

during this period the genes are 'turned on' to a large extent. They seem unselectively inhibited by treatment with histones. These effects could also be related to inhibition of other enzymatic systems by histones<sup>18-20</sup>.

<sup>18</sup> P. PERSON and A. FINE, *Science* 132, 43 (1960).

<sup>19</sup> B. S. McEWEN, V. G. ALLFREY, and A. E. MIRSKY, *J. biol. Chem.* 238, 758 (1963).

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*Riassunto.* Uova di Anuri (*Discoglossus pictus*) sono state trattate con istoni. Il trattamento con la frazione ricca in lisina (0,4-0,1 mg/ml) durante la segmentazione causa blocco alle prime divisioni. Con concentrazioni minori (0,05-0,025 mg/ml) lo sviluppo si arresta allo stadio di gastrula. La sensibilità al trattamento decresce notevolmente dopo la gastrulazione. La frazione ricca in arginina è risultata meno efficace.

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### Does Nitrogen Mustard Affect the Foetus Directly or Secondarily by its Effect on the Mother?

Since the teratogenic effects of nitrogen mustard on pregnant rats were first reported by HASKIN<sup>1</sup>, studies of these effects have been repeated by many investigators<sup>2-6</sup> and various kinds of malformation have been obtained in rat and mouse, especially skeletal ones. Developmental abnormalities in the rat were the most frequent when the compound was applied intraperitoneally 14 days after conception<sup>6</sup>.

It is not known whether the abnormalities arise from a direct interaction of the compound with foetal tissues, or whether they are an indirect effect due to the influence of nitrogen mustard on the mother<sup>7</sup>.

The purpose of the present experiment was to test whether nitrogen mustard (Antimit-'Pliva', Zagreb, N-methyl-bis( $\beta$ -chloroethyl)amine hydrochloride) directly affects the foetuses if applied as intrauterine injections into one uterine horn at the same period of pregnancy.

Female albino rats interbred at random in our laboratory were used. The animals were divided into two series. The first series of pregnant females was treated with 0.01% nitrogen mustard solution. The animals were subjected to laparotomy and the drug was injected early in the morning of the 14th day of gestation into the right uterine horn. It was usually injected in 2 or 4 conceptuses (0.05 ml per conceptus), exactly between the uterine wall and the foetal membranes. The left uterine horn was left untouched and served as the first control. The second series received 0.05 ml of sterile physiological saline in the same manner as described above and was used as the second control. Both groups of animals were killed on the 20th day of pregnancy. The foetuses were isolated and

fixed with Bouin's fixative for macroscopic investigation. The results of this experiment are summarized in the Table.

On the 20th day, autopsy showed in the untouched left uterine horns of the first series a number of normal foetuses (90), while in the treated right uterine horns 31 malformed foetuses were observed. All developmental abnormalities, such as exencephalia, brachygnathia etc., were similar to those obtained by intraperitoneal injection of the compound into the mother the 14th day after conception. In the second series of animals treated with saline solution, no malformation could be found among the treated foetuses. Only five foetuses of the treated right uterine horn were absorbed.

The results of these experiments demonstrate that direct injections of water soluble nitrogen mustard (0.01%) into one of the uterine horns of the pregnant rat induced remarkably gross abnormalities in the foetuses of the treated uterine horn only. The untreated horn of the uterus has never shown any malformed foetuses. Thus we may suppose that the compound directly affects the foetuses; this excludes one of our previous hypotheses that it primarily disturbs the normal functions of pregnant females and affects the foetuses due to this primary effect.

*Résumé.* On a analysé l'action locale de l'ypérite nitrée sur le développement embryonnaire du rat blanc. Cet agent a été administré par voie intrautérine et seulement dans une corne de l'utérus le 14ème jour de gravidité. A la base des déformations obtenues du foetus provenant de la corne traitée on peut conclure que l'agent affecte directement les foetus sans jamais porter atteinte à ceux de la corne non traitée.

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Series No.	No. of animals	Treated right uterine horn			Untreated left uterine horn
		No. of conceptuses on 14th day of gestation	No. of found foetuses	No. of malformed foetuses	No. of found normal foetuses
1	20	83	60	31 (51.6%)	90
2	15	76	71	-	80

<sup>1</sup> D. HASKIN, *Anat. Rec.* 102, 493 (1948).

<sup>2</sup> C. H. DANFORTH and E. CENTER, *Proc. Soc. exp. Biol. Med.* 86, 705 (1954).

<sup>3</sup> M. L. MURPHY and D. A. KARNOFSKY, *Cancer* 9, 955 (1956).

<sup>4</sup> H. NISHIMURA and S. TAKAGAKI, *Acta Sch. med. Univ. Kyoto* 36, 20 (1959).

<sup>5</sup> S. TAKAGAKI, *Acta anat. nippon.* 32, 248 (1957).

<sup>6</sup> M. MÜLLER and N. ŠKREB, *Experientia* 20, 70 (1964).

<sup>7</sup> M. MÜLLER, 4th Congress of Yugoslav Physiol. 49 (1965).