is due to direct effects and hence conclude that indirect effects in broth are relatively small.

This phase state phenomenon with supercooled suspensions offers a new parameter that is being used in connexion with other modifying agents to study the mechanism of cell damage by radiations.

This investigation was aided by grants from the U.S. Atomic Energy Commission and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago, and was carried out in the laboratory of Prof. R. E. Zirkle.

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A Technique for combining the Staining of Fixed Nuclear Material with the Subsequent Separation of Plant Tissue into Individual Cells

Following an earlier investigation[1], a further study has been made of the mode of action of *iso*-propyl-phenylcarbamate. In order that the observed inhibitory effects on the elongation of *Zea mays* roots could be interpreted in terms of cell division and cell extension, it was necessary to evolve a technique which coupled staining of the nuclear material with the separation of root tips into the individual component cells. The method, developed by Brown[2] for *Pisum sativum*, of gently bombarding carmine-stained root tips with glass beads, failed with maize roots to give adequate cell separation unless the period of bombardment was greatly prolonged, and then too many of the cells were damaged; also the differential staining of the nucleus and cytoplasm was not good enough to allow of the detection of the earliest stages of prophase. The alternative of using a pectinase[3] was then investigated, and eventually the following procedure for the separation of previously stained tissue was found to give excellent results if 'Pectinase' (Nutritional Biochemicals Company, Ohio) was employed.

The roots were first fixed in a mixture of 3 parts absolute alcohol and 1 part glacial acetic acid and stained by the Feulgen technique[4]. After washing in distilled water, the excised root tips were placed in a small volume (for example, 0·5 ml. for ten root tips 1 mm. long) of 2 per cent 'Pectinase' made up in distilled water, adjusted to *p*H 6·6 and saturated with toluol to minimize contamination by micro-organisms. The segments were left in the dark in the 'Pectinase' solution for 12 hr., and at the end of this period most of the 'Pectinase' was removed by a pipette and replaced by an equal volume of 3 per cent solution of a commercial pectin product ('Certo'). By repeated sucking and blowing the tips through a pipette with a fine orifice, the required state of division could be achieved; in order to prevent a loss of cells due to their sticking to the inside of the pipette a very slow intake was followed by vigorous ejection. When a drop of such a suspension was examined under high magnification (× 1,500), it was found that the cells were either completely separated or grouped into small masses of 2, 4, 8, 16 or, very rarely, 32 cells. There was no sign of damage to the individual cells, and all mitotic stages including early prophase were easily recognizable.

Since 'Pectinase' has also been found effective for the separation of fixed cells in both mature root tissue of maize and the meristems of *Vicia faba*, it is likely that this technique of combined staining and cell separation will be generally applicable.

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Histological Demonstration of Alkaline Phosphatase and Non-specific Esterase by Azo-dye Methods

Histochemical methods[1,2] already described are capable of greater sensitivity with reduced artefact formation when employed in a manner similar to that recently reported for the demonstration of acid phosphatase[3]. Increased sensitivity results from enhanced enzyme activity at an incubation temperature of 37° C., demonstration of weak foci of activity in tissues being greatly facilitated. Artefacts resulting from diffusion of enzymically liberated alpha-naphthol is avoided by increasing the speed of azo-coupling and by restriction where necessary of the rate of substrate hydrolysis. Hydrolysis is retarded preferably by reduction of the substrate concentration; this has the advantage over refrigeration of the incubation medium that fast azo-coupling is not prejudiced. For both the usual substrates, sodium alpha-naphthyl phosphate and alpha-naphthyl acetate, the most useful concentrations are 0·01–0·1 mgm./ml.

Accelerated azo-coupling results from incubation at 37° C. and from a high concentration of diazonium salt. Tetrazotized dianisidine (fast blue *B*), one of the most satisfactory compounds available, is best used at a concentration of 0·2–0·4 mgm./ml.; the high incubation temperature does not greatly increase its rate of breakdown or the concomitant tissue background staining. For tissues showing low residual enzyme activity the concentration of diazonium salt should be reduced to avoid excessive enzyme inhibition; otherwise, in the interests of fast azo-coupling the highest tolerated concentration is desirable.

Evidence of accelerated azo-coupling due to the catalytic effect of the quinoline derivative 'Percain' (2-butoxy-N-(2-diethylaminoethyl) cinchoninamide hydrochloride) is afforded by absence of the off-black shades of dye previously shown to be formed at a slow rate of coupling[3]. A further effect of 'Percain', present in the substrate medium at up to 0·4 per cent concentration, is considerably minimized tissue