EFFECT OF MATERNAL NITROBENZENE POISONING ON MORPHOLOGY AND HISTOCHEMISTRY OF HEMOCHORIAL PLACENTAS OF ALBINO RATS

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The effect of nitrobenzene poisoning on the placenta and fetus was studied in experiments on albino rats. Delay in development of the embryo and production of malformations and other disturbances of organogenesis were observed.

The histochemical and biochemical results are evidence of disturbance of oxidation-reduction processes in the placenta. A link is possible between the disturbance of placental functions and the appearance of malformation and other changes in normal development of the fetus.

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There is convincing evidence in the literature that certain toxic substances used in industry, together with pharmacological preparations, may have a harmful effect on the fetus during poisoning of the mother [1-4, 6-8]. These investigations have shown that such an effect may be expressed either by delay in development of the embryo as a whole or by disturbance of formation of certain organs. Meanwhile, changes in the placenta, the structure through which the harmful effect on the fetus is exerted, have received practically no attention.

In the present investigations histological, histochemical, and biochemical methods were used to study changes in the hemochorial placentas of albino rats during exposure of the mother to the widely used industrial poison nitrobenzene. Interest was concentrated on the respiratory enzymes (cytochrome oxidase, succinate dehydrogenase), acid mucopolysaccharides, glycogen, and the tissue aerobic respiration level of the placenta.

EXPERIMENTAL METHOD

Pregnant rats received daily subcutaneous injections of nitrobenzene in a dose of 125 mg/kg body weight starting from the 4th-6th or 9th-12th day of pregnancy. The number of injections varied from 7 to 13. On the 15th-16th or 20th-21st day the animals were sacrificed. The placentas and ovaries were fixed with 10% acid formalin, and sections were stained with hematoxylin-eosin, by Mallory's method, the PAS, and Hale's method, and with toluidine blue, with corresponding controls. Frozen sections were treated with tetrazolium salts to detect succinate dehydrogenase and with dimethylparaphenylenediamine to detect cytochrome oxidase. In parallel experiments, the tissue respiration level was determined in a Warburg's apparatus.

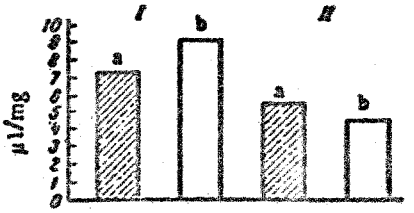

Fig. 1. Dynamics of oxygen absorption by placental tissues (in µl/mg) during maternal nitrobenzene poisoning I) control; II) experiment; a) without substrate; b) with sodium succinate.

EXPERIMENTAL RESULTS

To begin with, it is curious to note that nonpregnant rats are more sensitive to nitrobenzene than pregnant rats. With the same doses, they develop signs of severe poisoning sooner; cyanosis and signs of a hemorrhagic diathesis were observed.

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TABLE 1. MT in Submandibular Gland of Rats after Partial Hepatectomy (Series I)

Injury to embryo	Time of beginning of injections and their number	Experiment No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Slight delay in development (12)	Day of pregnancy			15	10			16		13	10						11	12			12	13	10	11	12						
	Number of injections			3	4			4		7	9						9	6			6	5	6	8	5						
Marked delay in development (4)	Day of pregnancy		5																							8			3	8
	Number of injections		7																							9			11	11
Complete arrest of development at time of implantation of fertilized ovum (10)	Day of pregnancy	4							3				4	4	3	3			4	3								4		
	Number of injections	3							7				14	14	10	10			8	4								8		
Gross developmental defects (malformations) (4)	Day of pregnancy					7	4					6															6			
	Number of injections					13	7					7															7			

Note. Number of fetuses shown in parentheses.

The character of changes in the normal course of pregnancy in the poisoned rats depended primarily on the stage of development at which nitrobenzene administration began (Table 1). If the poison was given to the mother on the 4th-6th day of pregnancy (before formation of the placenta began), as a rule severe delay in development of the embryo was observed. If pregnancy had reached 12-15 days, at autopsy changes characteristic only of the period of implantation of the fertilized ovum were found in the wall of the uterus with the naked eye and later histologically. These changes affected the embryo as a whole. Only if the period of pregnancy corresponded to 13-15 days was delay in development of the embryo combined with normal functioning of the placenta.

In 4 of 30 cases, (total number of observations) gross defects of morphogenesis were detected, with softening of the bones (2), hydrocephalus, and malformation of the trunk (1), and also with agenesis of the pelvic end of the trunk and the hind limbs (1). In all the animals the beginning of the injections and the period of marked cumulation of the poison took place at the stage of formation of the placenta.

If the poison acted on the 12th-14th day (stages of the formed placenta), no visible morphogenetic abnormalities were found. However, the embryos were usually smaller in size and weight than the controls at comparable periods of pregnancy. Severe retardation in development was again observed in cases when the injections began at the time of formation of the placenta.

Histochemical study of the placenta of a series of animals revealed a decrease in the glycogen content in the trophoblast with a parallel accumulation of glycogen in the decidual tissue (PAS reaction, control with amylase). However, these changes were not consistent, and at times were directly opposite, so that at

this stage we cannot identify a precise pattern in the dynamics of the glycogen content in nitrobenzene poisoning. Undoubtedly, however, it leads to essential fluctuations in the level of this energy-producing substrate of the placental tissues.

Histochemical detection of the respiratory enzymes showed that in the normal placenta succinate dehydrogenase activity is high. In the experimental placentas its activity was considerably reduced, particularly in the labyrinthine bands, but it remained high in the maternal placenta. The dynamics of the cytochrome oxidase content was similar.

Investigation of the intensity of oxygen uptake by surviving placental tissue, with or without the presence of substrates (sodium succinate and glucose) showed that the oxygen consumption of the control placentas averaged 7.3 μl/mg (without substrate) and 9 μl/mg (with sodium succinate), i.e., an increase of 1.7 μl/mg. In contrast to this, the placentas of the poisoned rats showed a marked tendency toward a lowering of the respiration level, both initially (mean value 5.4 μl/mg) and after addition of sodium succinate (mean value 4.4 μl/mg; 1.9 and 4.6 μl/mg below normal respectively, Fig. 1). These findings are equally probable evidence of a decrease in succinate dehydrogenase activity. In this connection, there are reports in the literature that some substances similar in structure to nitrobenzene, such as 3, 4-dinitrophenol, have a similar effect on activity of the hydrogenases [7]. In our experiments, after addition of glucose to placental tissue, the intensity of oxygen consumption was 33-50% lower than its initial level.

The results suggest that nitrobenzene causes essential changes in the mechanisms of aerobic respiration of the placental tissue, in particular by its apparently direct effect on respiratory enzymes. In turn, this adversely affects the vitally important placental functions, including the function of synthesis of organ-specific proteins required for building the tissues of the developing embryo. This may be a probable cause of the delayed development of the embryos and the appearance of developmental defects of varied severity.

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