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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chains was resuspended in sterile sucrose solution and centrifuged. The whole procedure was repeated several times. The supernatants from different centrifugations giving the same percentage of conidia on counting (90–95%), were pooled together and centrifuged at 3000 *g* for 20 min. The conidial pellet so obtained, was washed 3 times with sterile distilled water.

The mainly mycelial pellet of the 0.45 *M* sucrose centrifugation, on the other hand, was resuspended in 0.6 *M* sterile sucrose solution for liberation of conidia still retained in the mycelial mass and centrifuged as described above. The increase in the concentration of sucrose is necessary for the variation in density of mycelial fragments and conidia, after a stay in a first sucrose solution; this finally helps to obtain good separation between the diverse elements. This method when repeated many times produces a pellet with high percentage of free conidia (over 90%). This conidial preparation was mixed with the one obtained with 0.45 *M* sucrose solution ('Sucrose method').

The conidia so obtained constituted a starting material for the inoculation of fresh medium and for the analysis of enzymatic and proteinic activity. The following Table presents an average value of many counts and results from 6 experiments of conidial isolation.

The procedure is relatively simple and rapid, and permits the collection of free conidia in an uniform physiological and pregermination stage. This method is similar to the isopycnic gradient centrifugation, method used for obtaining yeast cells in their synchronous developmental state (MITCHISON[4], and Dr. J. DESHUSSES, personal communication). By dilution plate technique, a classical bacteriological method, we verified the synchronism of growth of isolated conidia.

With this method, we obtained a homogeneous inoculum, as good from the point of view of morphology and physiology for the experiments on the dynamics of *Neurospora* growth. On the other hand, we have now the possibility to analyze a population of conidia which does not present more than 10% of contamination from which half consists of conidia in pairs or chains. The fragments of mycelia represent only 5% of the entire population. Thus we can consider the results acquired as significant from the analytical point of view[5].

Results from preparation of isolated macroconidia

Type of cells	'Sucrose method' Number/ml	%	'Filtration method' %
Total of 'cells'	7.80×10^7	100	100
Free conidia	7.05×10^7	90.3	55–60
Conidial chains and mycelial fragments	0.75×10^7	9.7	45–40

Résumé. Une méthode simple d'obtention de conidies libres et isolées de *Neurospora crassa* est décrite ici. Après une culture de 4 jours du champignon sur milieu minimal solide additionné de glycine, les conidies sont récoltées; elles sont suspendues dans de l'eau stérile contenant un détergent non-ionique, puis filtrées et soumises ensuite à des centrifugations à basse vitesse dans une solution saccharosée. Les surnageants donnent alors une suspension à fort pourcentage de conidies libres et isolées.

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Reversion of α- into β-Hemolysis of *Diplococcus pneumoniae* by Low Concentration of Optochin

The stimulation of bacterial hemolysis by low concentration of some antibiotics is already known. STORY[1] reported his observations on the stimulation of hemolytic activity of *Diplococcus pneumoniae* by subbactericidal concentration of penicillin as early as 1953. Recently, LÖFSTRÖM, HALLANDER and LAURELL[2–4] as well as KIENITZ, RITZERFELD and GRÜN[5], and RITZERFELD, WINTERHOFF and KIENITZ[6], described the enhancement of the production of staphylococcal α-hemolysis by penicillin. The potentiation of hemolysis of some gramnegative bacteria by low concentration of penicillin and other antibiotics was noticed also. Recently, it was observed to happen also at low concentration of kanamycin and gentamicin (unpublished).

Here, the reversion of α into β-hemolysis in *Diplococcus pneumoniae* under the influence of low concentration of optochin is reported. The phenomenon was observed in 2 strains of *D. pneumoniae* isolated from sputum and in several strains isolated from liquor cerebrospinalis and pus. All strains appeared to be typical *D. pneumoniae*. The optochin test was made on 8% horse blood agar, the thickness of which was approximately 1 cm. 'Oxoid' optochin discs were used. The zone of inhibited growth around the disc varied from 2–3 cm in most cases. Following the usual overnight incubation at 37 °C, a narrow (ca. 2 mm in diameter) clear ring of β-hemolysis was visible at the border of the inhibition zone of optochin. Outside this ring of β-hemolysis, all colonies of *D. pneumoniae*, including those growing at the edge of the inhibition zone of antibiotics and sul-

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