

known investigations. Van der Stricht has written on the genesis and structure of the membrana tectoria and crista spiralis of the cochlea, and Duesberg on "la fécondation des ascidiens"—a study of chondriosomes. Cowdry likewise has dealt with the mitochondrial constituents of protoplasm and has supplied a shorter paper on the chromophile cells of the nervous system. Mitochondria in nerve cells are quantitatively considered by Madge D. Thurlow. The transitory cavities in the corpus striatum are described by Essick. Two papers deal with tissue cultures, the occurrence of binucleate cells being described by Macklin, and the development of connective tissue fibers by Margaret R. Lewis. Miss Sabin, through series of fine injections, strikingly reproduced, has traced the transformation of the posterior cardinal veins of pig embryos, and, in a second paper, the origin of the primitive vessels in the chick. Streeter has advanced the study of the cerebral sinuses, which have been beautifully drawn, and has described also the formation and spread of the periotic tissue spaces. Weed's important work on the development of the cerebrospinal spaces forms the whole of Volume 5. Clark interprets an extraordinary anomaly of the thoracic duct, and Cunningham describes the pulmonary lymphatic vessels of pig embryos. There are three monographic studies of normal human embryos, by Ingalls, Johnson and Watt; and a specimen with spina bifida is described by Miss Wheeler. Corner reports on the corpus luteum in the pig. Meyer has a statistical study of prenatal growth, based on obstetrical records, and Shipley and Wislocki jointly, interested in the chemical products of the poison glands of *Bufo agua*, a tropical toad, describe the histology of these epinephrin-producing glands. In the twenty-sixth and last contribution, Kunitomo deals with the retrogression of the caudal end of the spinal cord and the decline of the tail in human embryos.

The contributions are irregularly grouped in small volumes which are sold separately. Doubtless it would be appreciated if a limited number of the separate articles were offered

to embryologists, though every institution needs the complete file. Altogether it is a journal to be studied by those responsible for our anatomical publications. When the *American Journal of Anatomy* was founded and was being published in Baltimore largely under Mall's direction, it seemed that nothing better was likely to appear in this country. But as the *Journal* became securely established, losing—perhaps we imagine it—the enthusiasm of the earlier volumes, Mall's genius for publications sought new fields. His *Contributions* have caught in beautiful form and permanent record the spirit and purposes of current American investigations in embryology, and their future is full of promise.

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SPECIAL ARTICLES

NOTE ON THE TECHNIQUE OF SOLUTION CULTURE EXPERIMENTS WITH PLANTS

IN recent years a large number of sand and solution culture experiments have been carried out by various laboratories. It is becoming recognized that any complete understanding of soil fertility requires an insight into the absorption and metabolism of the plant as well as the nature of the soil solution. In connection with some investigations relating to the latter question, this laboratory has undertaken a series of studies on the effect of concentration and reaction of the nutrient solution on the growth and absorption of the barley plant. Incidental to this work it has been necessary to examine somewhat critically several phases of the technique employed in sand and solution cultures, and it is desired to present here a number of considerations bearing on the interpretation of these experiments.

Ordinarily the conclusions from such investigations have been based on the concentrations and composition of the solutions as originally prepared. In very few cases have analyses been made of the solutions after contact with the plant, nor of the plants themselves. It is not known therefore exactly what was the condition of the solution during the periods between changes. The percentage

variation in the solution for any given element will depend upon the total quantity absorbed, upon the concentration in the original solution, and also upon the volume of solution provided per plant. It is essential to differentiate between two sets of factors, the composition and concentration of the solution and the total quantities of the various elements present. The effect on the plant might be the result either of the concentration as found in the original solution, or of an insufficient total supply of one or more elements. In order to study the effects of concentration or of composition on plant growth, ideally a continuous flow of solution should be arranged so that the roots are always bathed in a solution of constant composition. Such a technique is ordinarily impracticable, and it is necessary to approximate the desired condition by providing a sufficient volume of solution per plant and by frequent changes. This is particularly true when the object of the investigation is to determine the relative effects of a series of solutions. To give a specific example, certain solutions may have only one tenth of their total concentration due to $\text{Ca}(\text{NO}_3)_2$. In such a case it is possible that all of the NO_3 might be absorbed before the solution was changed, or at least reduced to a very low level of concentration. Thus, if the interpretation of the experiment is based on three salt triangular diagrams, the effect, actually the result of insufficient NO_3 , might be correlated with a certain calcium magnesium ratio.

In some experiments small bottles (250 to 400 c.c.) have been used with three to six plants in each bottle, changes of solution being made every three days, or sometimes only every four or five days. In the sand culture series the size of the jars usually permits the use of only 250 to 400 c.c. of solution per jar. In our experiments (to be described elsewhere) from 500 to 2,200 c.c. of solution per plant (barley) have been used, with changes every two or three days in many cases. Actual determinations of the absorption of each element have been made by analyzing the solutions or the plants. It has

been found that under favorable conditions of light and temperature, more than 30 per cent. of the total electrolytes may be absorbed in three days, when 500 c.c. of a favorable nutrient solution of 2,500 p.p.m. concentration is provided for each plant. All of the elements are not absorbed in equal percentages, consequently not only the concentration but also the relation between the elements has been altered. In one experiment with solutions containing 100 p.p.m. NO_3 (500 c.c. solution per plant) barley plants six weeks old absorbed every trace of NO_3 from the solution in less than 72 hours.

In several experiments in which plants have been grown in solution and sand cultures the yields of straw and heads are fairly comparable with those of plants produced in the field, where an excellent crop is obtained. In some sand and solution culture experiments reported the yield per plant has evidently been much inferior to that for similar plants grown in the soil for an equal period. Some limitation of light, temperature, aeration or of the nutrient solution must therefore have existed. In many cases there is a strong presumption that the supply of nutrients may have been deficient, as noted above.

We do not desire, however to criticize any specific investigations. If plants are grown under sub-optimal light or temperature conditions, the total quantities of nutrients absorbed per plant may be much less than in our experiments. Moreover, in the first few weeks the plant has not reached its maximum power of absorption, so that short culture periods will require less quantities of nutrients. The point we desire to emphasize is that plants grown under the most favorable conditions may absorb or require much larger quantities of nutrients per plant than are ordinarily provided in sand and solution culture work. Each set of conditions should be tested by actual analysis of solutions and plants and results interpreted in terms not of the original solution alone, but also in terms of total supply and the varying condition of the solution in the periods between changes. It should also be noted that deficiencies in total

supply in the earlier stages of growth may stunt the plant so that absorption in the later stages is much less than would occur with a normal plant.

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UNHEATED EGG-YOLK MEDIA

For some years the writer has been using unheated egg-yolk media and has found them especially valuable in studying one of the fowlbroods caused by an organism (*Bacillus larvæ*) which offers considerable difficulty in its cultivation. In a paper "Further Studies on American Fowlbrood" to be published in the *Journal of Agricultural Research* reference is made to the employment of such media successfully in the study of this species. Believing that the fact might be of interest to those studying diseases caused by organisms for the cultivation of which unheated animal products are being employed and possibly also to those using heated egg media in their work, the technic used in the preparation of these media is given at this time.

These are prepared by adding simply a sterile aqueous suspension of egg-yolk to the different media commonly used in the laboratory. The egg suspension is obtained as follows: After being disinfected the shell of the egg is broken the white poured off and the yolk dropped into a flask containing about 70 c.c. of sterile water. By agitating the flask a uniform suspension of the yolk material is obtained. This is then transferred to sterile tubes by pipetting, and stored until needed. On standing the suspension separates into a more or less translucent supernatant fluid and an opaque lighter yellow-colored sediment.

In preparing the egg media about 1 c.c. of the egg-yolk suspension is added to each 5 c.c. of the base medium. If only the supernatant fluid is used a clearer medium will result. Egg agar has been the most useful of these media in the work referred to. The base should be at least 1.5 per cent. agar and after being liquefied should be cooled to between 45° and 50° C. before the suspension is added.

Tubes may be inclined and stored until needed. The medium may be inoculated and plates made, or sterile plates may be poured. Although the pipetting of the sterile suspension rarely results in contamination of the media, if convenient to do so, it is well to test them for sterility after this step is taken. The egg suspension itself is a medium of some differential value.

Eggs known to be recently produced are preferable for the egg-yolk suspension, although those obtained from the market labelled "strictly fresh" have usually been satisfactory. The shell is disinfected conveniently by immersing the egg in a suitable solution for a few minutes. A 1:1,000 mercuric chloride one is satisfactory for the purpose. Alcohol and solutions of carbolic acid and formalin have been used but the latter two unless gloves are employed are unpleasant to the hands. After removing the egg from the solution, the shell is broken about one end and removed with forceps sterilized conveniently in the direct flame. The white being poured off the limiting membrane of the yolk is broken and the yolk material is poured into the flask containing the sterile water. The degree of transparency of the supernatant fluid depends somewhat upon the amount of water used in making the suspension. Occasionally contaminations are encountered. These are usually detected by changes in the appearance of the suspension following incubation.

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