is important to point out again that instability can be detected only by tetrad analysis.) The genes controlling mating type, uracil synthesis, and copper sensitivity are of this type.

Geneticists prefer to work with stable genes, especially in map construction. Genes that occur with higher than the expected frequency in a population are called genes of "high penetrance." It is possible that deviations in "penetrance" may involve gene-conversion. Much of the early work in genetics was devoted to the selection of stocks carrying "good" genes. Although value judgments are rarely expressed in print, it is interesting to note that even the most astute and objective workers (*9*) have made them: ". . . it is a simple matter to determine the relation between different types of double exchanges, though this has not as yet been *satisfactorily* done, because of lack of *good* genetic characters localized within one arm of a single chromosome."

It is difficult to say how much of the attitude toward gene conversion has its origin in the preference of the geneticist for "good" genes that behave "properly." The designation genetical "marker" may provide a key to this psychological problem, since the term implies a relatively solid monument not subject to capricious change.

The theory of gene-conversion proposed by Winkler (*10*) is now known to be invalid in its original form, since it proposed to explain all recombinations by gene-conversion. The experiments presented by Stern (*11*) as refutation of Winkler's theory proved that crossing-over occurred but failed to prove that conversion did not. Stern showed that *all* recombination was not conversion, but his conclusion that this excluded the possibility of gene-conversion was incorrect. The climate of genetical thought in the 1930's was favorable to Stern's argument, and its fundamental fallacy was not discovered. The failure to evaluate it critically resulted in the persistence of the dogma of genic integrity and delayed the demonstration of gene-conversion.

Although geneticists have resisted acceptance of the simpler interpretation of the direct data contradicting the assumed 2 : 2 ratio at each meiosis, they have accepted evidence indicating that genes can be transformed from one type to another (i) by treatment with a nucleic acid extract from cells carrying the complementary allele or (ii) by infection with a virus that previously had infected a cell carrying the complementary allele. These phenomena of transformation (i) and transduction (ii) are gene-conversions in an operational sense (if genes are, in fact, involved) mediated indirectly through chemicals and viruses. The readiness with which these phenomena are accepted as evidence of gene-change and the resistance to the direct evidence that two genes present in the same nucleus can undergo conversion is evidence of the attitude that considers an old accepted concept more reliable than a new one.

The chronological sequence in which scientific discoveries are made has a direct bearing upon the way in which they are interpreted. Because the concept of genic stability preceded the discovery of direct data revealing true gametic ratios, the climate of genetical thought has not been receptive to direct evidence contradicting the concept of genic stability. Data that confirm a well-established theory are generally accepted without critical evaluation. The established concept serves as a fundamental point of reference, and new data are judged by the closeness with which they fit the established point of view. In genetics the stability of the gene is such a point of reference, and other data are evaluated by considering whether or not they support this fundamental concept. This has produced a system of circular reasoning in which experimental data are evaluated by the precision with which they confirm the concept of genic stability and this "confirmation" is taken as evidence for the reality of the concept.

CARL C. LINDEGREN
Biological Research Laboratory, Southern Illinois University, Carbondale

References and Notes

1. T. H. Morgan, *The Theory of the Gene* (Yale Univ. Press, New Haven, Conn., 1926), pp. 358.
2. This work was supported by a grant from the American Cancer Society administered by the Committee on Growth of the National Research Council.
3. Morgan and Cattell, from A. H. Sturtevant and G. W. Beadle, *An Introduction to Genetics* (Saunders, Philadelphia, Pa., 1939), p. 71.
4. C. C. Lindegren, *J. Genet.* 51, 625 (1953).
5. ——— and G. Lindegren, *J. Gen. Microbiol.* 5, 885 (1951).
6. H. Roman, D. C. Hawthorne, H. C. Douglas, *Proc. Natl. Acad. Sci.* 37, 79 (1951).
7. C. C. Lindegren, *Science* 121, 605 (1955).
8. ——— and G. Lindegren, *Genetica* 26, 430 (1953).
9. A. H. Sturtevant and G. W. Beadle, *An Introduction to Genetics* (Saunders, Philadelphia, Pa., 1939), p. 124.
10. H. Winkler, *Die Konversion der Gene* (Jena, 1930), p. 186.
11. C. Stern, *Biol. Zentr.* 50, 608 (1930).

16 January 1956

Localization and Quantitation of Water in Biological Samples by Historadiography

The development of the interference microscope has made it possible to determine the dry weight and water content of cells and parts of cells (*1*). Although this technique is especially well suited for work on isolated cells, it is perhaps less successful with larger samples, such as microtome sections of tissues fixed by the freeze-drying method. On the other hand, soft x-ray absorption has been applied with particular success to the measurement of dry weight in both tissue sections and cytological preparations (*2, 3*), but heretofore it has not been possible to use this technique for a comparable measurement of water. An instrumental improvement (*4*) by which the sample is permitted to remain at room conditions outside of the x-ray tube has now opened the way for an extension of this method to include the determination of water *in situ*. This report (*5*) is concerned with the theoretical basis for the measurement of the water and the experimental method for each of two procedures that have been devised.

1) If microradiograms are made by the conventional method (*3*), with the newer equipment (*4*), of a fresh-frozen biological sample before and after removal of water by freezing-drying, densitometric measurements of the photographic images will permit calculation of the respective masses and, therefore, of water content by difference. This is based on the following.

$$I_T = I_o e^{-[(\mu/\rho)_s \cdot m_s + (\mu/\rho)_w \cdot m_w]} \quad (1)$$

where the subscripts T, s, and w refer to total sample before dehydration, organic substance in the sample, and water, respectively, so that I_T and I_o represent the x-ray intensities transmitted and incident; and where (μ/ρ) is the mass absorption coefficient (that is, μ is the linear absorption coefficient and ρ the density), and m the mass per unit area. Since the extinction, E_T, equals $\ln(I_o/I_T)$,

$$E_T = (\mu/\rho)_s \cdot m_s + (\mu/\rho)_w \cdot m_w \quad (2)$$

After the removal of the water, the extinction becomes

$$E_s = (\mu/\rho)_s \cdot m_s \quad (3)$$

Likewise we can write

$$E_w = (\mu/\rho)_w \cdot m_w \quad (4)$$

and therefore

$$m_w = \frac{E_T - E_s}{(\mu/\rho)_w}. \quad (5)$$

The values of E_T and E_s obtained by measurement of I_o, I_T, and I_s (the intensity transmitted by the dried sample), permit the calculation of m_w, since $(\mu/\rho)_w$ can be calculated from standard tables.

For the experimental procedure, it would be preferable to freeze the fresh specimen suddenly in isopentane, dichlorodifluoromethane, or any of the other liquids commonly used for this purpose, which has been cooled by liquid nitrogen. The frozen specimen may then be sectioned in a cryostat or cold room and the sections used to obtain a microradiogram with x-ray apparatus inside of the cold chamber. It is essential that the

material does not thaw after the initial freezing, and that the microradiogram be taken before appreciable evaporation can occur. Specimens that do not require sectioning (for example, single nerve or muscle fibers) can be employed in a similar way. After the microradiogram has been taken, the sample is dried while it is still frozen to minimize morphological change (*6*), preferably with modern equipment (*7*), and the desiccated sample is used to obtain the second microradiogram.

2) If a microradiogram of only the dried sample is taken with 8 A or softer x-rays, the photographic positive can be taken as a true representation of the dry matter, since the microradiographic method of weighing cell structures gives the weight per unit area. The negative, however, will approximate a representation of the water: The "effective thickness" of the dry matter (t_S) in a given structure equals m_S/ρ_S, so that the "thickness" of the water present in the fresh material is

$$t_W = t_T - m_S/\rho_S, \qquad (6)$$

where t_T is the measured total thickness of the fresh sample. The mass of water (density equals 1) per unit area then is

$$m_W = t_T - m_S/\rho_S. \qquad (7)$$

It can be shown that the total x-ray extinction of a fresh tissue section of unit thickness changes relatively little with the varying proportions of water and dry substances common to soft tissues. Therefore, as was pointed out before, a photographic negative of a microradiogram showing the distribution of dry mass will show the distribution of water.

The application of procedure 2 is illustrated by the following. If earlier data on the dry weight of cells in dog gastric mucosa, obtained by microradiography of frozen-dried tissue sections (*8*), are substituted in Eq. 7, assuming $\rho_S = 1.3$, the approximate water content of the chief cells, epithelial cytoplasm, and parietal cells is 70, 76, and 85 percent, respectively. Similarly, if earlier data on the dry weight of ventral horn cells in the cat spinal cord (*9*) are used, calculations of the water content give 30, 65, and 75 percent for nucleolus, cytoplasm, and nucleus, respectively.

Procedure 1 is the more unequivocal, but it has the disadvantage of requiring a microradiogram of the fresh-frozen sample in addition to one of the dried sample. Although procedure 2 requires only the latter, the thickness of the fresh sample must be accurately known, since this will usually be 2 to 5 times the "effective" thickness of the organic material —that is, (m_S/ρ_S). A newer method permits the measurement of the thickness of a 5-μ section to within a few percent (*10*), while the older method of measurement by focusing on the upper and lower surfaces with a microscope usually involves an error of about 10 percent (*11*). Only a small error will result from the common deviations in the assumed value of 1.3 for ρ_S.

In both procedures it is necessary to consider the morphological effects of the drying process. Although freezing-drying minimizes these, the use of imbedding media may involve loss of substance on removal of the media. Whether or not such a loss is significant will depend on the nature of the specimen and of the medium. For frozen-dried gastric mucosa that is imbedded in paraffin, the deparaffinizing by xylol has no appreciable effect, owing to the low fat content of this tissue. Chemical fixatives are to be avoided, because they produce structural distortions and also affect the quantity of substance remaining.

ARNE ENGSTRÖM
Institute for Physical Cell Research, Karolinska Institutet, Stockholm, Sweden
DAVID GLICK
Histochemistry Laboratory, Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis

References and Notes

1. R. Barer and S. Joseph, *Quart. J. Microscop. Sci.* 95, 399 (1954) and 96, 1 (1955); H. G. Davies *et al., ibid.* 95, 271 (1954); R. C. Mellors *et al., Cancer* 6, 372 (1953).
2. A. Engström, *Acta Radiol. Suppl.* 63 (1946).
3. A. Engström and B. Lindström, *Biochim. et Biophys. Acta* 4, 351 (1950); B. Lindström, *Acta Radiol. Suppl.* 125 (1955).
4. B. Combée and A. Engström, *Biochim. et Biophys. Acta* 14, 432 (1954).
5. One of us (D. G.) received support from an institutional research grant to the University of Minnesota from the American Cancer Society, and research grant No. H-2028 from the National Institutes of Health, U.S. Public Health Service.
6. D. Bloom *et al., J. Histochem. Cytochem.* 2, 178 (1954).
7. D. Glick and D. Bloom, *J. Exptl. Cell Research*, in press; I. Gersh and J. L. Stephenson, in *Biological Applications of Freezing and Drying*, R. J. C. Harris, Ed. (Academic, New York, 1954), p. 329.
8. A. Engström and D. Glick, *Science* 111, 379 (1950).
9. J. Nurnberger *et al., J. Cellular Comp. Physiol.* 39, 215 (1952).
10. O. Hallén, *Acta Anat. Suppl.* 26, 1 (1956).
11. P. W. Lange and A. Engström, *Lab. Invest.* 3, 116 (1954).

17 February 1956

Biosynthesis of Pelargonidin-3-glucoside-C^{14}

In connection with current research conducted in this department on the stabilization of color in strawberry preserves (1, 2), it was desirable to obtain a quantity of C^{14}-labeled strawberry pigment to facilitate studies of its degradation (*3*). In view of the complex structure of the major strawberry pigment, pelargonidin-3-glucoside (3,5,7,4′-tetrahydroxyflavylium chloride 3-beta-glucoside), biosynthesis of the compound seemed more practical than its synthesis by conventional chemical means (*4*).

Several means of introducing C^{14} into the growing strawberry plants were considered. They included (i) exposure of the plants to $C^{14}O_2$ in a closed system, (ii) submersion of the roots in synthetic nutrient media incorporating C^{14} in an absorbable form, (iii) direct injection of glucose-C^{14} solution into stems of fruit during the final stages of berry maturation, and (iv) diffusion of glucose-C^{14} by direct immersion of the berries in a solution of radiosugar.

In view of the low activity of radiocarbon and the limitations on the amounts of tracer compounds used (microcurie levels were used in all preliminary experiments), only those methods appearing to channel the label most directly into the fruit were first tried. Injection of glucose-C^{14} solutions into either stems or fruits was unsuccessful owing to the absence of cavities in these that are capable of storing even small amounts of solutions. A slow, drip-feeding of glucose-C^{14} solution into the stems was equally unsuccessful. The diffusion of glucose-C^{14} into the green berries by direct immersion was not attempted because a preliminary trial, using an inactive glucose solution, resulted in a deleterious effect on the fruit.

However, two of the methods tried did result in the assimilation of the radiocarbon with no apparent injury to the plants. These methods were the exposure of strawberry plants to $C^{14}O_2$ and the application of crystalline, uniformly labeled glucose-C^{14} to freshly cut sections of fruit stems. In both instances, it was possible to extract, using suitable solvents, the pigments that were present and, upon chromatographic purification, to isolate the crystalline pelargonidin-3-glucoside-C^{14}. To eliminate the possibility that the pigment was merely contaminated with some of the radioglucose when the C^{14} was introduced in this form, tagged glucose was added to a portion of inactive juice from which the pigment was then also extracted. The absence of activity in pigment recovered from this sample is indicative of the effectiveness of the purification procedure used. Through acid hydrolysis of the active pelargonidin-3-glucoside to the pelargonidin, it was also established that the activity was uniformly distributed throughout the pigment molecule and not merely attributable to the sugar moiety.

The labeled sugar was introduced into the plant by placing the dry crystals in small, longitudinal, V-shaped grooves that were cut in the stems and allowing the plant juices to dissolve them. Forty grams of ripe strawberries was harvested from plants to which about 2μc (about 80 mg) of glucose-C^{14} was administered.