Paleozoic Pteridosperms and the recent Angiosperms, and thus they suggest a possible solution for one of the great outstanding problems of evolution.

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U. S. GEOLOGICAL SURVEY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MODEL OF MUSCULAR CONTRACTION[1]

IT is rather difficult to imagine the muscle-fiber shortening as a result of swelling and increase of internal tension. The following model, constructed on a suggestion of L. Wacker,[2] 1917, is useful to illustrate the possibility of such shortening to students. The muscle fiber is represented by a thin rubber condom. A cork disk, 3 cm in diameter and 1 cm high, is placed in the bottom of the condom, and a similar but perforated cork in the opening. This bears a glass tube for blowing into the condom. Outside the condom and over the corks are two rings of brass, between which threads are stretched, 1 cm apart. On blowing into the tube, the condom swells; but as the strings prevent it from lengthening, the sides bulge and so shorten the condom. The two positions are shown in the figures.

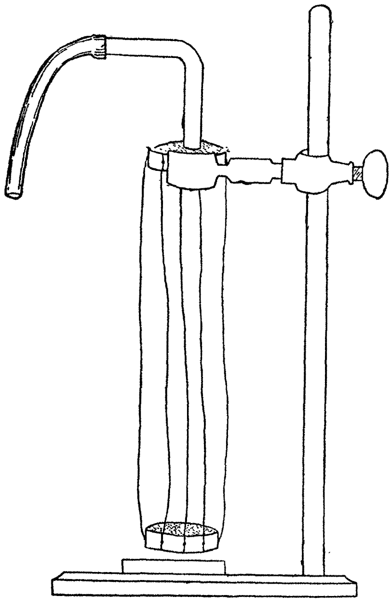

FIG. 1

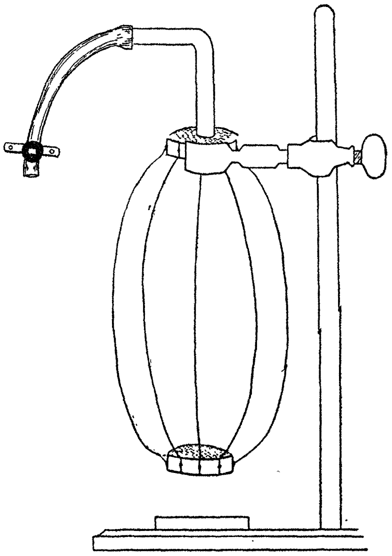

FIG. 2

The same phenomenon may be shown with dead small intestine from a rabbit or dog. A segment of intestine, about 10 cm. long, should be kept in normal saline solution for a day or two, at room temperature, to insure absence of vital tone changes. It is then arranged as in the Trendelenburg[3] peristalsis method. When the reservoir is raised, a centimeter at a time, the tracing lever shows progressive shortening, which disappears progressively when the reservoir is gradually lowered.

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SPECIAL ARTICLES

THE PRESENCE OF TREHALOSE IN YEAST

IN connection with studies on "Bios," an alcohol extract of Fleischmann's yeast was permitted to stand undisturbed for several months when a cluster of well-formed crystals was found clinging to the sides of the flask.

Preliminary tests indicated that it was a non-reducing, hexose containing di- or poly-saccharide. It was found to be exceedingly soluble in water and glacial acetic acid, either hot or cold, and somewhat less soluble in pyridine. It was insoluble in acetone. Unusual resistance to hydrolysis was shown by the fact that, after treatment with boiling glacial acetic acid, a Fehling's test was negative. Only when a solution of the crystals in N/2 HCl had been heated in a boiling water bath for one half hour was a positive Fehling's test obtained.

For more detailed investigation additional material was necessary. Securing these crystals a second

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[1] From the Department of Pharmacology of the Medical School of Western Reserve University, Cleveland, Ohio.

[2] Wacker, L.: Arch. ges. Physiol., 1917, clxviii, 158.

[3] Trendelenburg, Paul: Arch. f. exp. Path. Pharm., 1917, lxxxi, 55.

time did not prove a simple task. Finally, after several unsuccessful attempts with small quantities of yeast, forty pounds of compressed yeast were air-dried and then extracted in a large, continuous-extraction apparatus, first with ether for eighteen hours, followed by four portions of 90 to 95 per cent. alcohol for periods of eighteen to twenty hours each.

These combined alcohol extracts were stored in large glass bottles and seeded with small portions of the original material. After eight to ten days crystals began to form on the sides of the bottles. These were permitted to grow until there seemed to be no further increment in size. The alcohol was then decanted and the crystals dissolved in hot glacial acetic acid. After the solution had cooled ten to twelve volumes of acetone were added. The creamy, flocculent precipitate was filtered off and washed several times with acetone. A water solution of this precipitate was decolorized with charcoal, concentrated to a syrup and diluted with hot alcohol to make about 70 to 80 per cent. Care was taken at this stage to keep the solution hot and to add the alcohol slowly to prevent the reformation of a flocculent precipitate. Acetone was finally added until the solution just began to appear milky. It was then seeded with crystals and set under a bell jar to cool. This was to prevent any considerable absorption of moisture from the atmosphere. Within twenty-four hours large rock-candy-like crystals were found on the stirring rod and sides of the beaker.

The alcohol decanted from the crude crystals was evaporated in vacuo, the residue extracted with glacial acetic acid and further treated as described above. In this case the alcohol-acetone solution stood several weeks before the crystals began to form. The presence of impurities retards and may even prevent entirely the separation of this substance in crystalline form. When relatively pure it can be recrystallized from hot alcohol in a few hours.

After four or five recrystallizations from hot alcohol and acetone the substance was entirely colorless and odorless.

A melting point determination showed the characteristic behavior of trehalose, the crystals becoming fluid at 102.5° C., solidifying at 125° to 135° and melting again at 208° to 209°. Melting point determinations previously reported vary considerably. Mitscherlich[1] describes crystals of trehalose as losing water of crystallization at 130° C. and melting at 210° C. Later work by Lippmann[2] gives the melting point of the hydrated sugar as 103° C., loss of water of crystallization at 130° C. and a second melting point at 203° C.

Polariscopic readings were taken on a 20 per cent., a 10 per cent. and a 5 per cent. solution. The $[\alpha]_D^{20}$ values obtained from these observations were 184.6, 184.55 and 182.8, respectively. Correcting for the two molecules of water which trehalose contains, the $[\alpha]_D^{20}$ values for the anhydride were 204.02, 203.96 and 202.04. Various determinations quoted by other authors give the $[\alpha]_D^{20}$ value for the hydrated sugar as 167.4[3], 173.4[4] and 178.3[5]; for the anhydride as 197.3,[5] 198.6[6] and 201.5.[7] Our figures are higher than any quoted, suggesting a greater purity of the sugar.

Molecular weight determination by the freezing point method gave results of 354.6 and 358, considerably below 378, the molecular weight of the hydrated sugar. It is obvious that for substances of high molecular weight this method is not sufficiently delicate to obtain greater accuracy of results.

As has been stated before this substance is hydrolyzed with difficulty. To 20 cc of a 5 per cent. solution 2 cc of HCl, [sp. gr. 1.125,] were added and the mixture boiled under a reflux condenser for two and one half hours. The polariscope reading was then taken and again after an additional half-hour heating. Constant readings were obtained, and were such as would account for two molecules of glucose for each molecule of the original sugar. A glucose determination by the Munson-Walker-Bertrand method on an appropriate volume of the neutralized diluted solution confirmed this polariscopic reading.

A third portion of the neutralized solution was used for the osazone test. Only glucosazone was found. Distillation for furfural gave no phloroglucide in the distillate.

These observations all confirmed the original melting point determination, which identified the unknown crystalline substance with trehalose.

Trehalose is found with mannite in mushrooms. Bourquelot and Herissey[8] believe that *trehalose* and

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[1] Mitscherlich, Erdman's Prak. Chemie, Vol. 73, 65 (1858).

[2] von Lippmann, E., *Berichte der Deutschen Chemischen Gesellschaft*, Vol. 45, 3431 (1912).

[3] Wiggers, H. A., *Annalen der Chemie u. Pharmazie*, Vol. I, 173 (1832).

[4] Berthelot, *Annales de Chim. et de Phys.* (3), 55, 372 (1859).

[5] Harang, P., *Journ. de Pharm. et de Chim.*, Vol. 23, 16 (1906).

[6] Müntz, M. A., *Annales de Chimie et de Phys.*, 5e serie, Vol. 8, 56, 1876.

[7] Bourquelot, Em., *Compt. Rend. de l'Acad. des Sc.*, Vol. 108, 568 (1889).

[8] Bourquelot, Em., et Herissey, H., *Compt. Rend. de l'Acad. des Sc.*, Vol. 139, 874 (1904).

trehalase have the same function in mushrooms as *sucrose* and *invertase* perform in chlorophyll-containing plants, in that they may be involved in the hydrolysis or synthesis of the higher carbohydrates.

In 1876 Müntz[6] looked for trehalose in brewer's yeast, thinking that since it was so generally present in higher fungi it should also be found in the lower fungi such as yeasts. His several attempts to separate it from the water and alcohol extracts of yeast were a failure. He concluded that either this sugar does not exist in yeast, or that the large amounts and the diversity of other soluble substances present therein make its separation from the solutions impossible.

The present discovery of trehalose in an alcohol extract of baker's yeast was due to the comparative freedom from impurities, the relatively high concentration of trehalose and the fact that the solution stood undisturbed for a long time. It was under similar conditions that the same sugar was first found by Wiggers[3] in a water solution of rye ergot.

The alcohol extract of yeast from which trehalose was separated was slightly acid. It is a question whether it exists in the yeast cell as such or is a hydrolysis product formed during the long extraction process.

A similar extract of yeast added in small amounts to a medium in which yeast is growing increases to a marked extent the invertase content of the yeast grown.[6] It is possible that trehalose is responsible for such an effect on enzyme formation. This question is being investigated.

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THE ALKALINE REACTION OF THE DEW ON COTTON PLANTS

Since the presentation of a paper by me at the meeting of the American Chemical Society, New Haven, Connecticut, April, 1923, and its publication in the *Journal of Agricultural Research*,[1] under the title "Excretions from leaves as a factor in arsenical injury to plants," two communications upon certain points of this paper have appeared in Science, which seem to require on my part a few words of explanation.

The first point is one of chemical fact—whether the alkaline earth carbonates are or are not alkaline to phenolphthalein.

Mr. J. E. Mills (Science, September 19, 1924) states that, "Mr. Smith seems to think that there is something in his analysis of the dew, showing calcium and magnesium carbonates and bicarbonates, to account for the alkalinity observed. Since both acid and normal alkaline earth carbonates are neutral to phenolphthalein this would hardly seem possible." Power and Chesnut (Science, October 31, 1924) repeat part of this statement in the following words, "Mr. Mills has now noted (*loc. cit.*) that it would hardly seem possible that the alkalinity of the dew can be attributed to these compounds." The words "these compounds" as used are rather indefinite in meaning, but they must refer to the carbonates or bicarbonates or both, since those were the only salts to which Mills' statement applied. It is very easy to show that calcium carbonate, at least, is alkaline to phenolphthalein, as the reagent material, and even Iceland Spar, when powdered, will readily color an aqueous solution of that indicator.

Since calcium carbonate is a salt of a moderately strong base and a very weak acid, it hydrolyzes when dissolved in water, as the result of which the solution contains not only calcium and carbonate ions, but also bicarbonate and hydroxyl ions, the formation of which may be illustrated by the equation $\mathrm{CO}_3^{=} + \mathrm{H_2O} = \mathrm{HCO}_3^{-} + \mathrm{OH}^{-}$ The hydroxyl ions so formed are evidently sufficient in number to redden phenolphthalein. The question of equilibrium in solutions of lime and carbon dioxide has been thoroughly treated by Johnston and Williamson.[2]

Mills, after stating that the carbonates and bicarbonates of the alkaline earths could hardly explain the alkalinity, suggested that an alkali carbonate would suffice to do so. As a matter of fact I collected a sample of dew from cotton plants at Tallulah, Louisiana, subsequent to the work reported in my paper, and submitted it to the Insecticide and Fungicide Laboratory, Bureau of Chemistry, in July, 1923, for a more complete analysis than my limited facilities at Tallulah permitted. This sample was analyzed by Mr. J. J. T. Graham, who found potassium present equivalent to 252 parts of K_2O per million. The presence of potassium carbonate in the dew, along with other salts, can therefore be considered possible. In this connection it may be of interest to note an article, which later came to my attention, entitled "Die Secrettropfen an den Laubblättern von *Phaseolus multiflorus* Willd. und der Malvaceen," by A. Nestler.[3] Nestler found that *Phaseolus multiflorus* Willd. and several species of Malvaceae—cotton was not included among those investigated—secreted drops of liquid which were usually alkaline to phenolphthalein

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[9] Miller, E. W., *J. Biol. Chem.*, Vol. 48, 329 (1921).

[1] Vol. 26, No. 4, p. 191, October 27, 1923.

[2] *J. Amer. Chem. Soc.*, Vol. 38, No. 5, pp. 975–983.

[3] *Berichte der Deutschen Botanischen Gesellschaft*, Vol. 17 (1899), p. 332.