allowed to copulate with sexually mature males, and observations were continued thereafter for 14 days. One mouse of the experimental group

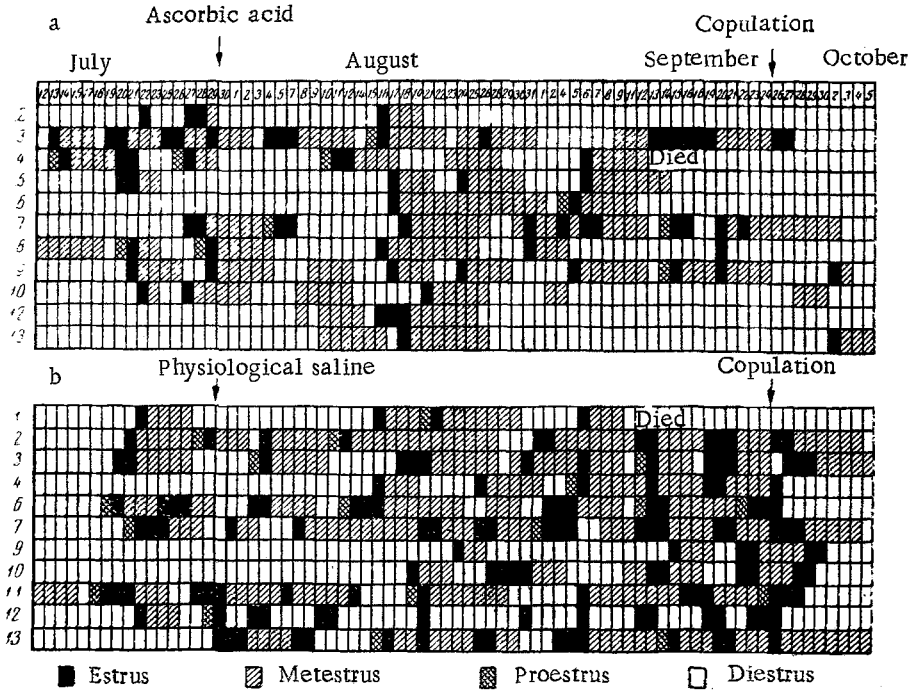

Fig. 1. Sexual cycles of mice of the experimental (a) and control (b) groups.

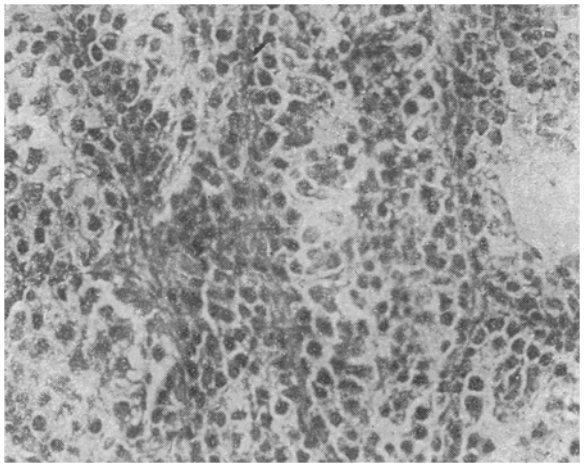

Fig. 2. Testis of an experimental guinea pig. Magnification 200×.

(No. 3) and seven of the controls were impregnated. The impregnated mice (the one experimental and three of the controls, Nos. 4, 6, and 11) were sacrificed 16 h after impregnation in order to determine the state of the ovum (the pronuclei are known to fuse after approximately 14-16 h [1]). Ova at the stage of two pronuclei were found in the oviducts of the experimental and control mice, demonstrating that fertilization had followed its normal course in both groups of mice.

The remaining mice were sacrificed on the 14th day after copulation. Post-mortem examination of the control group revealed six pregnant animals at different stages of pregnancy. None of the experimental mice was pregnant. The uterus, ovaries, and testes of the sexually immature guinea pigs receiving AA solution daily for 30 days were investigated histologically.

The uterus showed atrophic changes in the endometrium, mainly affecting the stroma. The glands were reduced in size and surrounded by a ring of connective tissue which compressed the lumen of the glands in places. The muscular and serous layers were unchanged. Proliferation of fibrous tissue and atrophic changes were slight in degree in the ovaries of the experimental group of animals.

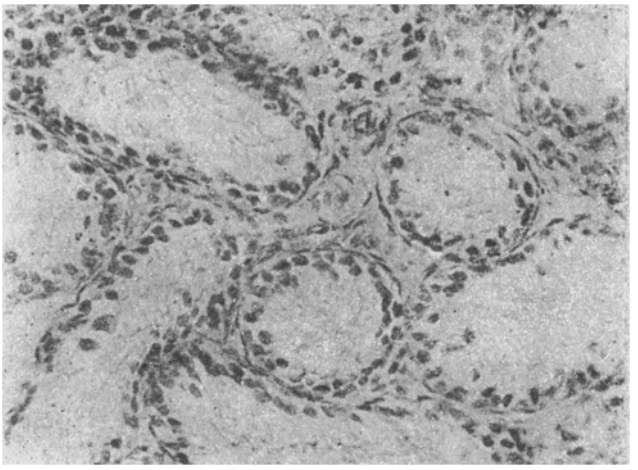

Fig. 3. Testis of a control guinea pig. Magnification 400×.

The histological structure of the testes of the guinea pigs differed in the two groups. The tubules of the testes of the experimental guinea pigs contained large numbers of reproductive and follicular cells, especially the latter. The interstitial tissue was very loose and the interstitial cells elongated, while here and there dystrophy of the cells was observed (vacuolation, accumulation of pigment), and the testicular tissue was ischemic (Figs. 2 and 3).

The results suggest that the parenteral administration of ascorbic acid, even in near-physiological doses, may not only be beneficial, but also harmful.

SUMMARY

Investigations, conducted on sexually immature female guinea pigs, demonstrated that subcutaneous administration of ascorbic acid in high doses and in doses approaching the 24 h vitamin C requirement lead to the reduction of weight of the reproductive organs. Ascorbic acid prolongs the intervals between the sexual cycles in mice, even completely arresting the rut in some of them. Connective tissue proliferation around the glands and atrophic changes in the uterine mucosa were detected in the animals who had received ascorbic acid subcutaneously. Individual foci of fibrosis were revealed in the ovaries. Parenteral ascorbic acid administration to immature male guinea pigs provoked excessive proliferation of cellular elements.

LITERATURE CITED

1. D. I. Genin, Investigation of the Importance of a Large Number of Survivors in the Fertilization and Development of Mammals. Author's abstract of candidate dissertation (Kiev, 1952).
2. L. P. Grigoliya, Byull. Éksper. Biol. 11, 369 (1950).
3. K. L. Kovalevskii, The Laboratory in Stock Breeding [in Russian] (Moscow, 1951).
4. A. L. Myasnikov, Atherosclerosis [in Russian] (Moscow, 1960).
5. S. M. Ryss, Vitamins [in Russian] (Leningrad, 1955).
6. P. I. Shilov and T. N.Yakovlev, A Reference Book on Vitamins [in Russian] (Leningrad, 1960).