capacity, and within several weeks, a wasting syndrome in neonatally thymectomized mice[8,9]. It is uncertain whether the demonstrated retardation of the ossification and the generalized bone atrophy, with the considerable alteration of the oxygenic processes, are the result of the depressed immunological capacity or of the complex and not exactly detected processes effecting the wasting syndrome. This defect in the growth indicates that neonatal thymectomy and the developing organism is suitable for the study of the antagonistic correlation[10] between the thymic function and the growth. On the other hand the lack of thymic function should be considered in the interpretation of those diseases, which are due to the defect of the epiphyseal plates.

Zusammenfassung. Es wurde eine Präponderanz der neutralen Mukopolysaccharide in der Epiphysealplatte der neonatal thymektomierten Mäuse beobachtet und das Phänomen diskutiert.

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Vertebral Malformations in Chicks Caused by X-Radiation During Their Embryonic Development

It is known that periods of higher sensitivity to the action of environmental factors may be stated in the development of an embryo as well as of buds of its particular organs. The periods of higher sensitivity of the embryo are often the moments at which hereditary information programming the development of the given organs are realized. Detection of those periods and the manner of the embryo's response to the acting factor is a necessary condition for understanding teratogenesis.

In the experiments presented the authors have decided to determine the periods of higher sensitivity in the development of the vertebral column in hens by means of X-radiation.

Newly laid eggs (150) of White Leghorn hens were divided into 5 equal groups (A–F) and incubated at 38 °C, air humidity being 65%. The eggs were turned 5 times in the course of 24 h. The separate groups of eggs were exposed to a single dose 500 r X-radiation (obtained from the apparatus DOF, 55 kV, 7 mA, without filtre) after 25 (group A), 42 (group B), 52 (group C), 65 (group D) and 72 (group E) hours of incubation, i.e. during the formation of somites[1]. In the 19th day of incubation the embryos were killed and fixed in alcohol.

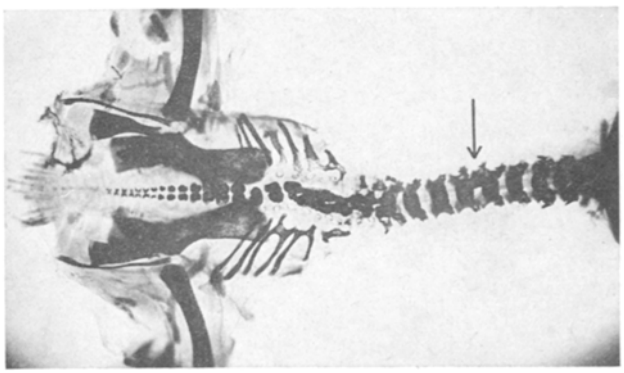

Fig. 1. An embryo of the group B. The arrow in cervical region points and additional half-vertebra. Thoracic vertebrae are deformed and fused. The ribs associated with them are either fused along their whole length or only at their base. The centra of the last thoracic and presacral vertebrae are split out.

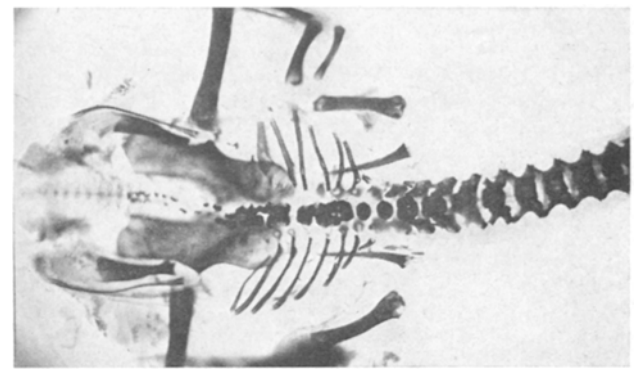

Fig. 2. An embryo of the group C. Thoracic vertebrae are deformed and interfused. In presacral region the centra and arches are not developed.

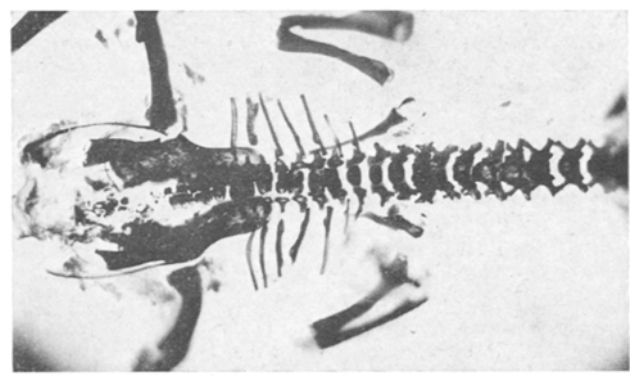

Fig. 3. An embryo of the group C. The centra of thoracic and two before last cervical vertebrae are fused. In presacral region the vertebrae are deformed and fused, the sacral and caudal regions are lacking the vertebral buds.

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The skeletons of the embryos were stained with red alizarin in 2% potassium hydroxide and cleared in a mixture of potassium hydroxide with glycerine.

The control series consisted of 40 skeletons of non-irradiated embryos incubated under identical conditions. No vertebral malformations have been stated in embryos of the control series.

Of 150 experimental embryos 92 died after irradiation and 48 continued their development. The highest mortality rate was observed in the group A (only 5 embryos survived); in this series no vertebral malformations were found, whereas they occurred in more than 60% of embryos belonging to the remaining groups. The malformations consisted (among others) in deformation of centra and arches and in fusion of the deformed vertebrae (Figures 1, 2 and 3). In some cases the vertebrae were fused to such an extent that it was difficult to determine their number. The ribs associated to those vertebrae were fused along their whole length (Figures 2 and 3) or only their bases were fused (Figure 1). The split out centra forming 2 separate ossifications were pretty frequently observed (Figures 2 and 3), the presence of wedge-shaped half-vertebrae without the symmetrical halves being less frequent (Figure 1). Sometimes the vertebral malformations were very extensive. In extreme cases the development of farther regions of the vertebral column was even stopped (Figure 3).

Results of these experiment show that the period of higher sensitivity to X-rays occurs in the development of hen embryos during the formation of somites causing vertebral malformations. It is probable that, in hens as in other vertebrates[2,3], it is also the period of sensitivity to other agents.

The period of sensitivity to X-rays observed in hens is more or less identical with analogical period occurring in mammalians[4–6].

As it follows from the experiments, the vertebral malformations obtained are chiefly the results of disturbances in formation and arrangement of the embryo's somites. Part of those malformations may, however, be due to the disturbances in the development of the notochord. If this development is disturbed or the notochord is broken, we observed irregular fusions and deformations of the vertebrae. In extreme cases farther region of the vertebral column do not develop.

Zusammenfassung. Bestrahlung befruchteter Eier des White-Leghorn-Stammes zwischen der 25. und 72. Bebrütungsstunde (38 °C). Einmalige Bestrahlungen mit einer Dosis von 500 r genügten zur Erzeugung charakteristischer Missbildungen der Wirbelsäule, wobei sich die Zeit der Segmentierung als eigentlich sensible Phase erwies.

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Preparation of Isolated Macroconidia of *Neurospora crassa*

In a kinetic study of the metabolism of conidiation in *Neurospora crassa*, it is necessary to obtain an inoculum as homogeneous possible, that is to say, to have an isolated conidial population in an uniform physiological condition. In effect, the simple collection by immersion of a conidiated culture gives a heterogeneous suspension sometimes containing mycelial fragments and isolated conidia in small chains. A gentle scraping of conidiated culture, followed by a filtration on muslin nylon does not eliminate sufficiently the mycelial fragments ('Filtration method').

One of the first obstacles in a good preparation of conidia is their strong hydrophobic character related to the presence of a peripheral lipoidal thin layer detected by electron microscopy[1]. To overcome this difficulty, we have adopted the use of a non-ionic detergent which offers the advantage without affecting, in any way, the viability of the conidia; this has been verified by different tests. The method is as follows.

The agarized minimal WESTERGAARD and MITCHELL[2] was supplemented with $10^{-3}M$ glycine. The addition of glycine favoured an intense and regular conidiation an entire surface of the petri plate[3]. The plates were spread with 0.2 ml of a conidial suspension (10^{-5} conidia/ml) and incubated for 4–5 days at 25 °C. The plates were then placed overnight at 4 °C. This thermal shock favoured the detachment of the conidia from their conidiophores, subsequently they were brought to atmospheric temperature and delicately covered with 10 ml sterile distilled water, followed by a gentle shaking of the submerged conidial mass. The immersion of the plates protect the dispersion of the conidia in air.

The conidial suspensions from plates were aseptically collected (about 4–6 plates gives a suspension quantitatively sufficient), added with 1/5000 of detergent (polyethoxyether d'alkylphenol) and agitated for 1 h at room temperature. The conidial suspension was then filtered through a sterile nylon cloth which was rinsed with 20 ml sterile water. The filterate containing conidia in suspension was centrifuged for 10 min at 3000 *g*. The supernatant was discarded and the conidial pellet was washed 5–6 times with 10 ml sterile water.

A little fraction of the conidial pellet was suspended in a known volume of sterile water and the number counted in a 'Thoma' hematocytometer; the other part of a conidial pellet was suspended in a sterile sucrose solution ($0.45M$). This suspension, containing 10^6 to 10^7 conidia/ml, was centrifuged horizontally for 30 min at 350 *g*. The supernatant was collected carefully and the pellet constituting mainly of mycelial fragments and some conidial

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