SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN IDEAL MOUNTING MEDIUM FOR MYCOLOGISTS

DURING the past three years the writer has used Amann's mounting medium with unusually satisfactory results. Although it is in rather general use abroad, this medium does not appear to be widely employed in this country, despite its many advantages over eosine-glycerine. In the study of dried material there is little delay, for this medium almost immediately restores the turgor of the specimens, while with fresh material there is no plasmolysis, especially in the higher fungi, and at the same time the medium serves as a killing agent. In addition to its application to mycology, the solution has been successfully used by Dr. Edgar Anderson, of this institution, in the course of his study of the pollen grains of *Iris.*

The formula, after Sartory,[1] is as follows:

Carbolic acid crystals	20	grams
Lactic acid, syrup	20	"
Glycerine	40	"
Distilled water	20	"

For greater rapidity in mixing the above materials, they may be heated over a low flame. When the solution has cooled, it may be used as made up, or should a dye be desirable, as is often the case with hyaline specimens, then .5 per cent. of cotton blue should be added. Frequently this concentration of dye is excessive, in which case the color may be made more dilute by the addition of varying proportions of the original medium.

Permanent preparations may be made by allowing the mounted specimen to stand a week in a desiccator to allow the water to evaporate, otherwise the ringing cement (preferably King's amber cement) will tend to run under the cover-glass. The lactophenol medium can also be used in connection with Diehl's[2] method of making permanent preparations if the same precautions (desiccation) are followed.

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NOTE ON PREPARATION OF COLLODION BAGS

FOR those interested in the use of collodion bags for dialysis, classroom experiments in physiology, etc., where uniformity and ease of preparation are important, I should like to call attention to two papers doubtlessly overlooked at this late date: Harris, N. M., *Centralb. f. Bak. und Par.*, I, 32, 74, 1902, and Gorsline, C. S., SCIENCE, p. 375, March 7, 1902.

Both authors describe the formation of a collodion bag on the outside of large gelatine capsules held by heated glass tubing thrust into one end, over which the collodion may form a narrow neck to the bag of perfect uniformity and free from flaws to any desired thickness. Warm water allowed to flow into the capsule after drying of the outer collodion coating dissolves the interior gelatine wall, leaving the perfect collodion bag. The glass tubing may be left in place or removed, as desired. This apparatus was originally intended to contain bacterial culture material for insertion into the peritoneum, allowing dialyzable bacterial products to diffuse out in immunity experiments, but it appears that this method may be diverted to other uses. I have found the method excellent. Harris gives a brief history of previous attempts to make such sacs on the ends of glass tubing.

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SPECIAL ARTICLES

ADAPTATION OF RICE TO FORTY CENTURIES OF AGRICULTURE

THE late Professor F. H. King in his book "Farmers of Forty Centuries" depicts the high level of crop production maintained for many centuries in some of the densely populated areas of oriental countries. Without the use of mineral fertilizers, depending almost entirely on occupational offal for purposes of soil improvement, Asiatic countries have maintained rice production in many sections sufficiently high to support their teeming millions. Various has been the comment or "explanation" on this phenomenon that appears as a paradox in the maintenance of soil fertility compared to the exhaustion of the soil by continued cropping of land to the cereals of occidental countries if practiced without fertilizers. Although the culture of rice (paddy) differs from that of wheat and barley in that the fields are usually submerged, the quantity of water applied annually usually does not contain sufficient nutrients to replace those taken from the soil by a normal crop of wheat or barley.

Results obtained in investigations to determine the smallest percentage of certain elements that can obtain in mature plants appear to throw light on the phenomenon of large yields of rice from lands cropped to this plant for many years continuously. The method employed in these experiments was to have test plants absorb varying quantities of a given element or elements by growth for varying lengths of time in a complete nutrient solution, subsequently

[1] Sartory, A. "Guide des manipulations de mycologie parasitaire," p. 100. Paris. Undated.

[2] Diehl, W. W. SCIENCE, N. S. 69: 276. 1929.

transferring them for the completion of growth into a culture medium devoid of the element for which the minimum was to be determined, but containing all others required for growth. By withholding absorption of the given element during the latter growth periods of the plant, data were obtained as to the maximum growth plants could make for a given quantity of an element absorbed during the early growth period. The identity of the same percentage of element in increasing magnitudes of yield and corresponding to increasing quantities of element absorbed was evidence that the maximum yield per unit quantity of element absorbed was obtained. The maximum yield of plant is the reciprocal to the minimum percentage of element that can obtain in the mature plant.

The nutrition of rice was studied with that of other cereals in the manner indicated above in order to obtain minima for various elements. Data were obtained as to the length of time (in terms of growth phase) elements were required. Furthermore, data were also obtained as to the length of time required for utilization of given quantities of materials after they were absorbed. The experiments are to be fully reported elsewhere, but brief account is given at this time as it appears that the data throw some light on the phenomenon of high yields of rice maintained for many years in oriental countries.

While chemical analyses are to be made on an array of varieties of rice treated to obtain the minimum for various elements required from the soil, nevertheless the results of the physiological experiments indicate that these will be very low for calcium, magnesium, phosphorus and sulphur. For example, seedlings grown initially in a complete nutrient solution for several weeks until they attained three grams dry weight per plant increased from eight to ten fold (dry weight) at maturity in a solution of KNO_3 only but containing iron. Similarly, seedlings grown initially four weeks in a complete nutrient solution and subsequently transferred for the completion of growth to culture media each devoid of one of the following elements—calcium, magnesium, phosphorus and sulphur—increased in weight from eight to fifteen fold at maturation. But seedlings grown initially for varying periods of time up to three months in complete nutrient solution upon transfer into media each devoid of one of the following elements—potassium, nitrogen and iron—produced markedly less crop at maturity than did any of the above treatments, or that of cultures grown entirely in a complete nutrient solution. Well-filled grain, but varying as to quantity depending on treatment employed, was produced from all treatments except those designed to determine the plant's requirement for iron. The absence of this element in available form in the media for relatively short periods as compared to that of other required elements was decidedly harmful to the growth of rice.

The complete nutrient solution used for the initial growth of rice was composed of equal concentrations of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, together with a trace of boron and manganese (each one p.p.m.) and iron supplied as tartrate. The experiments were thus designed in the belief that all the above-named elements were essential for the growth of rice, but the unexpected good yields obtained of fully developed plants grown four fifths of their lives in media devoid of one to four of the already indicated elements suggest the advisability of reconsideration of the evidence extant that rice requires the same elements for growth as does wheat or barley. While the results of the experiment did not prove that any one of the elements in the complete nutrient solution was unessential, they nevertheless did prove that if all were essential, exceedingly small, or relatively unimportant quantities, considered as fertilizers, of all elements named with the exception of potassium, iron and nitrogen were sufficient to produce large yields of rice. It is the markedly low minimum of calcium, magnesium, phosphorus and sulphur in the mature plant, different in each of the differentiated products—grain, straw, roots—that is cited as evidence of low requirement for these elements and is explanation for the maintenance of high yields from continuous cropping of land to rice without any apparent marks of soil exhaustion. It is held the data obtained show that the production of large yields of rice is not dependent on soil rich in the elements named. Comparison of the requirements of wheat or barley for these elements with that of rice indicate that the latter plant would produce large crops planted in soils which would be relatively (and in some cases practically) infertile to wheat or barley.

In addition to these features of rice, several others may be mentioned in view of certain conceptions concerning the nutrition of this plant. Ten types of markedly different nutrient solutions were used and several hundred cultures were grown to maturity with nitrates as the only source of nitrogen available. The yields in many instances were comparable to those of representative plants grown in the field producing large crops. Ammonium salts, contrary to the prevailing view, are not necessary for rice production in nutrient solutions or in soil as proven by Bartholomew[1] in a recent publication. The failure of investigators to obtain normal development of rice in nutrient media containing nitrate, and devoid of ammonium ions, appears to be due to the inavailability of the iron used, although the form used would be read-

[1] R. P. Bartholomew, "The Availability of Nitrogenous Fertilizers to Rice," *Soil Sci.*, 28: 85–100, 1929.

ily available to wheat or barley in such media. Another feature observed was that related to the conditions necessary for grain production. Repeated failure to obtain grain from apparently normal plants was explained in the condition necessary for the fertilization process to function. It was found that the diurnal changes in temperature and humidity play very important rôles in the process. Rice plants require dew or a fairly saturated atmosphere for pollination to proceed properly, and the absence of dew on the plants, although grown in water in the greenhouse, was found to be the cause of the failure for rice to set grain in the case above mentioned.

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THE MORPHOLOGY OF HAEMOPROTEUS LOPHORTYX SP. NOV.

EXAMINATION of 312 native quail in California showed that 45 per cent. of the birds were parasitized with a sporozoan, the gametocyte stages of which were to be found in the red blood cells of the birds.

The parasite, which belongs to the genus *Haemoproteus,* differs from *Haemoproteus columbae* Celli and Sanfelice of the pigeon and all other described forms. It is found in *Lophortyx californica* Shaw and all other species and subspecies of the genus *Lophortyx* inhabiting California, including Santa Catalina Island. It is herewith described as *Haemoproteus lophortyx,* the description being based largely upon the morphological characters of the mature gametocytes as they appear when the dried films are fixed with methyl alcohol and stained with dilute Giemsa's azur-eosin.

The diagnostic characters are as follows: Mature male gametocytes: Form and size: halter-shaped, partially encircling nucleus of blood cell but not in close contact with its nuclear membrane. Along greater part of its length, parasite extends out to periphery of blood cell. Diameter one and one half to two and one half microns; length up to eighteen microns when curving is taken into consideration. Both ends uniformly rounded, but the end containing the nucleus slightly broader. Instances not rare of the parasite completely filling the space formerly occupied by the cytoplasm of the host cell, in which case both ends of the nucleus-encircling gametocyte in contact.

Nucleus: elongate, ovate, almost always nearer to one end of the gametocyte, its broader end being nearer to the end of blood cell. Staining reaction, pale pink. Average size, one and one half by four microns. Becomes more diffuse and much larger, filling three fourths of volume of cell just preceding gametogenesis. Karyosome usually visible.

Cytoplasm: pale, almost hyaline.

Vacuoles: indistinct and diffuse, often a large one near one end of gametocyte with ring of pigment granules around its periphery.

Pigment granules: minimum number, eleven; maximum, thirty-nine; average, nineteen, with tendency to be deposited in two more or less terminal groups with a few scattered granules in between. Shape, from spherical to oval or rod-shaped. Size, from two tenths to eight tenths microns in greatest diameter. In fresh diluted blood, of carbon black appearance; in stained preparations, brownish. All granules highly refractive.

Mature female gametocytes: Form and size: like male gametocytes, halter-shaped encircling nucleus but not closely applied to nuclear membrane. Greater tendency for both ends of gametocyte to come into contact around nucleus of blood cell than in case of male gametocyte, in which case parasite loses characteristic halter-shaped appearance and fills entire space between nucleus and periphery of erythrocyte. Measurements in general same as for male gametocyte but tendency to produce greater hypertrophy of blood cell, especially when more than one gametocyte present in cell.

Nucleus: spherical to oval, more centrally located than in male gametocyte, average size up to one and one half or two and one half microns in the greater diameter. Staining reaction dark pink to red. Karyosome distinct.

Cytoplasm: staining reaction much darker blue, reticular appearance apparent.

Vacuoles: usually present, from one the size of the nucleus to two or more smaller ones irregularly placed.

Pigment granules: minimum number, fifteen; maximum, fifty-two; average, twenty-four. Tendency to be grouped less pronounced than in the case of the male gametocyte.

Experimental work has demonstrated that *Lynchia hirsuta* Ferris, an ectoparasitic louse fly, is responsible for transmitting the parasite from quail to quail. Transmission is biological, the sexual cycle of the parasite taking place in the fly.

Ordinarily a parasitized quail seems to be little inconvenienced, but numerous cases have been observed where the infected bird was weak and thin. Five fatal cases have been studied, the death of the birds being preceded by marked anemia due to the destruction of the red blood cells by the parasites.

The study of this parasite and its effect on the host is of especial interest since the California Valley quail is California's most prized game bird.

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