CHARACTERISTICS OF THE DISTURBANCE OF EMBRYOGENESIS UNDER THE EFFECT OF MYELOSAN IN THE EARLY STAGES OF RAT DEVELOPMENT

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It was previously demonstrated [1] that the antileukemic drug myelosan when injected into rats at early stages of pregnancy caused its termination. In this case some embryos were not implanted and many of the implanted embryos soon died.

Since the results pertain to the end of pregnancy (on the 17th day) it is difficult to solve the problem of the possible causes for the absence of implantation and early postimplantation death.

The purpose of this investigation was to study the changes in the embryo and extraembryonic formations a short period after the effect of myelosan before implantation and immediately after it.

EXPERIMENTAL METHOD

The work was performed on white rats weighing 150-200 g. Pregnancy was established by the presence of spermatozoa in the vaginal smears of females mated to males overnight. On the 1st, 2nd, 4th, and 7th day of pregnancy the rats were injected intraperitoneally with myelosan dissolved in persic oil in doses of 15 and 10 mg/kg.

On the 10th day of pregnancy the animals were autopsied, the uterus extracted, and the number of placentas counted. Then the uterus as a whole was fixed in Bouin's fluid for 1-1½ days and after transferring to 70% alcohol the blastocysts were opened.

For this purpose an incision was made through each placenta near the middle with a razor perpendicular to the longitudinal axis of the cornu of the uterus and the obtained "slice" (thick transverse section) was studied under a MBS-1 binocular microscope. For the characteristic of the degree of development of the decidual tissue and the blastocyst we measured, by an ocular micrometer, the length and width (two mutually perpendicular diameters) of the transverse section of the placenta and the length and width of the blastocyst.

The sample material was appropriately treated for a preparation of histological preparations (staining with Ehrlich hematoxylin and eosin).

EXPERIMENTAL RESULTS

The data of the experiments are shown in the table, from where we see that, after the administration of myelosan before implantation, the percent of implanted embryos decreased in comparison with the control, the effect on the 2nd and 4th day of pregnancy being somewhat higher than on the 1st.

Effect of Myelosan on Rat Embryos of Early Developmental Stages (results calculated on 10th day of pregnancy)

Day of effect (period of pregnancy)	Dose (in mg/kg)	No. of animals	No. of corpora lutea	No. of implantations		Blastocysts									
						macroscopically normal		underdeveloped						perished (resorption)	
								less than 0.2-0.5 mm²		less than 0.2 mm²					
				abs.	%	abs.	%	abs.	%	abs.	%			abs.	%
1st	15	12	124	93	75,0±3,9	34	36,6±5,0	19	20,4±4,2	12	12,9±3,5			28	30,1±4,8
	10	11	103	83	80,6±3,9	45	54,2±5,5	23	27,7±4,8	0				15	18,1±4,2
2nd	15	10	106	75	70,7±4,4	2	2,6±1,8	5	6,7±2,9	6	8,0±3,1			62	92,7±4,4
	10	9	91	79	86,9±3,6	6	7,6±3,0	11	13,9±3,9	14	17,7±4,2			48	60,7±5,5
4th	15	12	122	84	68,5±4,2	1	1,2±1,2	16	19,0±4,3	25	29,8±5,0			42	50,0±5,5
	10	8	84	66	78,6±4,5	5	7,6±3,3	36	54,5±6,2	7	10,6±3,8			18	27,3±5,1
7th	15	8	79	68	86,1±3,9	3	4,4±2,5	63	92,6±3,2	0				2	2,9±2,0
	10	7	63	58	92,1±3,4	33	56,9±6,5	24	41,4±6,5	0				1	1,7±1,7
Control		8	81	73	90,1±3,3	67	91,8±3,2	2	2,7±1,9	0				4	5,5±2,7

If there was no implantation the uteri of the experimental females were washed with Ringer's solution. In these cases we frequently noted in the contents blastocysts, many of which were degeneratively altered. It is interesting that on the uterine mucosa of the antimesometral side traces of decidual reaction were frequently observed at autopsy: loosening of the mucosa, swelling, and an enhanced local vascular picture. Apparently, the main cause of the absence of implantation is the nonviability of the blastocysts as a result of the action of myelosan.

As a result of the injection of myelosan before implantation an appreciable number of the implanted embryos died, the greatest percent of mortality being noted on the 2nd day of development. In such cases, we noted on the transverse section of the fixed placenta at the site of the blastocyst a slitlike cavity filled with blood and its disintegrated remains.

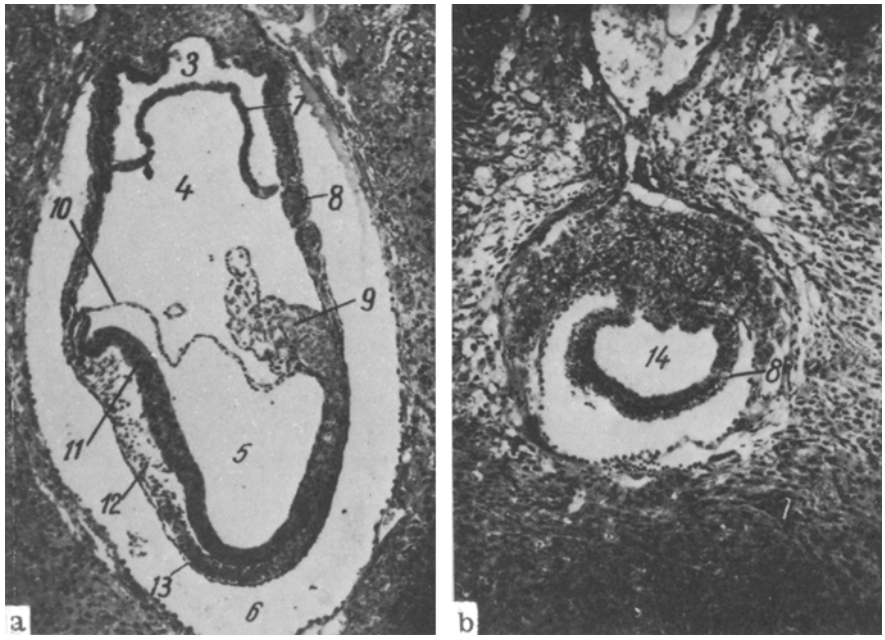
After the effect of myelosan we observed at all investigated periods of embryogenesis in many of the survived embryos developmental anomalies which were expressed most frequently in retarded development and deformation of the blastocyst; the embryos were improperly oriented in the blastocysts with respect to the lumen of the uterus.

After measuring the blastocyst the experimental embryos were ranked as macroscopically normal if the area of the cysts was not less than 0.5 mm^2, or underdeveloped which in turn were arbitrarily subdivided into retardation of the I degree (cyst area 0.2-0.5 mm^2) and of the II degree (cyst area less than 0.2 mm^2).

The most unfavorable results were demonstrated after the injection of myelosan on the 2nd and 4th day of pregnancy. The overwhelming majority of blastocysts were underdeveloped, a high percent of embryos severely retarded in development being noted at a dose of 15 mg/kg. In these cases, we saw on the histological specimens, as a rule, only the extraembryonic (yolk-sac) entoderm which bounded the proamniotic cavity (see figure, b). Sometimes the latter, having collapsed, acquired the appearance of an irregular folded slit. Along with this, among the cell elements of the yolk-sac entoderm and ectoplacental cone, we were able to see numerous atypical mitoses and degenerative changes which apparently were the result of the nucleotoxic effect of this pharmacological agent [16-18].

When the drug was injected on the 1st day of pregnancy, about one-half of the embryos (of those that survived) developed normally, one-third were underdeveloped (I degree). At a dose of 15 mg/kg we noted some (12 of the 65 living) were underdeveloped (II degree) which histologically did not differ from those described above.

An appreciable number of normally developed embryos was noted after myelosan was injected also on the 7th day of development in a dose of 10 mg/kg and only some in a dose of 15 mg/kg. In all remaining animals a I degree of underdevelopment (cyst area of 0.2-0.5 mm^2) was determined. The histological investigation of such placenta revealed, in addition to a decrease in the size of the blastocyst, finer disturbances in structure (figure, c). The blastocyst usually appeared deformed, especially its part where the embryo itself was situated (at the antimesometral pole). Differentiation of the germ layers of the latter was noticeably retarded: it was difficult to distinguish the ectoderm and thin streak of intestinal entoderm; the mesoderm did not develop.



Disturbance of the structure of blastocyst after the effect of myelosan. a) On 10th day of development (control); b) extreme degree of underdevelopment; proamniotic cavity is bounded only by the yolk-sac entoderm (4th day of development, dose 15 mg/kg); c) I degree of retarded development; deformation of blastocyst (7th day of development, dose 15 mg/kg); 1) decidual tissue; 2) ectoplacental cone; 3) ectoplacental cavity; 4) exocoelom; 5) amniotic cavity; 6) cavity of yolk-sac; 7) chorionic plate; 8) yolk-sac entoderm; 9) allantois; 10) wall of amnion; 11) ectoderm; 12) mesoderm; 13) intestinal entoderm; 14) proamnion. Hematoxylin-eosin. 83x.

The amniotic cavity had the appearance of a small narrow lumen or was totally absent. The exocoelom was markedly reduced, whereas the chorionic plate being, so to speak, reversed, passed deeply downward. The ectoplacental cone was developed relatively normally. In many respects an analogous character of anomalies was observed by the 10th day in rat embryos after various other effects [2, 6, 7, 12].

Of interest was the character of disturbance of the structure of the blastocyst of normal size: smoothness or a scarcely noticeable protrusion was noted frequently at the place from where the allantois grows.

A comparison of the data on the death and damage of embryos given in this work indicate that the greatest increase of mortality after the injection of myelosan occurs at the 2nd day of embryogenesis. This does not confirm the remarks of Thiersch [19] that myleran (myelosan) has the maximal pathogenic effect on rat pregnancy when injected on the 4th and 5th day: 50% resorption of embryos when injected in a dose of 10 mg/kg.

The works of P. G. Svetlov and cohorts also establish the high degree of injury at the stage of the 4th day of embryogenesis in rats under the effect of pathogenic agents in various nature both in experiments in vivo [11, 13, 14] and in vitro [10]. The discrepancy between our results and the cited data is probably due to the slower rate of manifestation of the effect of damage as a result of myelosan.

Adams, et al. [15] do not find any changes in the $6^1/_2$ day blastocyst of rabbit when myleran was administered on the 1st, 5th, or 4th and 5th day of pregnancy in doses of 4.8-12 mg/kg. The resistance of the embryos in this case is possibly associated with the species differences in the reaction to myelosan. The character of the injury to rat embryos after the effect of myelosan at early (pre-implantation) stages of embryonic development with consideration of the results on the 10th day of pregnancy shows that the sensitivity of the embryoblast is somewhat higher in comparison with the trophoblast, although the trophoblastic derivatives are seriously damaged. It is known that at later stages of embryogenesis (after the formation of the placenta) the trophoblast is highly resistant to the effect of various toxic agents [3-5], however, in the pre-implantation period of development the cells of the trophoblast are damaged no less than the cells of the embryoblast [8, 9].

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