[where $\zeta_0$, $\zeta_t$, $\zeta_\infty$ are respectively the relative viscosities immediately after irradiation ($t = 0$), at time $t$ and infinity] thus allowing the evaluation of the initial loss and the experimental rate constant governing the decay.

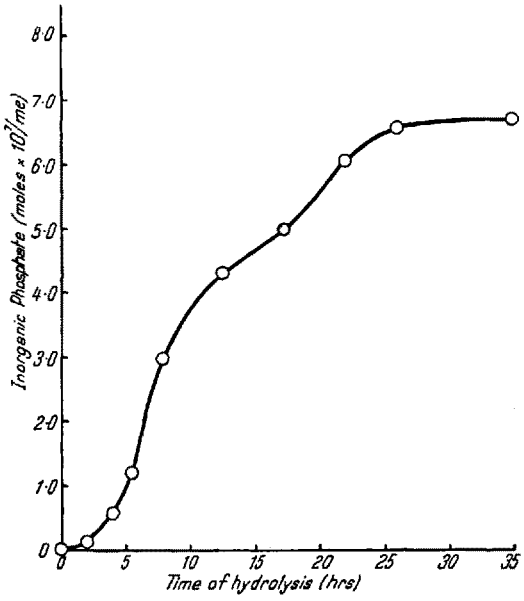


Fig. 3.–Irradiation of aqueous solutions of D.N.A. (0·1% w/v) with X-rays (200 kV) in the presence of oxygen (1 atm). Release of inorganic phosphate on hydrolysis (90°C; pH 6·8). Total dose = $3{\cdot}56 \times 10^4$ rep.

All these observations are not inconsistent with the assumption that irradiation in the presence of oxygen leads in part to a random labilisation of certain bonds in the polynucleotide chain (e.g. carbon-phosphate linkages) which may then undergo slow hydrolytic cleavage resulting in a post-irradiation viscosity loss. In the absence of oxygen, however, it appears that only immediate fracture of the chain takes place, this manifesting itself in the high initial viscosity loss.

In order to obtain some further insight into the chemical nature of the after effect, investigations have been carried out on aqueous solutions of some ribonucleotides. In particular, it has been found that, in addition to formation of inorganic phosphate on irradiation, hydrolysis at 25°C leads to the production of further amounts of phosphate at a measurable rate. This hydrolysis, which can be attributed to the formation of labile phosphate compounds during irradiation, was found to be of first order over the whole range of pH (0·5–13·0) and also exhibited a characteristic variation with pH. The rate and the extent of phosphate release was unaffected after freeze-drying. Hence, here again it is apparent that this process does not involve secondary oxidations by hydrogen peroxide. The pH-variation of the rate constant of the hydrolysis reaction suggests that the lability of the nucleotide is probably due to the formation of an activating carbonyl group in the sugar component[1]. When the irradiations were carried out in the absence of oxygen, some labile phosphates were still formed, though to a smaller extent; the rate constant of the hydrolysis was identical with that found in the oxygenated solutions.

[1] A. Desjobert, Thesis (Paris 1951); Bull. Soc. Chim. *111*, 809 (1947). – D. E. Koshland, J. Amer. Chem. Soc. *74*, 2286 (1952).

Similar experiments with D.N.A. and also with ribonucleic acid solutions have also shown some interesting features. Very little post-irradiation phosphate release could be observed at 25°C; this result is not unexpected since chain fission, following oxidative attack on a sugar component which is initially diesterified, would involve the formation of a phospho-monoester, except when attack occurs at an end group. On hydrolysis at 90°C, however, the yield of phosphate release from nucleic acids was increased to many times the initial value; this hydrolysis was acid and base-catalysed and exhibited a complex kinetic behaviour. A typical hydrolysis curve for an irradiated D.N.A. solution is shown in Fig. 3.

These observations demonstrate clearly the presence of labile phosphate bonds in irradiated solutions of nucleic acids.

A full report of this work is in course of preparation.

We are indebted to Professor R. Signer (Berne) for samples of D.N.A. Our thanks are due to the Northern Council of the British Empire Cancer Campaign and to the Rockefeller Foundation for financial support.

M. Daniels, G. Scholes, and J. Weiss

*University of Durham, King's College, Newcastle-upon-Tyne, 1, February 28, 1955.*

*Zusammenfassung*

Die Wirkung von Röntgenstrahlen (200 kV) auf wässerige Lösungen von Nukleinsäuren und Nukleotiden wurde unter besonderer Berücksichtigung des Einflusses von molekularem Sauerstoff und Wasserstoffsuperoxyd untersucht. Der so gefundene, nach der Bestrahlung einsetzende Abfall der Viskosität von D.N.A.-Lösungen (after-effect) kann als Hydrolyse der durch die Bestrahlung labilisierten Kohlenstoff-Phosphat-Bindungen angesehen werden. Untersuchungen über die Hydrolyse von bestrahlten Nukleotiden, -P.N.A.- und D.N.A.-Lösungen stehen in vollem Einklang mit obiger Annahme.

## Living Smears from Endosperm

Studies on the endosperm *in vivo*[1] show clearly that it is excellent material for experimental researches on mitosis in the living cell.

The advantages of endosperm in comparison with other plant material are great: the course of mitosis is much better visible than in other tissues, the lack of cellulose walls allows not only better penetration of chemical substances, but also polarization and micrurgical studies. It seems probable that it may be used as a modification of the *Allium* test *in vivo*. Among genera studied so far: *Iris, Leucojum, Haemanthus, Clivia* and *Colchicum* appeared to be the best, although *Ornithogalum, Zephyranthes, Helianthus* and *Daphne* genera are also very suitable. A longer list with some methodical notes is given elsewhere[2]. It is evident that the endosperm of some species appeared to be much better than that of others, e.g. *Allium cepa, Vicia faba* and *Secale cereale* are not suitable at all. However, hundreds of plants are excellent–so that the material with the exact properties required so far as chromosome dimensions,

[1] A. Bajer, Acta Soc. Bot. Poloniae *22*, 267 (1953). – A. Bajer and J. Molè-Bajer, Acta Soc. Bot. Poloniae *23*, 69 (1954). – A. Bajer, Acta Soc. Bot. Poloniae *23*, 383 (1954).

[2] A. Bajer and J. Molè-Bajer, Acta Soc. Bot. Poloniae *23*, 69 (1954).

their number, duration of mitosis, etc., are concerned, may be chosen.

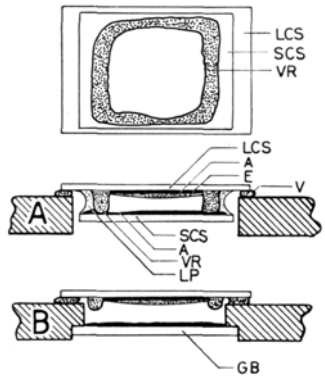


Fig. 1*A*.–Cross section of two cover slips and the holder and *B* of the chamber for observations of endosperm *in vivo*. Above *A* the top view of cover-slips with the pressed out endosperm. *VR* vaseline ring, *LCS* large cover slip (24 × 32 mm), *SCS* small cover slip (24 × 24 mm), *A* layers of agar, *LG* liquid paraffin (instead of liquid paraffin hot vaseline may be used), *E* endosperm with different quantity of liquid surrounding it, *H* metal holder to which cover slips are fixed with the vaseline *VGB* glass bottom. N.B.: *A* and *E* (agar and endosperm layers) are not drawn to scale. Actually they are much thinner.

The great value of endosperm is in the method of handling it, which is simple and quick. 3–6 weeks after pollination, according to the species, the endosperm is in a suitable stage of development, and, if the plant material is properly cultivated, may be available for several months in the year e.g. *Clivia* and *Haemanthus*. The preparation is made in the following way: on a large clean cover slip a 1–2 mm thick vaseline rectangle or ring of about 2 cm diameter is made and then the area inside the ring is smeared with hot agar with the proper sugar concentration. The endosperm is then pressed out with a small quantity of liquid surrounding it onto the agar to form a thin hanging drop and the whole is covered with the second cover slip also smeared with agar. The only reason for the agar on the second cover slip is to prevent the evaporation of the liquid surrounding the endosperm. The edges of the two cover slips are sealed with the hot vaseline or liquid paraffine (Fig. 1). If an inverted microscope is not used, the whole preparation is inverted and mounted on a suitably cut metal holder, 2–3 mm thick. The largest microscope magnifications may be used without difficulty. Agar 0·4–0·5 % and glucose 3–4·5 %, depending on the species, is suitable. The differences of 0·5 % glucose concentration are important, and the suitable one is found experimentally. If the hanging drop is very thin the endosperm begins to flatten and the degree of this process depends on the amount of the pressed-out endosperm. Living cells and mitotic spindles may be flattened almost to chromosome thickness, and, although they look like smears, this does not check the continuation process of the cell division (Fig. 2). It is evident that too severe flattening leads to disturbances and to death. The longer the process of mitosis, the slower should be the process of flattening, i.e. it should last 1–4 h depending on the species.

The same results as in the above-described two cover slip method are obtained if a special chamber with a glass bottom is used (Fig. 1). Oxygen atmosphere used previously[1] seems to be unnecessary. Some observations suggest, however, that oxygen as well as light may influence the mitosis in endosperm, especially the prophase, e.g. strong light stops the prophases of *Haemanthus* and usually all stages of *Crambe*. Probably there is also a correlation between light and oxygen. The above-mentioned difficulties, however, arose only in a few subjects. This method gives good results for most of the species investigated so far. The method, which has been improved during the last few years, suggests optimistic prospects. A short time ago, the author wrote[1]: "...the main defect of Haemanthus endosperm is that the normal course of mitosis can be observed only for a short time (usually 6 h) after preparation—and most cells die in prophase." Now cells entering and finishing the normal course of mitosis more than 60 h after preparation have been observed and most probably this time would be longer if sterile conditions could be attained. The author is deeply convinced that further improvement of this method is possible, even to a point where cell tissue cultures having the properties of animal tissue culture, i.e. creeping and growing in one layer, will be obtained from endosperm.

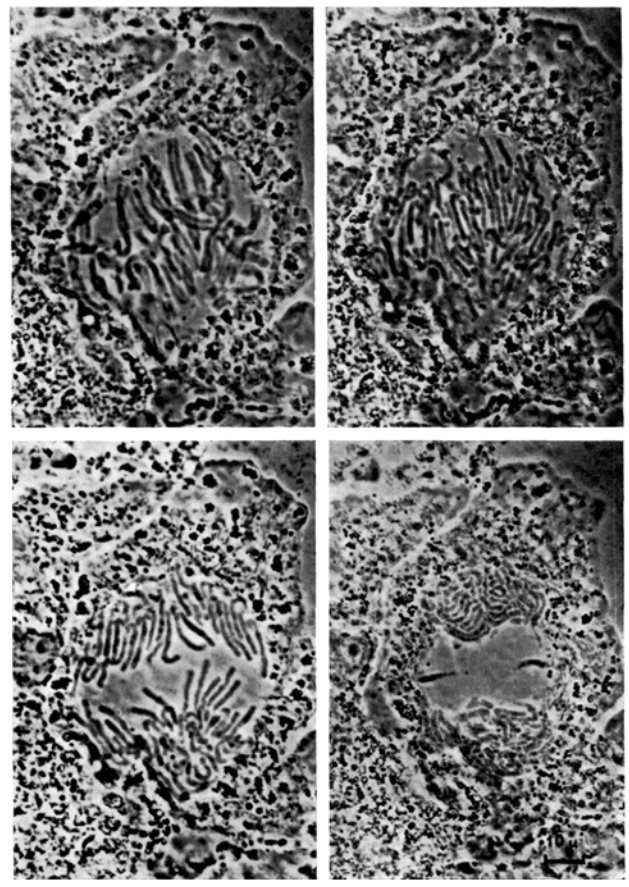

Fig. 2.–Anaphase in flattened cell of *Leucojum aestivum*. Chromosomes are arranged almost in one layer. Phase contrast illumination.

A. BAJER

*Laboratory of Plant Physiology, Jagellonian University, 53, Grodzka, Cracow, Poland, February 26, 1955.*

*Zusammenfassung*

Eine einfache Methode wird beschrieben, welche die Beobachtung der Endospermmitose *in vivo* erlaubt. Dabei ist es möglich, lebende Mitosespindeln stark abzuplatten und so den weiteren Ablauf des Teilungsprozesses in allen Einzelheiten zu verfolgen.

[1] A. BAJER, Acta Soc. Bot. Poloniae *23*, 383 (1954).

[1] A. BAJER, Acta Soc. Bot. Poloniae *22*, 475 (1953).