PATHOGENIC ACTION OF NEPHROCYTOTOXIC SERUM ON EMBRYONIC DEVELOPMENT OF ALBINO RATS

V. M. Mikhailov

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Very little information has been obtained concerning the participation of immunologic factors in the etiology and pathogenesis of developmental anomalies in mammals. Women with chronic thyroiditis, for instance, have been found to give birth to children without a thyroid gland [7], although the participation of autoantibodies against the thyroid in this pathological process developing in the fetus,have not been demonstrated experimentally [5, 10, 16]. When female mice are immunized with homologous brain tissue, 8-9% of the embryos develop anomalies of the nervous system [11]. Malformations have been observed in fetuses of rats and mice,if the mothers received injections of nephrotoxic serum during pregnancy [8, 9, 13]. However, the action of this cytotoxic serum had not been investigated throughout pregnancy, and the samples of sera used differed in their activity.

The object of this investigation was to study the pathogenic and keratogenic action of a nephrocytotoxic serum (NCTS) on the various stages of embryogenesis of albino rats and to compare the sensitivity of the embryos to nephrotoxin and to other pathogenic agents.

EXPERIMENTAL

To prepare the cytotoxic serum, rabbits were immunized with a homogenate of the corresponding organ of an adult rat mixed with Freund's adjuvant. The organ was first rinsed free from blood in situ. The cycle of immunization consisted of three weekly intramuscular injections of a mixture of homogenate and adjuvant in the ratio 1:1 in a dose of 2 ml per injection. The prepared serum was heated to 56° for 30 min and kept at 4°. The presence of antibodies was determined by the complement fixation reaction one week after the end of the cycle of immunization. In preliminary experiments several samples of kidney antiserum and brain antiserum, and also normal blood serum, were tested. These tests showed that the kidney antiserum was pathogenic and teratogenic against albino rat embryos when injected into the females on the 8th, 9th, and 10th days of pregnancy. The brain antiserum and the normal rabbit serum had no such action. Of the three methods of injection studied—intravenous, intraperitoneal, and subcutaneous—the last proved ineffective. The results of the intravenous and intraperitoneal methods of injection of the sera were indistinguishable [3].

In the present investigation a mixture of nine kidney antisera was used, so that a preparation of uniform activity at all times of its administration could be obtained. The experimental female rats received intraperitoneal and intravenous injections of the mixture in a dose of 1 ml/100 g body weight at approximately the same time of day. The control animals received normal rabbit serum in the same dose.

The experiments were carried out on noninbred albino rats weighing 120-200 g. The first day of pregnancy was taken to be the day when spermatozoa were found in the morning vaginal smear. The results of the treatment were determined when the animals were autopsied on the 17th-18th day of pregnancy. The sites of implantation in the uterine cornua and the living and absorbed embryos were noted. The postimplantation mortality was calculated from the difference between the number of implantation sites and the number of living embryos. The embryos were studied under the binocular loupe to discover malformations. The results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

In the experimental group, 528 living embryos were obtained from 101 females, and 130 of them were malformed. In the control group, 294 normal embryos were obtained from 44 females. In the various

Department of Embryology and Department of Microbiology, Institute of Experimental Medicine, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR D. A. Biryukov).
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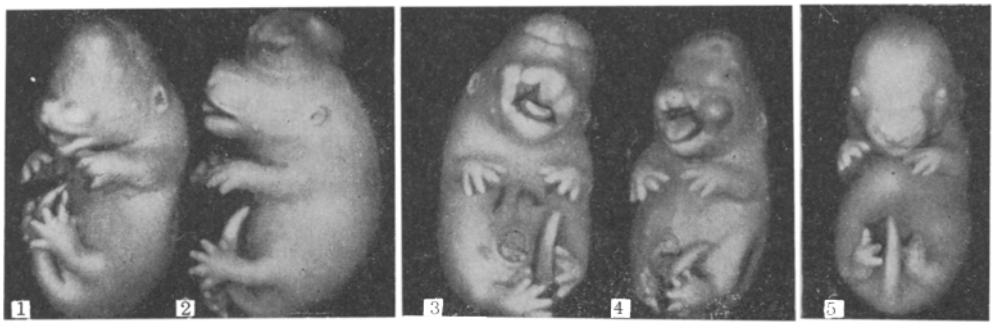
Lethal and Teratogenic Action of NCTS on Embryonic Development of Albino Rats (Autopsied on 17th-18th Day of Pregnancy)

Day of pregnancy	No. of rats in group	Implanted embryos		Embryo dying after implantation		Living embryos				
						total	normal		malformed	
		abs.	%	abs.	%		abs.	%	abs.	%
2	7	68	95,7±2,4	2	2,94±2,04	66	66	100	—	—
4	6	61	95,3±2,6	1	1,64±1,5	60	60	100	—	—
5	7	67	97,9±1,7	58	86,5±4,3	9	9	100	—	—
6	13	108	90,7±2,6	19	17,6±3,6	89	69	76,2±4,5	20	23,8±4,5
7	10	94	89,5±3,0	21	22,3±4,3	73	38	52,0±5,8	35	48,0±5,8
8	11	111	98,2±1,24	30	27,2±3,4	81	50	61,7±5,4	31	38,3±5,4
9	17	175	94,6±1,4	106	57,7±3,7	69	39	56,5±5,9	30	43,5±5,9
10	11	113	98,2±1,23	95	84,4±3,3	18	5	20,8±9,5	13	79,2±9,5
11	8	79	96,3±2,08	18	22,7±4,7	61	61	100	—	—
12	6	61	91,4±3,3	11	18,0±4,9	50	50	100	—	—
13	5	53	94,6±2,7	14	26,4±5,8	39	39	100	—	—



Developmental anomalies of rat embryos following injection of NCTS on the 9th day of pregnancy. Autopsied on the 18th day. 1) Meningocele, anophthalmia; 2) enencephaly, anophthalmia; 3) exencephaly, hair lip, bilateral anophthalmia; 4) meningocele, hair lip, bilateral anophthalmia; 5) control embryo on 18th day of development.

experimental groups 46 females died a few hours after receiving the injection of NCTS, and death was not related to the time of pregnancy, but to the method of injection of the preparation. As a rule the animals died after intraperitoneal injection of NCTS. Death of the females after injection of normal rabbit serum was not observed.

The mortality among the embryos following injection of NCTS on the 2nd and 4th days of pregnancy was 2-3%, i.e., the same as the mortality in the control group (see table). When NCTS was injected at later stages the mortality in the experimental group rose sharply.

The teratogenic action of the preparation was first manifested on the 6th day of pregnancy (see table). When injected on the 11th, 12th, and 13th days of pregnancy no malformed embryos were found. Hematomas and areas of edema were found in the embryos after treatment with NCTS from the 4th to the 13th days of pregnancy inclusive. The predominant malformations were developmental anomalies of the eyes, most frequently anophthalmia. Developmental disturbances of the brain (anencephaly, microcephaly, meningocele), were found when the NCTS was injected on the 7th, 9th, and 10th days. Developmental anomalies (of the facial skull hypognathus failure of fusion of the maxillary processes) were observed in a few cases when the preparation was given on the 6th, 7th, 8th, and 9th days of pregnancy. Hernias of the liver were seen only in embryos of females treated with NCTS on the 10th day of pregnancy (see figure).

It follows from these results that the sensitivity of albino rats embryos to NCTS differs at different stages of embryonic development. The embryos were not injured when NCTS was injected on the 2nd and 4th days of pregnancy, i.e., when the dividing cocyte was still in the fallopian tube and maximally sensitive to the action of agents such as x-rays and radiomimetic substances (pyrimethamine, Myleran) [1, 2, 4]. The sensitivity of the embryos to NCTS rose sharply when the blastocyst entered the uterine cavity, i.e., on the 5th day of pregnancy. The peak of increased sensitivity to the action of agents such as maternal

hyperthermia and ether anesthesia is on the 4th day of pregnancy [4]. The first peak of increased sensitivity of albino rat embryos to NCTS was on the 5th day of pregnancy. In the implantation period (6th and 7th days of pregnancy), the sensitivity of the embryos to NCTS fell sharply, but in the period of placentation it rose again and reached a second maximum on the 10th day of pregnancy. On the 11th day and later the sensitivity of the embryos to NCTS fell.

Nephrotoxin shows certain special features of its teratogenic action. For instance, malformations appear when the preparation is injected after the 6th day of pregnancy, i.e., before the beginning of the period of organogenesis. The curve of teratogenic activity includes the 6th, 7th, 8th, 9th, and 10th days of pregnancy, without forming a statistically significant peak. When NCTS was injected on the 11th, 12th, and 13th days of pregnancy no malformations appeared, i.e., the teratogenic activity of the preparation had ceased, at a time when other teratogenic agents still continue to form developmental anomalies.

A differential sensitivity of embryos at the various stages of embryogenesis thus exists toward the injection of kidney antibodies into the mother, and is shown by two peaks of increase of embryonic mortality, on the 5th and 10th days of pregnancy. During the analysis of the teratogenic action two facts stand out: the earlier onset of teratogenic activity and the comparative uniformity of the observed developmental anomalies. When these findings are discussed, the mechanism of action of NCTS must be borne in mind. Is the observed teratogenic defect connected with the immediate action of the injected antibodies or with chronic effects from the injured mother, or on the other hand, are these effects the result of the direct action of antibodies on the embryonic cells or the result of physiological changes in the mother as the result of the injected NCTS? That this last suggestion is possible is shown by the high mortality among the females from the dose of the nephrotoxin used.

Reports in the literature suggest that the antibodies have a direct action on the embryonic cells immediately after injection of NCTS. According to Brent [8], compression of the vessels of one cornu of the uterus for 30 min at the moment of injection of the antiserum protects the embryos in that cornu from injury. Determination of the circulation time of the kidney antibodies in the blood stream showed that the antibodies disappeared from the blood 40 min after injection of NCTS [15]. The kidney antibodies consist of two components, the concentration of the first of which falls in the blood by half within 4 min, and of the second, within 80 min after injection [14]. It is also known that nephritis among female sheep [17] and female rats [12] does not cause developmental anomalies in the embryos. These results, and also the differential sensitivity of the embryos at different stages of embryogenesis to administration of NCTS, favor the view that the teratogenic action of NCTS is due to its direct action on the embryonic cells. However, special investigations are needed before this problem can be finally solved.

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