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.

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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.

young sporangia showed a slight flash of color, but no reaction could be observed in the hypha under the microscope. Portions of young mats of *Aspergillus niger* and of *Fusarium* sp. showed brilliant color. Water extracts of these and of colonies of *Erythrobacillus prodigiosus* responded to the reaction. The hypha is probably so small that the amount of reduced glutathione present does not give sufficient color to be discernible. Portions of fresh yeast cake gave a brilliant color, yet no color could be seen in the individual yeast cell.

No color reaction was observed in either *Spirogyra* or *Zygnema*. This may be due to the small amount of the dipeptide in the slender filaments, or it may be that the color was disguised by the large chloroplasts.

(*b*) *Bryophytes.*—Liverworts and ferns were used. Sections through the expanded tops of the male and female gametophytes of both *Marchantia polymorpha* and *Reboulia* sp. showed color in the antheridia and archegonia. The reaction occurred in the antheridial contents in crushed antheridia of *Mnium* and in the region of the spores in the crushed immature capsule.

(*c*) *Pteridophytes.*—Young gametophytes of *Pteris aquelina* showed color in the antheridia and archegonia and a suggestion of color in the young rhizoids. In sections of the rhizome and uncoiling fronds of *Woodsia* sp. a flash of color showed in the meristematic regions and in the vascular bundles. The color, though not the characteristic pink or purplish-red because of the presence of pigment, was evidently, from its behavior, the same type of reaction obtained in other tissues.

(*d*) *Spermatophytes.*—Sections of the seed of *Pinus edulis* showed pronounced color in the embryo, especially at the tip of the hypocotyl, but no color in the megagametophyte. In the Angiosperm, inflorescences, embryos and parts of the flowers, stems and roots responded to the test. In *Brassica oberacea* var. *botrytis* and in *Asparagus* sp. vivid color occurred in those spots where the new floral parts were forming. A paler pink marked the region of the vascular bundles. Sections through the grains of *Zea mays* and *Hordeum* sp. gave no color in the endosperm, but color showed throughout the embryo. The cotyledon was a faint pink, but the plumule, the primary and the secondary radicles were a vivid color.

In the flower the most vivid color occurred in the ovules and throughout the pollen grains. No color was observed elsewhere in sections of the ovaries and anthers except a faint pink in the vascular bundles.

Herbaceous and woody stems, bulbs and tubers were tested. *Lycopersicon esculentum* and *Asparagus* showed color in the region of the phloem. In the latter the color quickly spread throughout the vascular bundle. Twigs of *Prunus Persica* one, two and three years old showed color in the region of the phloem and the cambium. In twigs from staminate and pistillate trees of *Salix,* color occurred in the phloem and cambium, but there was no apparent difference in the intensity of the color in the twigs from the staminate and the pistillate trees. *Gladiolus* bulbs showed faint color throughout the bulb with a more vivid color in the vascular bundles of the leaves and a brighter color in the flower buds. In tubers of *Solanum tuberosum* a well-developed color appeared in the region of the vascular bundles and an intense color in the tip of the bud. A section through the terminal bud of the sprout showed vivid color in the axillary buds with faint color in the phloem.

Longitudinal sections through the apex of primary and adventitious roots showed no color in the root cap. In the embryonic region the color was vivid but became very faint in the region of elongation. In the stele, however, the color extended back into the younger portion of the region of differentiation. The secondary root tips also showed a vivid color. A cross section of the stem of *Lycopersicon esculentum* through an embryonic adventitious root showed color in the vascular bundles of the stem and a much more vivid color in the cone of the embryonic root.

The results obtained by the writer on the distribution of reduced glutathione in plant tissues as indicated by the nitroprusside test show that it occurs throughout the plant kingdom with the exception of the algae. The apparent absence in the latter may be due to the methods of examination and the difficulty of observing color in the thin filaments and highly pigmented material. The substance occurs in those cells which have the capacity of producing new cells—in the primary and secondary meristematic regions of the roots and stems and in the reproductive organs. In addition to these regions it is found in the vascular bundles of those plants which possess vascular systems. In the Angiosperms this substance occurs in the phloem. With the exception of the phloem, the areas which react to the nitroprusside test are rapidly growing areas. There is apparent correlation, therefore, between meristematic tissues which have a high respiratory rate and the areas giving the reaction for reduced glutathione.

Sections of viable corn grains and corn grains which had lost the ability to germinate because of age were tested with nitroprusside. There was no apparent difference between the color obtained in these.

The embryos[2] of corn grains exposed to X-rays[3] at 120 KVP, 4 ma, 18 cm for 94 minutes showed a more intense color throughout than the embryos of un-

[2] Tunnicliffe, *Biochem. Jour.*, 19: 149–8, 1925.

[3] The writer is indebted to Dr. L. J. Stadler for treating the corn and barley grains with X-rays.

treated corn grains. Exposure for 144 minutes increased the intensity of the reaction.

The embryos of soaked barley grains exposed to X-rays for one, two, four and eight minutes showed an increase in the intensity of color in the one-minute exposure with a change in the quality, and decreased intensity of color in the two- and four-minute exposures. The eight-minute exposure showed a very faint pink color.

Attempts were made to find a method for a quantitative determination of reduced glutathione in the higher plant tissues. Tunnicliffe's[2] quantitative method was tried with yeast with results somewhat lower than those obtained by him. The same method was applied to the tissues of higher plants, but the attempts were unsuccessful because of the writer's inability to eliminate the pigments and at the same time retain the substance which reacts with nitroprusside.

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ON THE RECOVERY FOLLOWING LESIONS IN THE CEREBRAL CORTEX

THE recovery of functions following lesions in the cerebral cortex is a common "observation" which has received little or no thorough study. In the literature on this subject that was accumulated during the World War, Hollander has found numerous cases in which the patient, after having lost large quantities of nervous tissue, is said to have "entirely recovered his mental faculties." In many cases the patient demonstrated this complete recovery by sticking out his tongue and walking across the room when told to do so by the attending physician. Obviously a little more testing is necessary before we shall care to place much confidence in such reports.

In the work with experimental lesions the same error has been not quite so obvious. Complete recovery of function has been reported to be found in experimental animals when there have been either no tests at all, or only tests of gross movements such as locomotion. Employing such methods, the major question is, "How great a lesion may be made without producing loss of function?"

If adequate tests are used, if those reactions are tested which the animal finds difficult to make, the interest undergoes a reversal. The question now becomes, "How small a lesion may be made in the cortex and still produce a measurable loss in the animal's reaction capacity?"

In our investigations we are employing cats. The animals are tested before and after the operations on such situations as climbing a vertical screen, to which they hold with three paws while they stretch for food with the fourth; high jumping; climbing a vertical ladder; crawling through small holes; jumping up to catch a rope and hanging there by the forepaws while the food is captured by the head; stretching down from a platform; walking across a narrow bar on which there are obstructions; reaching through a small hole at various angles in both the vertical and horizontal planes; removing a bag from the head, and a few others of a similar caliber.

The potentialities of such an attack may be illustrated by some of the results obtained on three animals.

We removed from the parietal region of No. 32 an amount of tissue which we judged to be about equal to all that forward of the cruciate fissure. Six weeks after the operation our tests could reveal no loss of motor reactions. In the tests made before the operation No. 34 used his left forepaw much more often than he used the right. A lesion about a quarter inch wide and three eighths inch deep was made in the arm area of the right motor cortex. No tissue was removed. Six weeks later the animal was completely right handed. In those situations which demand skilful use of the left forepaw to obtain food the animal goes hungry. All the tissue forward of the cruciate fissure was removed from No. 35. Six weeks later the animal was unable to perform any of the required reactions.

It is interesting to note that at the time of the second test all these cats were "normal" to a casual observer.

There is little evidence here that there is "segmental localization," "equipotentiality of the cortex," "vicarious assumption of function," or that the "brain acts as a whole." Our work, so far, has produced results which indicate that there is organization in the central nervous system. In any organization certain parts have certain functions.

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