Growth tests with Xanthomonas pruni. A strain of the plant pathogen *X. pruni* has been studied and found to respond to all of the postulated nicotinic acid precursors as far back as anthranilic acid(7). It seemed likely that if 3,4-dihydroxyanthranilic acid were an intermediate between 3-hydroxyanthranilic acid and nicotinic acid, it should support growth at levels equal to or less than those at which 3-hydroxyanthranilate is active.

Whereas 3-hydroxyanthranilic acid supported optimum growth at a concentration of 1.2 μM and quinolinic acid at about 200 μM with measurable growth at much lower concentrations, 3,4-dihydroxyanthranilic acid did not show significant activity at levels from 0.12 to 240 μM. Addition of similar concentrations to cultures containing sufficient nicotinic acid (.04 μM) for sub-optimum growth (60-70% transmission) resulted in no growth promoting activity. Some inhibition of growth under these conditions was noted when the 3,4-dihydroxyanthranilic acid concentration was above approximately 100 μM.

To eliminate the possibility that all of the 3,4-dihydroxyanthranilic acid was destroyed during the lag phase of growth, it was added to cultures already actively growing on sub-optimum concentrations of nicotinic acid. Again no growth promoting activity was noted.

Discussion. The explanation of the wide discrepancy between these results and those presented by Makino *et al.*(5) is not known. If 3,4-dihydroxyanthranilic acid were an intermediate as suggested by their data, the intact animal would be expected to utilize it for growth and to excrete nicotinic acid and quinolinic acid as it does when given tryptophan, kynurenine, 3-hydroxyanthranilic acid and quinolinic acid. Likewise, the bacterium tested should respond. The ease with which 3-hydroxyanthranilic acid is converted to quinolinic acid *in vitro* would suggest that any intermediate involved should give rise to large amounts of quinolinic acid when added to such systems. The intermediate reported by Bokman and Schweigert is not 3,4-dihydroxyanthranilic acid‡(10). This intermediate shows an absorption band at approximately 315 mμ in acid solution and 360-375 mμ in buffer at pH 7.4. In trichloracetic acid or ethanol, 3,4-dihydroxyanthranilic acid has two absorption maxima; one at 268 mμ and one at 322 mμ.

Summary. 3,4-dihydroxyanthranilic acid was not converted to quinolinic acid or nicotinic acid by rat or hog liver preparations. Spectrophotometric evidence indicates that this compound is not the transient intermediate formed in the conversion of 3-hydroxyanthranilic to quinolinic acid by liver enzymes. It was not utilized by *Xanthomonas pruni* for growth, or by the rat for growth or formation of urinary nicotinic acid or quinolinic acid.

‡ In a private communication, Dr. Schweigert states that rat liver preparations failed to form quinolinic acid or nicotinic acid when incubated with 3,4-dihydroxyanthranilic acid supplied by Dr. Makino.

10. Bokman, A. H., and Schweigert, B. S., *Fed. Proc.*, 1951, v10, 164.

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Mechanics of Cell Division. I. The Living Spindle. (19098)

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The destruction of the longitudinal half of a mitotic spindle by X irradiation has been described(1). Chromosomes in the irradiated area of the spindle became pycnotic whereas those in the structurally intact area maintained their separate identities (Fig. 1A).

1. Tahmisian, T. N. and Gasvoda, J. D., Quarterly Report, *Biological and Medical Divisions,* Argonne National Laboratory, August to November 1948, eds., A. M. Brues and H. Lisco, ANL-4227., p154-180.

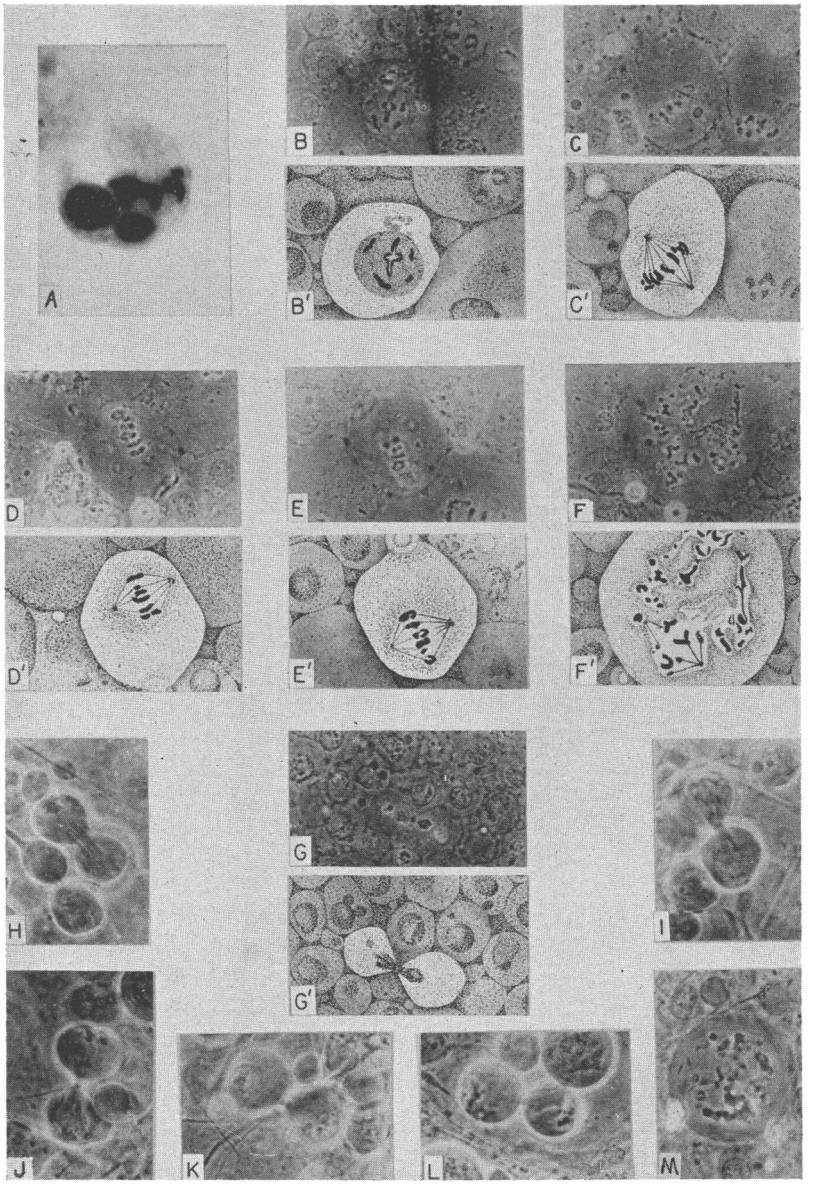

FIG. 1. A. Cell from prediapause embryo that was irradiated with 250 rep emanating from SR^{89}. Note the pycnotic chromosomes in the area where the spindle has been destroyed while the chromosomes that are attached to the remaining portion of the spindle appear intact (× 600). B-G. Phase contrast photomicrographs of living germ cells showing various stages of meiosis and birefringence of the spindle (× 600). B'-G'. Drawings of photographs B, C, D, E, F, and G showing the relationship of the intracellular components with the spindle (× 750). H-M. A phase contrast series of photomicrographs showing a germ cell undergoing meiotic division (× 600). M. A cell that was pretreated with colchicine 24 hr previous to study. Note the absence of a spindle.

Because this work and that of other investigators(2) indicates that a functional spindle is necessary to maintain the orientation of the chromosomes, it was thought important to determine whether the cell and spindle are living when observed under conditions of the

2. Brues, A. M., Stroud, A. N., and Svihla, G., *Anat. Rec.*, 1951, v109, 411.

technics used and to observe the presence of the spindle in the normal cell and its absence in the colchicine-treated one.

Material and methods. Testes from third instar *Melanoplus differentialis* nymphs were used. The nymphs were immersed in 70% ethyl alcohol and then wiped dry with Kleenex. The wings and legs were removed and a dorsal slit was made in the abdomen. The testes were removed under a dissecting microscope with a pair of forceps and placed in Belar(3) solution. The constituents of Belar solution were either autoclaved or passed through a sterile Berkefeld filter, porosity N, prior to mixing. In both cases the calcium and, when used, magnesium solutions were added after almost complete dilution in order to avoid precipitation with phosphate and carbonate. The carbohydrate content was kept at or below 0.2% because cells in their normal environment are bathed in saline of balanced salts in which the carbohydrate content seldom exceeds this value. The pH of the final solution was checked with a glass electrode Beckmann pH meter. The pH of Belar was 6.8. (The pH of grasshopper blood is 6.8 as determined by the Beckmann one-drop glass electrode). After immersion into Belar solution the testicular follicles were placed on a glass slide, and the proximal ends cut and discarded. The distal ends of the follicles, containing cells in the primary and secondary meiotic stages of division, were gently ruptured by mild pressure on the coverslip. Sufficient solution was used to fill the coverslip area without any overflow and the coverslip was then sealed to the slide with paraffin to eliminate evaporation. The observations and the photomicrographs were made with the aid of an Ortholux microscope. This microscope was adapted to the American Optical Co. phase contrast objectives as well as to the phase contrast substage condenser. Spindle birefringence was best observed and photographed approximately 5000 to 6000 A. This range of the visible spectrum was obtained by lowering the substage condenser until a yellow-green ring surrounded the retardation plate of the phase contrast ocular telescope.

Living nymphs were injected with 50 γ of colchicine in 0.1 cc of H_2O in the abdominal cavity 24 hours previous to sacrifice. The approximate concentration of colchicine per nymph was 1.1×10^{-5} g. In no case was a spindle present in the colchicine-treated testes (Fig. 1M). If colchicine was added to the coverslip preparation after the spindle was formed dissolution of the formed spindle did not occur.

Results. The meiotic spindle of the first and second maturation divisions in the grasshopper testis are shown in Fig. 1, B-G and H-L. The spindle fibers are shown to be attached from the centrosome to the chromosome (Fig. 1C, D, F, and F'). This condition is readily observed but difficult to photograph. In order to see the spindle it was necessary to find portions of the slide in which the cells were only one layer thick.

The cells that appear in the photographs (Fig. 1, B-G) were under pressure. Meiosis in these cells progressed slightly beyond metaphase. Fig. 1, H-L shows that cells that were under less pressure divided; the interzonal fibers and the *Zwischenkörper*(4) can be seen in these pictures. The mitochondria can also be seen as long strands occupying positions lateral to the spindle axis. In late anaphase or early telophase they were seen to move across the spindle to a position located centrally between the two newly-forming nuclei and become aligned along the axes of the spindle fibers. Such conditions are shown in Fig. 1G and H-L. Colchicine-treated material observed under identical conditions did not show spindles (Fig. 1M).

Discussion. Although most cytologists agree that the spindle is a real entity in the living cell(5-11), there are those who believe it is

3. Belar, K., *Arch. Entwicklungsmech. Organ.*, 1929, v118, 359.

4. Wassermann, F., *Wachstum und Vermehrung des Lebendigen Masse Handbuch des Mikroskopischen Anatomie Des Menschen*, 1929. Vol. 1, Pt. 2, p143-146, Julius Springer, Berlin.

5. Schmidt, W. J., *Biodynamica*, 1936, 22.

6. Wyckoff, R. W. G., *Cold Spring Harbor Symposia on Quantitative Biology*, 1934, v2, 39.

of an artificial nature. Several recent reviews on the subject are available(12,13). Under conditions approximating as nearly as possible the *in vivo* situation, the spindle was photographed in an uninjured condition in living material, and the cells were seen to complete division. It is assumed that spindle formation is not an artifact produced by pressure, since colchicine-pretreated cells observed under similar conditions did not have spindles.

Rosza and Wyckoff(14) showed that in acid- or Flemming-fixed material the interzonal fibers were apparent in onion root tip, whereas they were absent in neutral formalin-fixed material. On the basis of our observations their findings on acid-fixed material may be considered "realfacts"(15) and the dissolution of the fibers by neutral formalin may be considered as an artifact. For the same reason the electron micrograph of the spindle prepared by Beams *et al.*(16) may be considered to be a "realfact" and not an artifact. Since we have observed and photographed the centriole, spindle fibers, and interzonal fibers, in living material, we conclude that a spindle in fixed tissue is a "realfact."

Summary. The observation that exposure to X irradiation causes partial dissolution of cell spindles and that chromosomes in the destroyed area become pycnotic led to this study of the living spindle. Photographs of grasshopper testis, living cells that have been immersed in Belar solution show spindle fibers and the attachment of some of these fibers from the centrosome to the chromosome. The reality of the spindle in fixed tissue, as revealed by phase contrast microscopy, is emphasized as a result of these studies. Prior to this experiment, Schmitt and Belar were also able to show the birefringence of spindle fibers.

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12. Schrader, F., *Mitosis*, Columbia University Press: New York, 1944.

13. Miner, R. W., ed., The mechanism of cell division, *Ann. N. Y. Acad. Sci.*, 1951, 1279.

14. Rosza, G., and Wyckoff, R. W. G., *Bioch. et Biophysica Acta*, 1950, v6, 334.

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Detection by Tissue Culture of an Organism Resembling *Histoplasma capsulatum* in an Apparently Healthy Horse.* (19099)

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In this laboratory we have been concerned with the cultivation of the virus of equine abortion (E.V.A.) in certain tissues of the horse maintained *in vitro*(1). During the course of the investigation, intracellular organisms, resembling *Histoplasma capsulatum*, were observed in supposedly healthy horse tissue grown in tissue culture. As a result of these findings, Randall and McVickar(2) demonstrated the experimental development of *H. capsulatum* in tissue culture.

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1. To be published.

2. Randall, C. C., and McVickar, D. L., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 150.