reaction can be produced by replenishing aceto-acetic ester or dehydro-gallic acid to desoxypentose nucleic acid. Noteworthy is the fact that this fixation solution causes desoxypentose nucleic acid to precipitate and pentose nucleic acid to melt (*5*).

Ketoenolic substance in a raw tissue is unstable, and when the tissue is immersed in water at 37°C for 1 hr the ketoenolic substance disappears. However, if it is immersed longer than this period, a degradation of nucleal desoxypentose nucleic acid occurs, because of autolysis, and a new kind of ketoenolic substance is produced (*6*).

Cold perchloric acid and trichloracetic acid will extract ketoenolic substances out of a raw tissue, but such is not the case with a tissue that has undergone the chromate-mixture fixation. However, when the extract is warmed, it is successful. When the carbol-fuchsin-iodine method is applied to an extracted tissue section and observed under a microscope, there can still be found a few large granules of violet color remaining in the tissue (Fig. 1). These are not ketoenolic substances but lipids. Since unsaturated fatty acids are especially well stained by this method, differentiation of lipase from ketoenolic substances can be made.

With regard to ferments, pepsin will not affect ketoenolic substances. Although the action of trypsin cannot be clearly ascertained, because the ketoenolic substances are dissolved by baryta water used in the preliminary procedure of trypsin digestion especially, the ketoenolic substances attached to the nuclear membrane or in the nucleus disappear.

The nucleotidase taken from beef liver digests these substances in a raw tissue, but action upon the tissue that has undergone the chromate-mixture fixation is difficult. Thus, unless it is drastically freed from fat beforehand, such action will not occur. With acid phosphatase that has been taken from a human prostate, similar action will be noted. Ribonuclease cannot make ketoenolic substances disappear from either a raw tissue or a fixed tissue.

Crystalline desoxyribonuclease (Worthington Chemical Laboratory) cannot digest ketoenolic substances. On this occasion, it is very interesting to note that a remarkably large amount of ketoenolic substances appear when the nucleotides are produced, owing to the degradation of the desoxyribonucleic acid in the nuclei. The Feulgen reaction proves negative to ketoenolic substances, while the improved Feulgen reaction (Hamazaki) (*2*) gives rise to a positive reaction. The reason for this is that, when hydrolysis is performed by warm HCl, ketoenolic substances are extracted (*7*).

When tissue was fixed with the chromate mixture, under a 2600-A ultraviolet-ray microphotograph, ketoenolic substances were found to be very absorbent. As previously mentioned, the ribonucleic acid extracted by this fixation has no part in absorption. When the carbol-fuchsin-iodine method is applied to the same section, violet-stained granules identical to the figure of absorption appear. These granules were not extracted by cold perchloric acid or digested by ribonuclease, but they disappeared by treatment with hot perchloric acid, and the absorption figures of ultraviolet rays totally disappeared.

The liver of a dog fixed by the chromate mixture was homogenized and extracted by 0.25-percent baryta water, which is an adequate solvent for ketoenolic substances. The extract underwent tests for ribose and desoxyribose by orcinol and cystein-sulfuric acid reactions, respectively. Not only were both reactions positive but the Feulgen reaction of the same extract was also slightly positive.

It can be concluded that Hamazaki's ketoenolic substance is a material that is chiefly made up of desoxyribose nucleotides (perhaps nucleosides mixed) which combine with certain lipids. Therefore, nucleic substances containing desoxyribose component can be demonstrated in cytoplasm outside of the nucleus.

References

1. Y. Hamazaki, *Trans. Soc. Pathol. Japon.* **24**, 91 (1934).
2. ———, *Japan. J. Med. Sci. (V) Path.* **3**, 85 (1938).
3. ———, *Trans. Soc. Pathol. Japon.* **38**, 1 (1949).
4. Y. Hamazaki and K. Sano, *Trans. Soc. Pathol. Japon.* **38**, 5 (1949).
5. Y. Hamazaki, *Physiology and Pathology of Cell Nucleus* (Osaka, 1952).
6. ———, *Japan. Med. J.* **2**, 212 (1949) ; **3**, 195 (1950) ; **3**, 315 (1950).
7. S. Ichikawa, *Tokyo-Iji-Shinshi,* in press.

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Exchange of Incompatibility Factors between the Nuclei of a Dikaryon

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Most of the Basidiomycetes have an obligate sexual union in their life-cycle. This sexual process is peculiar in that the cells of the fruiting-body do not contain diploid nuclei but pairs of haploid nuclei of complementary incompatibility types. The whole fruiting-body and a varying amount of mycelium represent a part of the life-cycle between plasmogamy and karyogamy, the latter occurring just before meiosis and the formation of basidiospores.

The two nuclei of a dikaryotic cell divide synchronously, and in most species this is accompanied by the formation of a clamp connection, which provides a useful indicator of dikaryotization. Dikaryons are normally formed whenever two monokaryons, or haploid mycelia, of appropriate incompatibility types meet.

In *Schizophyllum* and other Basidiomycetes the incompatibility type is determined by two series of incompatibility factors, the A series and the B series, individual factors of each series being designated by superscripts. For two monokaryons to be compatible, they must differ in both A and B factors. Thus, the matings $A^1B^1 \times A^3B^3$ or $A^1B^2 \times A^2B^1$ are compatible

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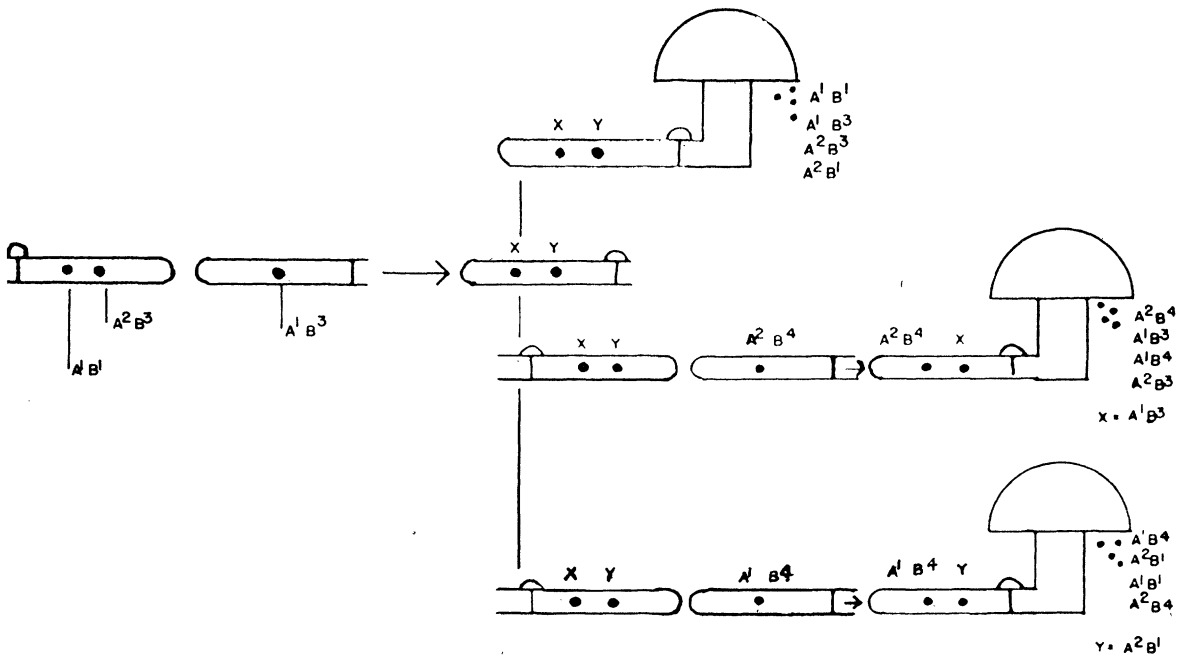

Fig. 1. Method used by Quintanilha to demonstrate formation of new nucleus. Arrows indicate diploidization; lines indicate subcultures. Symbols have been altered to conform with present usage. The fruit at the top indicates that no new mutant factors are present in X or Y.

and will form the dikaryons $A^1B^1 + A^3B^3$ and $A^1B^2 + A^2B^1$.

In 1931, A. H. R. Buller (*1*) described a phenomenon by which a monokaryon was dikaryotized by a dikaryon neither of whose nuclei were compatible with it.

In such non-compatible di-mon matings (*2*) where neither of the nuclei of the dikaryon is compatible with that of the monokaryon—for example, $(A^1B^1 + A^2B^2) \times A^1B^2$—there is evidence from two different experimental approaches (*2, 3*) that one of the nuclei of the newly formed dikaryon has an incompatibility factor derived from each of the nuclei of the old dikaryon.

Quintanilha's method (Fig. 1) involved the use of three incompatibility factors of the *B* series and relied upon the performance of two hemi-compatible di-mons, where one of the nuclei, X or Y, of the unknown dikaryon was expected to be compatible with the monokaryon. The factors present in the resulting dikaryon were found by allowing it to fruit. In Papazian's method, the composition of the unknown dikaryon was determined without forming fruiting-bodies by making use of the visible difference between noncompatible and hemi-compatible di-mons. In two out of 13 different combinations, there was evidence of an exchange of factors

A more satisfactory analysis could be made if the two nuclear components of a dikaryon could be separated. Such a separation would be easy in a species whose dikaryon produced homokaryotic oidia (*4*), but such an occurrence is apparently rare.

Microsurgical operations (*5–7*) have been used sucessfully, but they are very tedious and are ac-

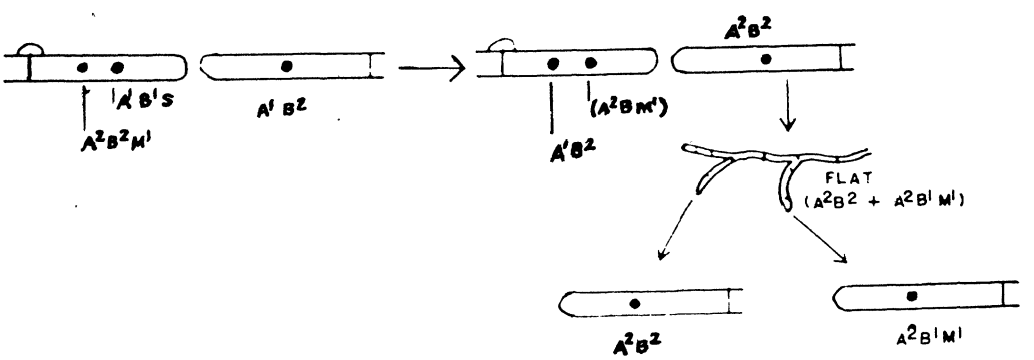

Fig. 2. Method of separation of components of a dikaryon by the use of "flat" heterokaryons.

companied by other complex phenomena. Prolonged blending in a Waring blender will also produce some portions of hyphae that grow into monokaryons, but the same objections apply here as do to microsurgical operations.

In *S. commune* another method has been devised; this relies upon the fact that two monokaryons that differ only in their *B* factors will form a peculiar heterokaryon called "flat," which is easily separated into its components by isolation of single hyphal tips. The formation of "flat" mycelia takes place also in noncompatible di-mons before the formation of the new dikaryon. Thus $A^1B^2 \times (A^1B^1 + A^2B^2)$ will give rise to a region of "flat" of constitution $A^1B^2 + A^1B^1$, and hyphal tips of the latter will give rise to two types of monokaryons, A^1B^2 and A^1B^1

Ten duplicates of the noncompatible di-mon $(A^1B^1s + A^2B^2m^1 \times A^1B^2$ were made. Streak (s) is a morphological mutant linked with the *A* incompatibility factor, and m^1 is a biochemical mutant, requiring uracil, unlinked to any other known factor. In six cases, a new dikaryon was formed on the A^1B^2 side of the mating, and this was then mated with an A^2B^2 monokaryon, $[(A^1B^1s + A^2B^2m^1) \times A^1B^2] \times A^2B^2$.

In all six cases, a region of "flat" developed. These were subcultured and ten single hyphae from each were mated with the tester strains A^1B^1, A^2B^2, A^1B^2, and A^2B^1.

In three cases, only A^2B^2 types were recovered, but in the other three, some of the single hyphae cultures gave all the reactions with the testers consistent with an incompatibility type A^2B^1; they were all $s+$ and m^1 (Fig. 2).

A monokaryon of type A^2B^1 had not entered into any of the crosses, and it is felt that this constitutes good evidence that the $A^2B^1m^1$ monokaryon had been formed by an interchange of genetic material between the nuclei of the original dikaryon $(A^1B^1s + A^2B^2m^1)$. The *A* and streak factors remained in their parental combination; thus, there was no evidence for crossing over.

Although this phenomenon by which a nucleus of different incompatibility type is formed through exchange of factors between two nuclei in a vegetative hypha is not widely recognized (*8*), the evidence presented here should make it more acceptable. Unfortunately, little is known of the mechanism responsible.

Cytological studies have been made but proved unrewarding. Under phase contrast, the paired nuclei of the dikaryon in *Schizophyllum* can be seen very clearly. They consist of a large spindle-shaped outer membrane with a large dark sphere (nucleolus?) inside. During nuclear division, however, no mitotic apparatus can be seen. The dark spheres and the membrane disappear, and filamentous mitochondria concentrate in the region, but no metaphase chromosomes or spindle can be seen. Some 20 min later the daughter nuclei appear.

Work is now in progress with *Coprinus lagopus*, which appears to be a more suitable organism for such studies.

References

1. A. H. R. Buller, *Researches on Fungi*, IV (1931).
2. H. P. Papazian, *Botan. Gaz.* **112**, 143 (1950).
3. A. Quintanilha, *Compt. rend.* **129**, 730 (1938).
4. K. A. Gilmore, *Botan. Gaz.* **81**, 419 (1926).
5. R. Harder, *Z. Botan.* **19**, 337 (1927).
6. N. Fries and K. Aschan, *Svensk Botan. Tidskr.* **46**, 429 (1952).
7. H. P. Papazian, unpublished paper.
8. L. S. Olive, *Botan. Rev.* **19**, 439 (1952).

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Communications

Farmdale Drift

Recent field studies indicate that a portion of the glacial drift in northern Illinois formerly mapped as Illinoian is Farmdale in age, the earliest substage of the Wisconsin stage. The drift sheet consists of till and widespread deposits of water-laid materials in the form of kames, eskers, and kame terraces. So far as is known, this is the first reported occurrence of glacial drift of Farmdale age.

This drift is the uppermost drift in the northern half of Boone County, in all but small areas in southeastern and northwestern Winnebago County, in southeastern Stephenson County, in northern Ogle County, and in small areas in eastern Carroll and northern Whiteside counties. The loess (Peorian) cover which varies from a few inches to about 5 ft is usually leached.

Farmdale drift is recognized in auger borings and cuts to lie beneath discontinuous deposits of younger drift (Shelbyville and Bloomington?) in eastern Boone County; west, central and southern Ogle County; and eastern Whiteside County.

There are several indications that the drift is older than Iowan; the Farmdale loess [Leighton and Willman, *J. Geol.* **58**, 602 (1950)] which lies stratigraphically below Iowan drift is absent, although it occurs in the surrounding areas; the drift is more deeply weathered than Iowan drift at the same latitude in Iowa; the Farmdale drift passes beneath Shelbyville drift, which appears to be essentially contemporaneous with the Iowan of Iowa. [Shaffer, *Bull. Geol. Soc. Am.*, in press].

The Farmdale till differs markedly in color and texture from the younger tills that overlie it on the east, southeast, and south. Unaltered Farmdale till is usually light pink to salmon in color (7.5 YR 8/4 dry) and sandy textured. The color of the till resembles that of the Farmdale loess. The Farmdale till