other chemicals may be removed. One of the chief obstacles to success is the amount of osmic acid employed.

In the first place, most of the osmic acid on the market is of inferior grade and, what is perhaps just as important, not always of the weight labeled. Best results are obtained with the best quality of the acid. If a given tube supposed to contain one gram of osmic acid actually contains more, then what the worker makes up as a 2 per cent. solution is obviously of greater strength. Working with some of these inferior grades of osmic acid, tubes of which frequently contain more than one gram, I have found that I could get very satisfactory results by using as little as 0.75 cc of osmic acid in Fleming or Meves solution. Indeed, in some cases, the amount of acid used was less than half this amount. The cytoplasm is very well fixed, and the staining with iron hematoxylin leaves little to be desired. Such small amounts of osmic acid have been successfully employed in the fixation of most diverse types of cells. The criterion for the amount of osmic acid necessary for good cytoplasmic fixations is that which will blacken the oil drops in the centrifuged uninseminated egg of *Arbacia*. One need simply to prepare a solution of 1 gram of the acid in 50 cc of distilled water, then take 4 or 3.5 cc of this with chromic and acetic acids as used in Fleming or Meves solution, and prepare also other solutions using instead 2, 1.5 and 0.75 cc of osmic acid. Eggs after centrifuging are placed in these solutions for thirty to sixty minutes, after which they are washed in several changes of tap or distilled water and examined under the microscope. If the disk of oil drops, the so-called gray cap in the living egg, is blackened in that solution containing the least amount of osmic acid, this constitutes sufficient evidence that enough of the acid has been used. After the use of these solutions containing small amount of osmic acid, the cells are perfectly preserved, both as regards nuclear and cytoplasmic structures. After iron hematoxylin, the cytoplasm is a clear pale blue, mitochondria are stained dark blue and the chromosomes after breakdown of the nucleus are stained black. Results with this method are infinitely superior to those obtained by the so-called weaker Fleming. That is to say, in my experience at least, it is better to alter the amount of osmic acid alone, leaving the chromic and acetic acids in the proportions originally given by Fleming and Meves.

E. E. JUST

SPECIAL ARTICLES

VARIATION IN THE PERCENTAGE OF PROTEIN IN THE GRAIN OF A SINGLE WHEAT PLANT

THAT a plant may vary markedly in composition is common knowledge to all engaged in plant investigations. Causes and significance of such variations, particularly of the percentage of given proteins in wheat, have been the subject of much study, because of the relation this property of grain has to the quality of bread. As information was desired on the probable range of variation in the percentages of protein in the grain of single wheat plants, experiments were performed that were designed to obtain marked differences in this character of wheat. Some of the data which were obtained are given in the following table. The values given are those of the percentages of protein in the grain of different heads on the same plant. They were chosen as representatives of the lowest and the highest protein grain grown on an individual plant, but are not to be considered as the lowest or highest values that possibly could have been obtained.

TABLE I

VARIATION IN THE PERCENTAGE OF PROTEIN IN THE GRAIN OF DIFFERENT STALKS OF THE SAME WHEAT PLANT

Variety	Percentage of protein		Difference	
	Low	High	Actual	Percentage
Bunyip	13.6	17.6	4.0	30
Cedar	12.3	18.6	6.3	57
Dart's Imperial	10.9	11.1	.2	2
Early Baart	10.4	12.2	1.8	17
Fulcaster	8.2	11.4	3.2	39
Hard Federation	11.8	17.3	6.5	55
Sonora	6.4	14.0	7.6	119
White Australian	10.2	13.0	2.8	27

Variation in the percentage of protein in wheat is directly related to that of the supply of nitrogen available to the plants at different growth periods—the later in growth a given supply is absorbed the higher the protein content of the grain. Thus an essential feature of the experiment was that of providing conditions whereby a given supply of nitrogen would become available late in the growing period of some stalks and early in case of others. This required that each plant have two distinct crops of stalks, one that arose early in its life and the other late. The requirement was obtained by planting seed in soil deficient in nitrogen in order to restrict stalk formation of the early growth period to one culm per plant. But later in growth—ninety days after planting—an ap-

plication of 250 mgs of nitrogen per plant was added to the soil in the form of $NaNO_3$ in order to induce new stalk formation. Soon after this treatment tillers arose on various plants and thus two distinct crops of stalks, each capable of bearing grain, were produced. Because of the differences in time of the inception of these two crops of stalks on a plant, marked differences were obtained in their ripening. Usually, but not invariably, the grain of the parent stalk ripened before that of the tillers. As the amount of nitrogen supplied to each plant was more than the parent stalk could absorb but less than that which the tillers could utilize, the required conditions were met, namely, that of providing an ample supply of nitrogen during the later growth period of some stalks of the plant and early in case of others. The former produced high protein grain, the latter low protein grain.

The length of the interval between the ripening of the grain of two stalks on a plant or that of different plants grown under similar conditions appears to be of considerable importance and related to variation in the protein content of wheat. The correlation appears to be: the larger this interval, the larger the differences in the protein content of the grain. From these circumstances it follows that uniform ripening of all heads is an essential condition for the production of wheat of low variability. Inspection of the datum shows that a difference of one day or less in the ripening of two heads of wheat of some varieties can be detected in the composition of the grain. For example, the differences between the high and the low values of Cedar, Hard Federation and Sonora are 6.3, 6.5 and 7.6, respectively, which is equivalent to the accumulated change (difference) of 0.1 per cent. per day of the indicated amounts for 63, 65 and 76 days, respectively. But as the difference in time of ripening of the two crops of Cedar was only 36 days, the total difference of 6.3 is equivalent to a daily change (difference) of .17 per cent., a figure far above that of the experimental error in the method commonly employed for the determination of protein in wheat grain.

That a difference of one day in the ripening of two heads of wheat, or even of a few hours as happened to be the case in some varieties, can be detected in the composition of the grain appears at first consideration inconsistent with the general observation of the relationship between these circumstances. Mere change in the rate of desiccation or hardening obviously can not affect the ultimate percentage that a given constituent thereof has to the whole. As the protein content of wheat grain resolves itself into a relationship between two variable factors: (*a*) the amount of nitrogen available for grain, (*b*) the amount of grain in which a given quantity of nitrogen will be stored, so the correlation found in the variations in time of ripening of wheat with that of the protein content of the grain indicates that the time between final ripening and the period when the causes for variation can be operative is a fixed interval. In the case at hand the relationship is explained that the supply of nitrogen available for absorption was less than the potential quantity which would have been absorbed were it present, and the percentages of nitrogen found in two different heads of wheat indicate the quantities which are proportional to the proximity to harvest that comparable rates of the absorption of nitrogen were maintained.

W. F. GERICKE

LABORATORY OF PLANT NUTRITION,
UNIVERSITY OF CALIFORNIA

GLUTATHIONE IN PLANT TISSUES

GLUTATHIONE, because of its apparent function in the fundamental process of respiration, has become a substance of great interest. In 1927 Fink[1] published a micro method for the determination of glutathione in insect tissues. This method has been used by the writer in an attempt to determine the distribution of the dipeptide in plant tissue.

In making the determinations thin free-hand sections of the tissues were made. Immediately a solution of hot dilute acetic acid (approximately 20 per cent.) was poured over these. After a few seconds the acid was drained off and the sections were covered with 5 cc of a saturated solution of ammonium sulphate, $(NH_4)_2SO_4$, and 6 to 10 drops of a 5 per cent. solution of sodium nitroprusside, $Na_2Fe(CN)_5(NO) \cdot 2H_2O$. The tissue was allowed to stand for at least twenty minutes to insure the penetration of this solution. The section was then removed with enough of the solution to keep it moist. Several drops of dilute ammonium hydroxide (one part in three parts of water) were added. On the addition of the ammonium hydroxide a color, pale pink to a vivid purplish-red, flashed up through the tissues. This color lasted for a few seconds only, although longer in some tissues than in others. The difference in the intensity of the color is thought to be due to the difference in the amount of reduced glutathione present in the tissues.

Representatives of the different divisions of the plant kingdom were tested. The following examples are typical of the results obtained.

(*a*) *Thallophytes.*—Both Fungi and Algae were used. In sections of fresh sporophores of *Coprinus,* vivid color showed in the lamellae and extended gradually through the stipe. In *Rhozipus nigricans* the

[1] Fink, SCIENCE, 65: 143, 1927.