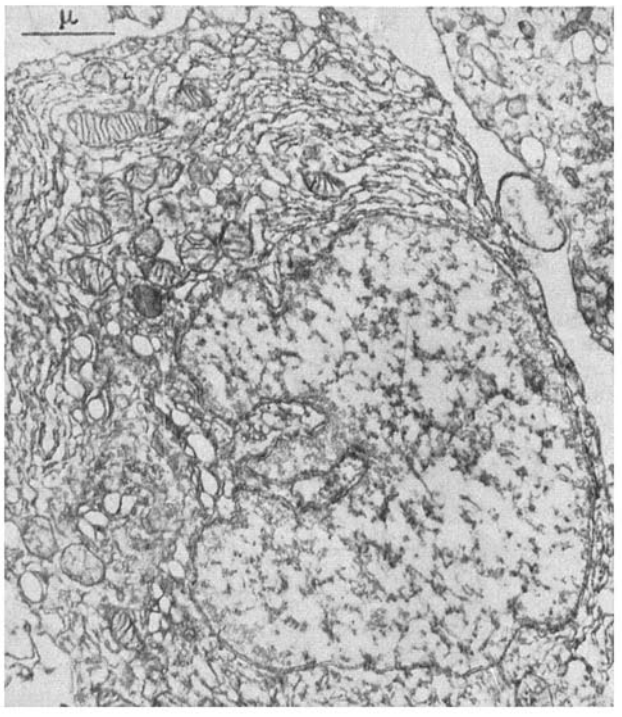

Fig. 2. Retikuläre Zellen nach Inkubation mit RNS aus Kalbsmilz. 40% der retikulären Zellen wiesen am Ende der Inkubationszeit ein solches morphologisches Bild auf.

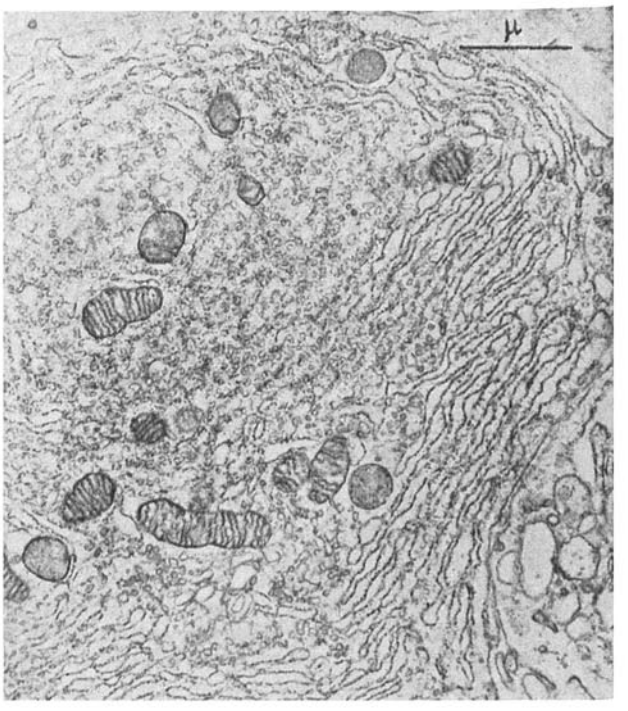

Fig. 3. Bild des endoplasmatischen Retikulums und der Mitochondrien in einer retikulären Zelle am Ende der Inkubationszeit in Anwesenheit von RNS.

Mitochondrien hatten unausgebildete Cristae, viele Ribosomen erschienen unregelmässig im Cytoplasma verstreut, das endoplasmatische Retikulum fehlte fast vollständig, und schliesslich fanden sich im Cytoplasma grössere Abschnitte von homogenem Material, oft von gut sichtbarer Membran umgeben (Figur 1).

Das Zellmaterial aus Kulturen ohne RNS bestand nach 72 h aus 93% indifferenzierten retikulären Zellen, 6% Granuloblasten und 1% Erythroblasten. Morphologisch stimmten die 750 elektronenmikroskopisch überprüften retikulären Zellen völlig mit denen vor Beginn der Kultur überein.

Nach Kultur in RNS enthaltendem Medium bestand das Zellmaterial am Ende der Inkubationszeit aus 97% retikulären Zellen, 2% Granuloblasten und 1% Erythroblasten. Von den 750 überprüften retikulären Zellen erschienen 28% identisch mit denen vor Anlage der Kultur, 32% zeigten unbedeutende Änderungen, während die restlichen 40% klare morphologische Abänderungen vom Ausgangsstadium aufwiesen. In diesen Zellen waren stark ausgebildete ergastoplasmatische Bildungen vorhanden (Figur 2). Die Mitochondrien waren in zwei Hauptformen vertreten: einige von ihnen hatten regelmässige und deutlich ausgebildete Cristae, die bei anderen kaum angedeutet waren (Figur 3).

Die in RNS-freiem Medium kultivierten leukämischen retikulären Zellen zeigten somit keine Fähigkeit zu einer Umwandlung oder Entdifferenzierung. Bei einem Teil der in Anwesenheit von RNS aus Kalbsembryonenmilz kultivierten Zellen wurden morphologische Aspekte festgestellt, die, wenn auch nicht identisch, so doch dem Bild der retikulären Zellen des normalen hämopoetischen Gewebes sehr ähnlich waren.

Riassunto. Vengono descritte le modificazioni morfologiche, rilevate al microscopio elettronico, delle cellule reticolari leucemiche coltivate in presenza di ARN estratto dalla milza di embrioni di vitello.

S. ESPOSITO

Clinica Medica dell'Università, Pavia (Italia),
10 ottobre 1963.

Does Nitrogen Mustard Mimic the X-Ray Effects in any Case?

The effects of mustard gas on mammalian embryogenesis has been described many times[1–4] and attention has been called, especially, to malformations of the feet. As far as other animals are concerned, BODENSTEIN[5] has made a detailed analysis of its effects on amphibian[6] and insect development. Since this compound is often called radiomimetic and 'mimics malformations produced by radiation'[7], we thought it useful to analyse its effect on brain development in rats and to compare with the data of HICKS[8] following X-ray irradiation. According to HICKS[8], the precursors of the neurons seem to be very radiosensitive, while the neurons and cells that are actually in mitotic stages are selectively spared. Therefore the so-called rosettes of the mitotic cells start to form rapidly after radiation. The same regenerative process, whereby rosettes were formed, was observed by one of us ((N. Š.) after heat shock in rat embryos[9]. Most attention will be given to histological analysis of the brain development in experiments to be reported here.

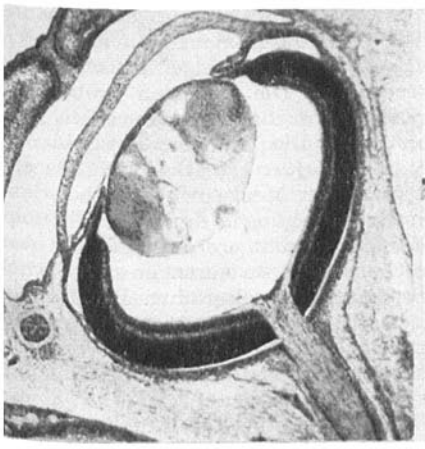

Fig. 1. Frontal section of the eye. The centre of the lens is highly necrotic.

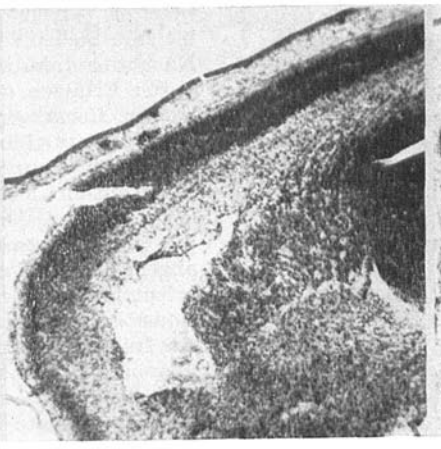

Fig. 2. Frontal section. Well limited cavity after necrosis in telencephalon. (Treatment on day 12.)

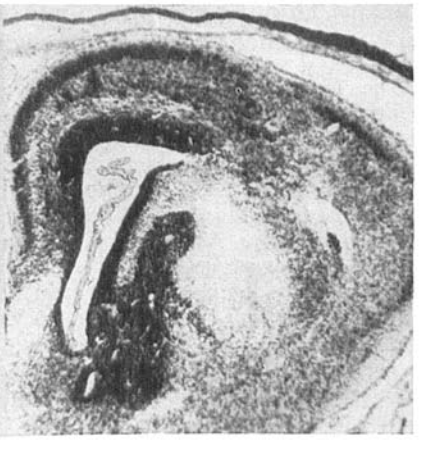

Fig. 3. Frontal section of the brain. Necrotic centre in striatum. (Treatment on day 14.)

Nitrogen mustard (Antimit–'Pliva', Zagreb, N-methyl-bis-(β-chloroethyl)amine hydrochloride) was administered by a single intraperitoneal injection in an amount equivalent to about 1 μg/g of body weight on the 12th and 14th day of gestation. All female rats were killed on day 20, and foetuses isolated, fixed, and serially sectioned.

Although many malformations of the feet and the head (cleft palate and exencephalia), which confirm the data of other investigators, have been observed, only the changes of the eye and the brain will be analysed here.

On day 12 the number of resorbed embryos was still very high so that the percentage of malformation seemed to be greater than on day 14. For this analysis only such specimens were chosen which had no visible malformation of the head. Therefore these foetuses can be considered as only slightly affected by the agent used. The same principle was followed when the histological analysis of the head after heat shock has been performed[9]. It is possible to show by histological analysis that the brain and the eye had been damaged but in a manner different from that reported after X-rays and heat shock. The lens showed more or less developed necrotic centres and the necrosis in the brain formed some well limited cavities. These necrotic centres were present in the telencephalon in the anterior and lateral walls of the lateral ventricles. On day 14 the lens was normal, while the brain was injured in the same way as on the previous day, only the sites of the injury were in the majority of cases in the striatum. Sometimes necrosis was observed even in the diencephalon.

We were unable to find any formation like the rosettes and regeneration seemed not to occur after the doses mentioned of nitrogen mustard. The neuroectoderm seemed to be inhibited, contrary to the effect of X-rays, and could not restore the brain damage.

As far as the eye was concerned, both X-rays and heat shock caused rosette formation in the retina. Nitrogen mustard did affect the lens and caused necrosis, but did not elicit any rosette formation.

The absence of rosettes after mustard gas is the most striking difference compared with the effect of X-rays. As regards the sites of necrosis they were similar in both cases, but the subsequent processes appeared to be quite different. Regeneration, which occurs after X-rays, seems to be absent following a single administration of nitrogen mustard.

The differences in biological effect between the two agents mentioned have been noted also in other systems, especially on megakaryocytes developing *in vitro*[10].

Summarizing, we may say that the nitrogen mustard mimics the X-ray effects only roughly, and that there are many dissimilarities in their effects on brain development. As far as the rosette formation is concerned, the heat shock is more 'radiomimetic' than the nitrogen mustard.

Day of gestation	No. of females	No. of found embryos	No. of malformed embryos	%
12	25	66	54	81.8
14	25	172	54	31.3

Résumé. Les effets de l'ypérite nitrée sur le cerveau et les yeux de l'embryon du rat blanc ont été analysés. Seulement les embryons présentant un aspect normal ont été coupés et colorés. De larges centres de nécrose ont été trouvés sur les coupes tandis que les «rosettes» n'ont pas pu être décelées.

Bien que connue comme radiomimétique, l'yperite nitrée ne mime pas les rayons X en ce qui concerne la formation des «rosettes» dans le cerveau.

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Institute of Biology, Faculty of Medicine, Zagreb (Yugoslavia), September 30, 1963.

[1] D. Haskin, Anat. Rec. *102*, 493 (1948).
[2] C. H. Danforth and E. Center, Proc. Soc. exp. Biol. Med. *86*, 705 (1954).
[3] H. Nimishura and S. Tagakaki, Acta School. Medicin. Univers. in Kioto *36*, 20 (1959).
[4] A. Jurand, J. Embryol. exp. Morph. *9*, 492 (1961).
[5] D. Bodenstein, J. cell. comp. Physiol. *43*, Suppl. 1, 179 (1954).
[6] K. Montagu, Exp. Cell Res. *21*, 626 (1960).
[7] S. P. Hicks, J. cell. comp. Physiol. *43*, Suppl. 1, 151 (1954).
[8] S. P. Hicks, Physiol. Rev. *38*, 337 (1958).
[9] N. Škreb and Z. Frank, J. Embryol. exp. Morph. *11*, 445 (1963).
[10] M. C. Berenbaum and M. Calley, Nature *196*, 656 (1962).