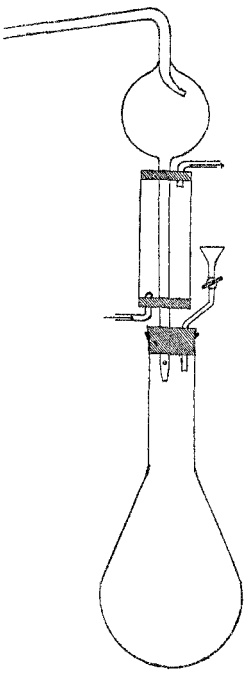

opening the system, a separatory funnel is inserted in the stopper. In using this apparatus 12.5 grams of potassium hydroxide and a few pieces of pumice stone are placed in the Kjeldahl flask. Twenty-five cc. of the "bases" is then added and the apparatus fitted upon a Kjeldahl distillation rack with a receiver containing 0.0714 *N* standard acid, as in an ordinary Kjeldahl determination (bringing exit of tube as near the surface of the standard acid as possible). During the 6 hours' digestion with potassium hydroxide a stream of water is directed through the condenser. The condenser is then drained and the system allowed to cool. From 100 to 200 cc. of water is then added through the separatory funnel, together with a small amount of zinc dust. The final distillation is now conducted as in a regular Kjeldahl determination until 100–200 cc. (depending on the volume of water added) of distillate has been collected.

The titration and calculation of the "arginine" nitrogen are carried out in the usual manner.

The advantages of this apparatus over that described by Van Slyke have already been given.

(*a*) There is no danger of loss due to "bumping."

(*b*) No break in the system or transfer of material is necessary.

(*c*) The rather expensive "Folin bulb" is eliminated.

ST. PAUL, MINN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE KENTUCKY AGRICULTURAL EXPERIMENT STATION.]

THE SIGNIFICANCE OF THE PEROXIDASE REACTION WITH REFERENCE TO THE VIABILITY OF SEEDS.

By J. S. McHARGUE.

Received January 19, 1920.

Among the various enzymes that occur in living animal and vegetable tissue perhaps none is more vital to the life process than those concerned with oxidation within the cells. This oxidation is brought about by the oxidases and the peroxidases which are widely disseminated through the living tissues of both animals and plants. During the life and growth of plants oxidases are abundant in all the tissues; however, when maturity is reached, peroxidases predominate in the seeds of most species of plants. A few species contain both oxidases and peroxidases. Sound, well-matured, fresh seeds of all the species thus far examined have shown a pronounced

peroxidase reaction after having been crushed and finely ground in the presence of air.

Kastle,[1] in his memoir on the oxidases, states that the peroxidases and catalases seem to be even more widely distributed in various living tissues of the plant and animal than the oxidases. He further states that to such an extent is this the case that the properties of these substances might almost be turned to account as a general chemical test for vital activity. He also states that it can certainly be said of any tissue or organ that it is dead when it fails to show the reactions of the peroxidases and catalases. However, the writer has recently observed certain specimens of dead grains in which little or no peroxidase reaction could be obtained, yet they contained a substance which decomposed hydrogen peroxide, thus showing the presence of a catalase in the absence of a peroxidase. This observation, therefore, indicates that the peroxidase reaction may be a specific test for viability of seeds, although catalase activity may not be.

Brocq-Rosseu and Gain[2] have tested for peroxidases in samples of wheat varying in age from 2 years to 5000 years. They state that all viable seeds tested showed the proxidase reaction. They report finding peroxidases in a sample of wheat 2000 years old and came to the conclusion that peroxidases existed in the wheat a hundred years after it had lost the power to germinate. The latter statement appears quite remarkable since the writer has obtained seeds of several different species of plants, which showed zero germination and did not give a peroxidase reaction. In every case where the seeds have shown a weak or zero germination by methods in use in seed-testing laboratories, it has been found that another portion of the same lot of seeds showed a weak or zero peroxidase reaction, thus showing a close parallelism between the germination and the peroxidase test. Seeds showing zero germination, which gave no peroxidase reaction when tested with guaicum solution and hydrogen peroxide are corn, hemp seed, tomato seed, tobacco seed, oats, cowpeas, soy beans, castor beans and two samples of lettuce seed.

The peroxidase test has been applied to a considerable number of seeds of different species of plants upon which no germination test has as yet been made, but from all external appearances the germination test would run 90%, or above, and in every case where sound seeds were ground in air a deep blue color has been obtained.

In order to determine the relative proportions of the peroxidase enzyme in the germ and the endosperm, the germs were dissected from

[1] J. H. Kastle, "The Oxidases and Other Oxygen-Catalysts Concerned in Biological Oxidations," *U. S. Public Health Ser. Hyg. Lab. Bull.* 59.

[2] Brocq-Rosseu and Gain, "Sur la durée des peroxydiastases des grains," *Compt. rend.*, 146, 545–548 (1908).

several grains of sound corn and the two different portions ground separately in a clean porcelain mortar and each part tested for peroxidases. The germs gave a deep blue color, whereas the endosperm gave only traces of blue coloration, thus showing that the substance which gives the peroxidase reactions is contained in the germ.

In another experiment, sound grains of corn were immersed in strong alcohol in a shallow vessel and the germs removed and ground beneath the surface of the alcohol. The alcohol was then diluted with 3 volumes of distilled water and the peroxidase reagents added. Only a pale blue color was obtained, thus showing either that the enzyme substance had been destroyed by the strong alcohol or that oxygen had been excluded to such an extent that but little peroxidase was formed under these conditions. The germs from other grains of corn of the same sample were dissected from the endosperm and ground, beneath the surface of distilled water, and tested for peroxidases in the usual way. A depth of color somewhat comparable with that obtained when the germs were removed under alcohol, was obtained, thus showing that the germs of grains of corn contain a substance which, on exposure to oxygen, absorbs the latter rapidly and a peroxidase is formed in this process.

It is interesting to note that among the 20 or more different species of seeds which have been tested, only 3 have contained both oxydases and peroxidases. Soy beans gave a pronounced oxidase and a peroxidase reaction. Alfalfa seed showed a weak oxidase reaction and a strong peroxidase reaction. Lettuce seed gave an intense oxidase and a moderate peroxidase reaction. Lettuce seed that showed zero germination gave an oxidase reaction of equal intensity to that given by seeds which showed a high percentage of germination. However, upon destroying the oxidase reaction by carefully heating until the blue color was destroyed, cooling to room temperature and adding hydrogen peroxide, no peroxidase reaction was obtained upon the seed that showed zero germination, whereas the sound seed showed a strong peroxidase reaction after this treatment, thus showing that the peroxidase reaction was quite specific as a test for seed viability, in this particular case.

On applying the test for peroxidases to 3 different samples of oats which had a germination of 80, 44 and 0%, respectively, a deep blue color was obtained with the sample showing 80% germination; a decidedly lighter blue color was obtained with that showing 44% germination and only a light blue was obtained with the sample showing zero germination, thus showing a close and unmistakable agreement between the germination test and the peroxidase reaction. The temperature at which the blue color was destroyed increased with the viability of the seeds. The light blue color or the zero viability was destroyed between 60–65, the 44% viability at 65–70 and the 80% between 70–75°. Experiments

are under way with known mixtures of viable and non-viable seeds for the purpose of establishing a color scale from which the approximate percentage of germination of a given sample of seed may be estimated by means of the peroxidase test.

Tests on the temperature at which the oxidase and the peroxidase reactions are destroyed show some variation with different species of seeds.

Conclusions.

1. From the results obtained, the writer is convinced that the peroxidase reaction can be made use of in seed-testing laboratories for detecting non-viable seed and for distinguishing between seed of high, medium and low viability.

2. That lettuce, alfalfa and soy-bean seeds are unique in that they contain both oxidases and peroxidases.

3. That the vital property of seeds is contained in a substance (presumably an oxygenase) which has the power to activate molecular oxygen, when exposed to the air, peroxidases being formed, and that when this power is lost the seed loses its power to germinate.

4. That the peroxidase reaction may be further turned to account in determining the rate at which seeds lose their viability.

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THE IDENTIFICATION OF PHENOLS. II.

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In a former article[2] it was shown that *p*-nitrobenzyl bromide reacts readily with alkaline solutions of phenols to give well characterized ethers which may be used for the identification of the phenols. It was thought desirable to extend this work to other phenols.

Materials and Methods.

The *p*-nitrobenzyl chloride was made by nitrating benzyl chloride and separating the *para* compound by crystallization. The *p*-nitrobenzyl bromide was made by brominating *p*-nitrotoluene.

Into a 100 cc. flask was measured 25 cc. of 0.2 *N* sodium alcoholate and a nearly equivalent amount of the phenol. Except in the case of the salicylic acid esters the mixture was left slightly alkaline to avoid contamination of the product with the phenol. The reagent, usually 1.0 g. in amount, was added and the solution heated one hour under a

[1] The experimental work except that on salicylic acid esters was done at Pomona College by J. A. L.

[2] THIS JOURNAL, 39, 304 (1917).