most of the studies have been made a long time after the stenosis was performed. To our knowledge, the only study made after a short time is that of MEERSON et al.[3], two days after the cardiac functional overload started. Swelling of mitochondria and vacuolization of the matrix have been observed after several degrees of ischaemia[5–7]. In this case, no increase of mitochondrial size was reported.

The modifications described in this paper are similar to those found after functional overload and ischaemia. It is probable that they are the result of both kinds of stimuli. However, what was striking was the rapidity of the alterations. Although some of them, such as the swelling, could reasonably occur in a few minutes, the fusion and appearance of giant mitochondria indicate in our opinion a true increase in mitochondrial mass, with active reproductive phenomena. To our knowledge, no biological system has yet been reported where such a rapid increase in mitochondrial mass occurs.

Apparently, the myocardial mitochondria possess the ability to reproduce in a short time, in response to stimuli that produce an acute functional overload.

Zusammenfassung. Eine deutliche Vermehrung der Mitochondrienzahl und -grösse in den Herzmuskelzellen wurde in Hunden durch erzwungenes Schwimmen (rasche und erschöpfende Leistung) festgestellt.

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Inhibition of Amphibian Egg Development by Histones

The histones are the basic nuclear proteins of animal and plant cells. They seem to be involved in the regulation of the gene activity[1,2]. However, some recent reports suggest a more important role in this respect for the non-basic nuclear proteins[3]. Biochemical evidence shows that histones inhibit DNA-primed RNA synthesis in pea seedlings, mammalia and bacteria[4–8]. Moreover, the activity of DNA-polymerase is diminished by the addition of histones at high concentrations[9–11]. This inhibitory effect is probably related to the formation of a DNA-histone complex, which is unable to act as 'primer' for the nucleic acids synthesis[4,12]. On the other hand, selective removal of the histones from the nucleus by mild trypsin treatment enhances *m*-RNA synthesis[5]. Very similar effect is obtained by acetylation and methylation of histones, and this reversible mechanism could be responsible for the regulation of genes in vivo[13].

MOORE[14] suggests that, during early embryonic development, before gastrulation, most of the structural genes are 'turned off' because they are closely associated with histones. After gastrulation, the genes are 'turned on' by changes in the DNA-histone association. In this respect the treatment of amphibian eggs by histones greatly inhibits gastrulation[15].

We treated the eggs of Anurans (*Discoglossus pictus*) with lysine-rich (HL) and arginine-rich (HA) fractions of calf thymus histones[16]. The eggs treated with 0.4–0.1 mg/ml of HL fraction at two blastomeres are blocked during cleavage; with 0.05–0.025 mg/ml the development stops at the gastrula stage.

In the first case the results are likely to be related to the inhibition of DNA-polymerase reaction. At lower concentrations, however, only the DNA-primed RNA-synthesis could be affected, and development stops at gastrulation, when new molecules of *m*-RNA are required for the differentiation[17]. After the gastrula stage, the eggs become relatively insensitive and the effects of the treatment are unimportant. The HA fraction has the same effect but at higher concentrations. The protamine from salmon milt is as effective as HL fraction.

With shorter treatment (2 h) between two blastomeres and late blastula stages, 0.05–0.025 mg/ml HL is able to inhibit the development: only a few eggs gastrulate and form abnormal tadpoles.

The developmental abnormalities are remarkable in every embryonic apparatus (microcephaly, cyclopia, adhesive organs fused, olfactory organs absent, vacuolar notochord, spina bifida and external intestine). At gastrulation the inhibitory effects begin at concentrations of 0.2 mg/ml, and the tadpoles have greatly reduced tails. After this stage only 10 h treatment can produce functional abnormalities in the tadpoles. The marked inhibitory effect on morphogenesis produced by brief treatment with histones from cleavage to the blastula stage, suggests that

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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[18–20].

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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[1], studies of these effects have been repeated by many investigators[2–6] 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[6].

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[7].

The purpose of the present experiment was to test whether nitrogen mustard (Antimit-'Pliva', Zagreb, N-methyl-bis(β-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 laparatomy 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.

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

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 embryonaire 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 fœtus provenant de la corne traitée on peut conclure que l'agent affecte directement les fœtus sans jamais porter atteinte à ceux de la corne non traitée.

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