Where populations tend toward a negative binomial form, as is often true with insects, serious *bias*, as well as higher variance, must be expected from the indirect estimation of $\bar{x}$. The negative binomial is more complex in its algebraic expression than the Poisson, the usual equation for fitting involving both mean and variance (*2*). Sufficient to show bias in the indirect method is the fact that the proportion of zeros is always higher for a given mean in the negative binomial than in the Poisson. Hence, estimation of the mean from the proportion of zeros, in negative binomial material mistakenly regarded as Poisson, will give too low a value. The expression $\bar{x} = -\ln(q)$ can readily be seen to have this tendency if q is higher than expected in proportion to $\bar{x}$. This will give a definite negative bias to such population estimates in many populations.

The tendencies that variance would show, if unbiased estimation of the mean from the proportion of zeros in negative binomial material could be carried out, may be studied briefly. The expression used for fitting the negative binomial is complex and does not lend itself to such a study. However, for selected levels of excess of variance over mean (for example, variance equal to twice the mean), the expression is simplified and can easily be shown to have the same tendencies as with the Poisson.

Thus, it is shown that in populations agreeing with the Poisson, the method of estimating mean density from the proportion of zeros loses much information at higher densities as compared with direct counts, although it is practically unbiased. With populations tending toward the negative binomial conditions, a strong bias also appears. These factors should be considered in appraising this method.

References

1. F. M. Wadley, *J. Am. Stat. Assoc.* **40**, 85 (1945).
2. ———, *Ann. Entomol. Soc. Amer.* **43**, 581 (1950).
3. L. H. C. Tippett, *Proc. Roy. Soc.* **137A**, 434 (1932).
4. M. Bowen, *U.S. Dep. Agr. ET 225*, 6 (1945).
5. C. Eisenhart and P. W. Wilson, *Bacteriol. Rev.* **7**, 57 (1943).
6. W. G. Cochran, *Biometrics* **6**, 105 (1950).
7. C. I. Bliss, *Conn. Exp. Sta. Bull. 518*, 12 (1948).

Received September 8, 1953.

Histochemistry of Ketoenolic Substances (Hamazaki)

Yukio Hamazaki and Masataka Shiraishi

Department of Pathology,
Okayama University, Okayama, Japan

Ketoenolic substance was first demonstrated in 1934 (*1*) by Y. Hamazaki, who completed the ordinary morphologic research in 1938 (*2*). This substance was disclosed as fine granules in the normal cytoplasm and rarely in the nucleus in tissues treated with the special fixation and staining method described here. Whether or not any substance containing desoxyribonucleic acid exists in cytoplasm outside the nucleus of normal cells is still unknown both morphologically and chemically (*3*). Though the significance of this substance in the living organism is still unknown, it has a function almost similar to folic acid and, moreover, it can be a carrier of molecular oxygen. When any chronic disturbance of metabolism of this substance occurs, wear and tear pigments are formed (*4*).

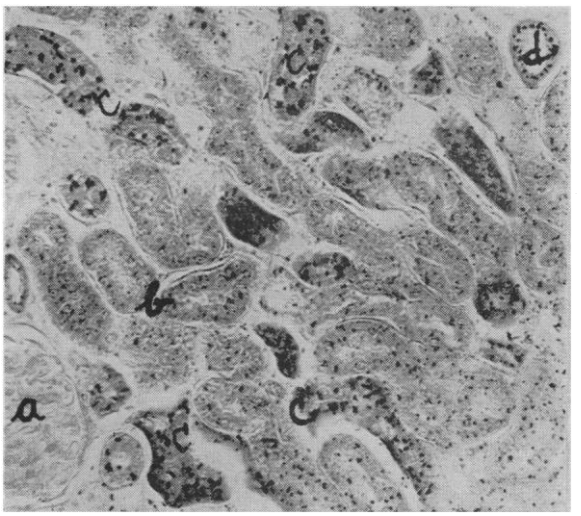

Fig. 1. Ketoenolic substance in the kidney of albino rat. Fixation with chromate mixture; carbol-fuchsin-iodine method. (*a*) Glomerulus; (*b*) proximal convoluted tubules; (*c*) intermediate portion; (*d*) intercalary portion.

For fixation, a chromate mixture originated by Hamazaki is applied; other fixation methods previously used were not successful. A mixture of 2.5 g of potassium bichromate, 1.0 g of sodium sulfate, 100.0 ml of distilled water, and 6.0 ml of glacial acetic acid is prepared, in which tissue is fixed for 48 hr. Paraffin or carbowax methods should be employed for making tissue sections. The carbol-fuchsin-iodine method invented by Hamazaki is applied as the staining method, because with other routine methods heretofore used the ketoenolic substance is not stained. Five-tenths gram of crystalline basic fuchsin is dissolved in 5.0 ml of absolute alcohol, into which 95 ml of 3-percent aqueous solution of carbolic acid is added. Tissue sections are stained in this solution for 1 hr and, after being washed in water, they are placed in 1-percent HCl for 10 min. After being washed in water, they are immersed in Lugol's solution for 30 min and later immersed in 1-percent sodium hyposulfite for 1 to 2 min to remove the iodine color. After being washed in water for several minutes, they are again placed in 3-percent HCl for 15 min. They are thoroughly washed in tap water, dehydrated with alcohol and xylol, and sealed with balsamum. This staining is a chemical color reaction.

When tissues are fixed with the chromate-mixture, the desoxypentose of nucleic acid is oxidized into ketone and then changed into enol, the OH of which reacts with fuchsin and iodine to produce a new kind of acidproof dye that is violet in color. Thus, the same

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.

Received March 10, 1954.

Exchange of Incompatibility Factors between the Nuclei of a Dikaryon

H. P. Papazian

University of Chicago[1]

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

[1] Present address is Osborn Botanical Laboratory, Yale University, New Haven, Conn.