forceps until it and the attached membrane are breached, and then the hole is carefully enlarged until it is roughly 1 cm. long and 3–4 mm. wide, running parallel with the margin of the air space. Next, the shell without the attached shell membrane is broken away for 3–4 mm. beyond the margin of the air space—that is, over the chorioallantois. In doing this, care must be taken to lift the fragments of shell slowly outwards with the forceps and not to allow them to hinge back and pierce the chorioallantois. The exposed outer layer of shell membrane is then torn away, and the inner layer of shell membrane is found to have a fold about 1 mm. wide marking the margin of the air space. A fine pair of forceps is used to tear the shell membrane along this fold. Up to this stage of the operation the egg is held in the left hand in an approximately horizontal position; it is now tilted with the air space downward. With 11-day embryos the chorioallantois usually falls readily, giving an artificial air space in the same position as with the standard Burnet technique. With 12-day embryos it is usually necessary to tap the egg with the fingers to commence the separation of the chorioallantois from the shell membrane. When the separation commences, the egg is returned to a horizontal position. The inoculum is placed on the chorioallantois with a Pasteur pipette, the hole in the shell sealed with cellophane adhesive tape, and the egg incubated in a horizontal position.

Amniotic inoculation. After preliminary incubation with the air-space end uppermost, in most of the eggs the embryo is found close to the margin of the air space, which is essential with this technique. The eggs are candled and the hole commenced as for chorioallantois inoculation, but it is not extended beyond the margin of the air space. A sharp, pointed pair of forceps is thrust through shell membrane and chorioallantois, close to the margin of the air space, and the hole thus made extended by blunt dissection to about 1 cm. As the air enters, the embryo enclosed in the amnion is presented at the hole. It may be necessary to remove some of the shell membrane adhering to the chorioallantois. Sometimes an air bubble blocks the hole; this may be disposed of by touching it with a needle heated in the flame. The amnion is grasped with forceps and inoculated with a Pasteur pipette drawn to a fine point and beveled, or with a syringe and needle. The egg is sealed with cellophane adhesive tape and returned to an upright position for incubation.

Allantoic and yolk sac inoculations. The current technique in many laboratories is to pierce the shell with a needle, and it is mentioned here merely to complete the series of techniques not requiring a dental drill. The hole is made in the shell with a surgical needle mounted by driving it through a rubber bung, after which the standard techniques are followed.

Position of the egg during incubation. Although it is well known among embryologists that the position of the egg during incubation determines the position of the embryo within the egg, this fact does not appear to be generally known by virus workers.

After 4 days incubation the embryo often moves freely within the shell, rising immediately to the uppermost point as the egg is rotated. Between the 5th and 9th days movement is slower: if an egg lying horizontal is rotated 90°, the embryo and membranes rise to the top after several hours. Between the 10th and 12th days the position of the membranes becomes fixed, but the position of the embryo may still change to some extent if the egg is turned.

If eggs incubated horizontally for 6–9 days are turned 180° so that the embryo and membranes are on the lower side, about 25 per cent die overnight. On the other hand, among eggs that are not turned at all during incubation up to the 12th day I have observed very few deaths—no more than occurred in eggs turned slightly once or twice a day.

If eggs have been incubated in the horizontal position, it is often not easy to harvest the allantoic fluid because, on reflecting the membrane under the air space, one encounters yolk sac and egg white as well as the allantoic cavity. If an egg has been incubated with the air space down, it is impossible to harvest either allantoic fluid or the yolk sac via the air space, because under the membrane in the air space there is only egg white. The embryo will be located in the "sharp" end of the egg, and the egg white is always in the lowermost point.

When cultivating influenza virus for vaccine production by red cell concentration technique, it is especially desirable to have the embryo centered under the air space. Furthermore, unless it is in this position, inoculation via a hole in the air-space end of the egg will often fail to infect the allantoic cavity.

It is of considerable importance that vaccines produced from the developing egg should be free of egg white; therefore, for the production of vaccines from allantoic fluid, yolk sac, or embryos, the eggs should be incubated before and after inoculation in a nearly vertical position with the air space upwards. They may be inclined 30°–40° from the vertical and turned by tipping back and forth once or twice a day. The only advantage in turning in this way is that it results in a more symmetrical development of the chorioallantois and allantoic cavity. On the other hand, for inoculation on the chorioallantois it is somewhat of an advantage to have a large area of chorioallantois on one side of the egg, so the eggs are better not turned before this type of inoculation. For intracerebral inoculation or amniotic inoculation by the standard Burnet technique it is better to incubate eggs in a horizontal position before inoculation.

Reference

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Use of Trisodium Phosphate With Herbarium Material and Microfossils in Peat

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A brief article recommending the use of dilute aqueous solutions of trisodium phosphate for reclaiming dried zoological specimens (*1*) suggested the possibility of using the same technique for restoring herbarium specimens to a condition for sectioning or clearing. In one test, for example, two leaves and a twig of dried *Viburnum acerifolium* L. were placed in 25 cc. of the solution and kept in an oven at 60° C. for 2 hours. The leaves assumed the external appearance of fresh material (except for a slightly less brilliant green color) and approximately the natural flexibility, toughness, and turgidity of the fresh state. Free-hand sections of the leaves showed the par-

enchyma cells to be in a state approximating normal turgidity and the protoplasts with a distended, although not truly life-like, appearance. The chloroplasts of the mesophyll were distributed approximately as in the living condition. The twig also resumed its flexibility and turgidity. It was possible to cut free-hand sections of the twig with nearly the same ease as with fresh material; and the cells of the pith, wood parenchyma, and cork resembled very closely the forms of those in the living tissues.

Prolonged treatment is to be avoided; for after 18 hours at 60° C., or upon standing for several days at room temperature, certain tissues begin to show areas of brown discoloration (due presumably to the breakdown of chlorophyll and the diffusion of lignin derivatives). After 24 hours at 60° C., treated leaves become uniformly brown and begin to lose resiliency.

The success in restoring herbarium specimens led to the application of this method to the preparation of peats for analysis of microfossils. After trying concentrations which varied from 0.25 per cent to 10 per cent and temperatures ranging from 24° to 60° C., the best results again were achieved with 0.25–0.5 per cent solutions heated for 2 hours at 60° C. The peat sample is placed in a 15-cc. centrifuge tube, covered with approximately 10 times its volume of solution, and placed in the oven. If the peat has been stored in the wet condition, it can be gently broken up in the centrifuge tube with the tip of a camel's-hair brush before heating. If, however, the peat has been dried in storage, this operation is best delayed until the solution has reached 60° and has thoroughly moistened the peat. The addition of a wetting agent, e.g. Nacconol (National Aniline Company), to the solution accelerates the action on dried material. After heating, the material is twice washed with distilled water, centrifuged, and decanted. An amount of glycerine equal to the volume of the sediment is then added, and sufficient quantities are pipetted out to make slide mounts.

Although the trisodium phosphate preparation of peat samples has the disadvantage of removing very little of the materials which may tend to make the identification and counting of pollen and spores difficult, it seems desirable because it restores the sediment to a condition similar to its state when freshly deposited. The particulate matter is well deflocculated. Pollen, spores, fungal hyphae, trichomes, and most tissues of a more gross nature are re-expanded to approximately their normal shape in life. In addition to the re-expanding of the pollen grains, the exine layers appear to become turgid, so that characters such as the arcuate bands between pores of the *Alnus* grain, the hyaline bodies beneath the pore of the *Fagus* grain, and the "fingerprint" markings on the surface of the *Acer* grain stand out very clearly. The most delicate features of waxy excrescences on certain fern spores were entirely undamaged. The method causes no apparent change in the preserved diatoms, insect chitin, exoskeletons of planktonic crustacea, etc.

The study and identification of small fragments of tissues and microfossils other than pollen will prove to be of increasing significance in peat studies, particularly in contributing new lines of evidence concerning the biota and the ecological factors relating to particular strata or deposits. The use of the trisodium phosphate method is recommended as an aid in the study of this whole fossil biota of peats.

Reference

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Three-dimensional Graphs

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With respect to the construction of three-dimensional graphs (*1*), it has occurred to me that a simple procedure I have used for this purpose will be of interest. On a sheet of ordinary graph paper a base is laid out by drawing lines at angles of 45° from the horizontal. On this are plotted independent variables (x, y),

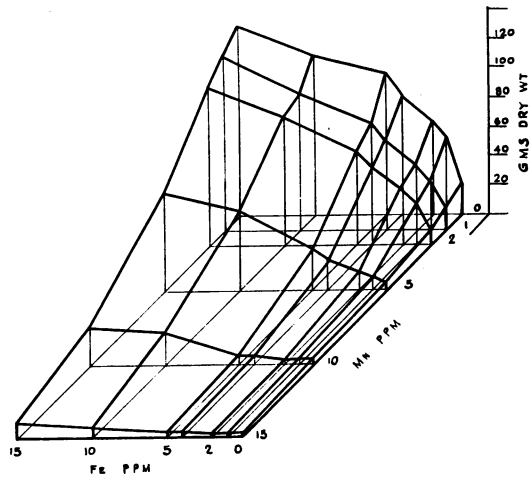

Fig. 1. Effect of simultaneous variation of manganese and iron on the total dry weight of bean plants.

while the dependent variable (z) is plotted on verticals from the base. Spaces on the graph paper serve for all three dimensions, and, if desirable, logarithmic values may be used. By connecting the points in two directions and to the base a solid figure is shown in perspective. The best effect is obtained if the graph is oriented so that the smaller values of z are in the foreground. Heavier lines should be used for the "roof" and front and right "walls" to give the correct optical appearance. For publication the graph may be copied on tracing paper and photographed.

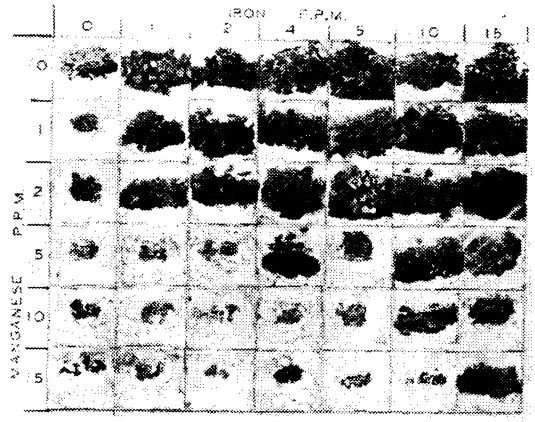

Fig. 2. Photographs of cultures of bean plants represented in Fig. 1.

On Fig. 1, which was constructed as described above, are