

The use of the model as an explanation of the red cell form requires that the red cell be in contact with a cylindrical surface as is the mercury drop. While passing through the gill capillaries one elliptical surface of the red cell is usually applied to the capillary wall although this is not the case in larger blood vessels. It is also required that capillary diameter be no greater in triploids since, other factors remaining constant, the larger the diameter of the cylinder the less eccentric will be the fluid drop. Measurements of gill capillary diameters disclosed no differences between diploids and triploids. FANKHAUSER reports constant diameter for another tubular structure, the kidney tubule¹.

The model lacks the bulging nucleus characteristic of the amphibian red cell. However, salamanders of the genus *Batrachoseps* are characterized by anucleate blood cells (erythroplastids) formed by cytoplasmic division from circulating nucleated erythrocytes². As EMMEL's figures show, these blood elements are clearly elliptical in form with larger cells tending to be more eccentric but no greater in thickness. The presence of a nucleus does not alter the basic similarity between red cell form and the equilibrium form assumed by a fluid drop in contact with a cylindrical surface.

No. of Cells	$\frac{2n}{42}$	$\frac{3n}{40}$
Area/cell μ^2	$560 \pm 102^*$	843 ± 123
Eccent. a/b	1.55 ± 0.17	1.82 ± 0.21
	Area $3n = 1.49$	
	Area $2n = 1$	

* S.D. = $[S(\text{dev})^2/N]^{\frac{1}{2}}$

By maintaining the cell thickness constant the oxygen exchanging properties of the red cell undergo little change with increased cell volume. As already noted skin epidermis as well as lens epidermis remain of constant thickness. Considered as a diffusion barrier the properties of the epidermis are probably not altered by polyploidy. Similar considerations apply to the kidney tubule which remains constant in diameter and wall thickness. Stated in general terms: *cell dimensions perpendicular to physiological surfaces remain unaltered*. Consequently, the physiological properties of the polyploid animals themselves probably undergo little if any change.

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Zusammenfassung

Der Flächeninhalt der elliptischen Erythrozyten des triploiden *Triturus viridescens* ist bei gleichbleibender Dicke der Zellen ungefähr 50% grösser als bei diploiden Kontrolltieren. Als Ellipse betrachtet, zeigt die triploide Zelle eine grössere Exzentrizität als die diploide. Im Kontakt mit der Wand eines horizontal gelagerten Zylinders vergrössert sich die Exzentrizität elliptischer Quecksilbertropfen mit zunehmendem Volumen; sie gleichen den Blutkörperchen auch insofern, als ihre

Dicke weitgehend konstant bleibt. Diese Beobachtungen unterstützen unsere Ansicht, wonach die endgültige Form der Erythrozyten weitgehend durch die physikalischen Kräfte bestimmt wird, welche während ihrer Reifung in den zylindrischen Blutgefässen, vor allem in den Kapillaren, wirksam sind.

Evidences for the Bipartite or Diploid Nuclei in Conidia of *Streptomyces griseoflavus*

Two kinds of mycelia, primary and secondary, are known in the life cycle of *Streptomyces fungi*¹. In 1947, KLIENBERGER-NOBEL proposed in her study of strains of *Actinomyceles* that the primary mycelium might correspond to the haploid and the secondary to the diploid phase of her strains. But, owing to the technical limitation of studying such minute organisms by cytological means, conclusive evidences were not presented for the state of ploidy in the mycelia or in the conidia. In our study, the conidia of one strain of *Streptomyces griseoflavus* proved to be apparently uninucleate, but the nucleus seemed to be bipartite or diploid.

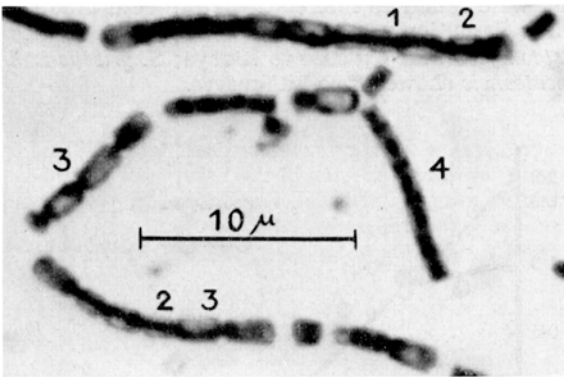


Fig. 1. A late stage of conidia formation in the order 1 to 4.

For cytological study, the strain was cultivated on a modified Krinsky's medium, supplemented with 0.25% yeast extract and 1% peptone, and the nuclei were stained by Robinow's technique. After 24 h at 30°, so-called initial cells (corresponding to the nest-like structure in KLIENBERGER-NOBEL's paper) were distinctly observed under a microscope. When the secondary mycelia developed from the initial cells, the behaviour of nuclei in the mycelia was as follows: at the beginning the chromatinic substances were fragmented, but later, they turned into threads or slender dumbell forms and finally into thick rods. Upon spore formation, the rod-shaped substances divided into smaller parts so that every conidium was charged with two units. The figure soon after the segmentation is twisted and dumbell-like; occasionally, figures are apparently showing two stainable bodies (Fig. 1). As time passes, the spheres of the dumbell unite and, after maturation, only one nucleus is found in each conidium (Fig. 2).

¹ E. KLIENBERGER-NOBEL, J. gen. Microbiol. 1, 22 (1947). – J. F. MCGREGOR, J. gen. Microbiol. 11, 52 (1954).

² V. E. EMMEL, Amer. J. Anat. 33, 347 (1924).

X-ray inactivation tests of the mature conidia gave 'two-hit' curves. With the assurance that more than 90% of the conidia were single, a saline solution containing about 10^6 of conidia per ml was exposed to X-rays (200 kV, 20 mA, filtered through a 1 mm aluminium plate) at a distance of 18 cm (1000 r/min). The frequency of survivors after irradiation was determined by routine methods. As the examples in Figure 3 show, survival

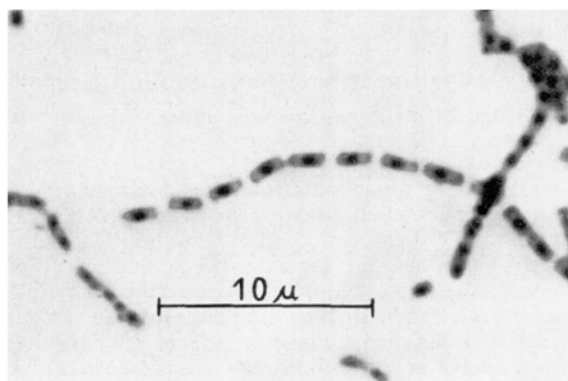


Fig. 2. — Mature conidia.

curves having a shoulder resulted. In a series of experiments with other mutants of the strain, the extrapolations were found to intercept the ordinate around 2.0 with a deviation of 0.3. In parallel, other species of *Streptomyces* were exposed to X-rays; *S. griseus* and *S. kitasatoensis* showed 'one-hit' curves.

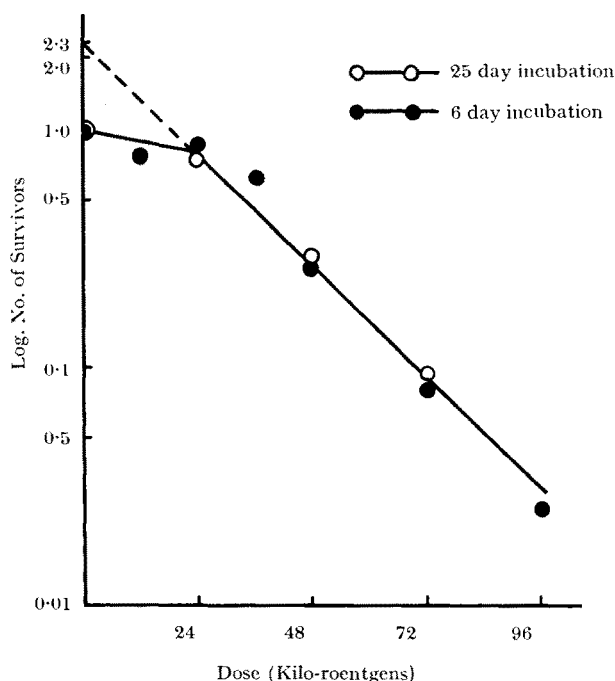


Fig. 3. — Survival of conidia after X-irradiation

In an attempt to isolate biochemical mutants among the conidia irradiated by ultraviolet light, we happened to gain another evidence supporting our view. When U. V. irradiated conidia were incubated in minimal medium and subsequently filtered through a glass filter,

the frequency of mutants among the colonies on complete medium was about 0.1%. However, if conidia were preincubated on complete medium for a week before letting the newly formed conidia stand in minimal medium, the frequency rose to 1%. These results are readily understandable if the nuclei in the conidia are bipartite or diploid, because the preincubation on complete medium would be favorable for the induced heterozygous nuclei to segregate out their components, the biochemical mutants.

In order to test the hypothesis more extensively, the following experiments were performed. By the use of a mutant requiring glutamate and methionine for growth, the segregation pattern of its reversions was studied in detail. First, a suspension of conidia was irradiated with a 15 watt germicidal lamp at a distance of 40 cm, and then was plated on methionine-supplemented agar. As a result, the frequency of back mutation among survivors with regard to glutamate dependence was found to be 10^{-7} at zero time and 10^{-5} after 2-min-irradiation (about 1% surviving). When the colonies became large, hundreds of conidia were carefully picked up from each colony and streaked on complete medium in order to examine the requirement of the resulting colonies by the replica technique. Out of 18 back mutants thus examined, 16 gave two types of segregants, glutamate requirers and non-requirers, in ratios from 200:3 to 3:50. Every attempt to find a constant segregation ratio ended in failure because of technical difficulties. In this connection, it must be noted here that the technique adopted for picking up conidia free from the back-ground cells was reliable and justified by reconstruction tests with auxotrophic and prototrophic strains. Anyhow, the fact that most of the colonies appearing on minimal medium were heterogenous with regard to conidial type suggests that the conidia forming them must have had two genetic units. The same conclusion was drawn from the reversion tests with other types of biochemical mutants.

For the reasons mentioned above, we wish to propose that the conidia studied here seem uninucleate but the nucleus is really bipartite or diploid.

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Résumé

1° L'étude cytologique a montré que les conidia de *Streptomyces griseoflavus* sont uninucléaires et que dans un conidium la substance chromatique a deux sphères durant la période qui précède sa maturation. 2° L'inactivation par rayons-X des conidia donne des courbes du type « deux-coups (two-hit) ». 3° Dans le cas d'isolation des mutants biochimiques de conidia irradiés par rayons ultraviolets, on a trouvé une apparence de mutation retardée probablement à cause du délai de ségrégation. 4° Le conidium de mutant venant d'auxotrophe par réversion comprend des unités génétiques hétérogènes.

Ces quatre faits indiquent que les conidia de cette souche ont les nuclei bipartites ou diploides.