focus. This type of hanging drop culture is described below.

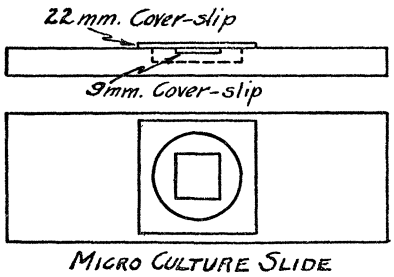

FIG. 1

A suspension of mold spores is made in any suitable liquid culture medium. A small drop of this suspension is transferred with an inoculating loop to the center of a 22 mm cover-slip. On top of the drop is placed a 9 mm cover-slip, easily made by quartering an 18 mm slip. The large cover-slip, carrying the smaller one, is then inverted and placed over the hollow chamber of a micro culture slide and the edges sealed with petrolatum to prevent evaporation of the medium. The smaller cover-slip is held firmly by the surface tension of the film of medium. Two views of the culture slide are shown in the accompanying diagram.

This technique has proved very satisfactory for the observation of growing molds over relatively long periods of time. With practice one can obtain films containing few or many spores at the start, depending upon the density of the original inoculation and the size of the drop used. The size of the drop also determines the thickness of the film which can be varied from 5 to 40 microns. If care is used in cleaning the cover-slips, uniform films free of air bubbles can easily be obtained. If desired the entire operation can be carried out aseptically.

If a hollow chamber about 15 mm in diameter and 3 mm deep is used, there appears to be enough oxygen present to support normal growth of the spores near the edge of the smaller cover-slip. Spores near the center of the smaller cover-slip at times fail to germinate, probably because of the lack of sufficient free oxygen. Since the total amount of medium present is relatively small for the amount of growth which takes place, the supply of foodstuff is more quickly exhausted and concentration of the waste products increases more rapidly than in the normal test tube or plate culture. Only the vegetative hyphae of such molds as the Penicillia and Aspergilli remain in the film of medium; the fertile hyphae grow in the air at the edge of the small cover-slip.

By the use of this type of culture, it has been possible to take a motion picture of mold growth during a five-day period. Rapid motion of small granules within the hyphae has been observed. With some types of molds, immiscible metabolic products separate in the medium. Variations in morphology caused by varying amounts of available oxygen can be observed. The writer has found this type of culture very satisfactory for photomicrographic work.

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SPECIAL ARTICLES

THE ORIGIN OF THE CELLULAR DEBRIS IN VAGINAL SMEARS OF THE GUINEA-PIG

THE mammalian vaginal discharge ordinarily contains at some time or other in the oestrus cycle large and small non-cornified epithelial cells, large cornified epithelial cells, leucocytes (mainly polymorphonuclear), erythrocytes and mucus. It is generally known that the leucocytes reach the vaginal lumen by diapedesis through its epithelial lining and that the large non-cornified and cornified cells are sloughed from its epithelium. There has also been every reason to believe that the erythrocytes come from mucosal hemorrhages of the uterus, and possibly in some cases by way of the oviduct, following the rupture of the ovarian follicles. The only cell type over which there seems to be much question is the small non-cornified epithelial cells which often occur in clumps or sheets in the vaginal secretions. These have been assumed to be of uterine origin.

We used eight adult guinea-pigs, ranging from oestrus through postoestrus to the dioestrus condition. None were prooestrus. A normal vaginal smear was made from the living guinea-pig. The animal was then killed by illuminating gas, the abdominal wall opened, and the vagina, uterus and oviducts exposed. A horn of the uterus was cut across near its upper end, and, by means of an eye-dropper, physiological saline was gently injected into and sucked back from the uterus above the section. A drop of this was allowed to evaporate on a slide. Another transverse cut was made below the previous one and the process repeated, the discharge again being obtained from that part of the uterus above the section. Smears were thus made from the uterine cornu, uterine fundus, the vagina near the cervix and the vagina. Since, however, no differences were observed in smears taken in different parts of the uterus, or in smears taken in different parts of the vagina, we continued to make

smears only of the uterine cornu, uterine fundus and vagina.

Cornified epithelium was found in the normal control vaginal smears of the living. In all these animals after death cornified epithelium was found in smears of the vagina as high as the cervix, and was invariably absent above the cervix. Except in animals that were in oestrus, large non-cornified epithelial cells were found in vaginal smears before and after killing. In no cases did we obtain large non-cornified epithelial cells in the uterus. Small non-cornified epithelial cells were found throughout the genital system in those animals not in heat. Clumps of small non-cornified epithelial cells, such as observers have noticed in normal external vaginal smears, were found in the uteri of all. Polymorphonuclear leucocytes were found abundantly in both uterus and vagina in those animals not in oestrus, whereas those in heat showed none in the vagina and a decreased number in the uterus. Mucus occurred in both uterus and vagina, in several cases being more abundant in the uterus. Some erythrocytes were present in all smears taken after cutting open the tract, and were therefore assumed to be due to unavoidable contamination resulting from the cutting.

Before drawing any conclusions, it is well to keep in mind that we used only eight animals and that only a rough quantitative estimate of the cells was made, so no attempt to point out cyclic variations is justified. Furthermore, the method allowed the accurate determination of the *highest* origin of a constituent only. For instance, we found leucocytes in both the uterus and vagina. We can say with reasonable certainty that they do arise in the uterus, but our only indication that they arise in the vagina is their greater abundance there. Of course from the work of others where sections of the vagina have been made, it has been clearly demonstrated that they do arise in large quantities in the vagina by diapedesis. Then, too, there is a possibility that the oviducts contribute to the débris. The oviducts of the guinea-pig are very slender; their products, if any, must be very slight in amount, so we feel justified in disregarding them.

In the guinea-pig, then, the origin of the large non-cornified and cornified epithelial cells is the vagina. Small non-cornified epithelial cells, often in clumps and sheets, come from the uterus. Leucocytes arise in considerable quantities from the uterus as well as from the vagina. Mucus also arises mainly in the uterus, but of course may possibly also come from the vagina.

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AVAILABILITY OF VITAMINS IN PLANT TISSUES[1]

AFTER a careful consideration of the natural occurrences of vitamins, it is quite evident that most plants have the ability to produce either vitamins or their precursors. By some means, not yet clearly understood, plants can bring together non-living materials and transform them into the organic compounds of living matter, and undoubtedly during this process, the vitamins or their precursors find their origin.

According to their nature, vitamins are classified as fat-soluble or water-soluble, and within each class are found several individuals. When these vitamins are produced by plants, each species of plant is genetically capable of establishing certain individual vitamins within its tissues. However, the quantity, potency or available amount of a certain vitamin in a plant of a given species is not always uniform. This appears to vary with the variety[2] of the plant, its degree of maturity or the conditions of soil and climate under which it grew, and with seasonal differences.

After the plant produces its vitamins, either for self-defense and protection, or to serve as hormone-like regulators, it stores them in its tissues. They appear to be kept within the plant cell. Carotene, now recognized as a precursor of vitamin A, has already been associated with the chloroplasts of the cell.[3] The occurrence of droplets of fat in the cytoplasm offers a location for fat-soluble vitamins, or they may be connected with the lipoids, that seem to possess great significance in the activity of the cell, by forming thin films at the interfaces between the continuous and disperse phases. As for water-soluble vitamins, they undoubtedly would be found in the watery sap that fills the vacuoles, or in the aqueous part of the cytoplasm of the cell.

Not all the plant cells, however, are equally supplied with vitamins. In some cases, vitamins seem to be stored in that portion of the plant most exposed to sunshine.[4] House[5] and associates have also found the periderm of the carrot root to be a better source of its vitamins than the cortex.

If the cytoplasm of the plant cell is then recognized as the place where the vitamins are located, their

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper No. 18, Journal Series.

[2] M. F. Bracewell, E. Hoyle and S. S. Zilva, *Biochem. Jour.*, 24: 82–90, 1930.

[3] L. S. Palmer, "Carotinoids and Related Pigments," American Chemical Society Monograph No. 9, Chemical Catalog Co., Inc., New York, 1922.

[4] V. C. Heller and R. R. St. John, *Jour. Nutr.*, 4: 227–33, 1931.

[5] M. C. House, P. M. Nelson and E. S. Haber, Research Bulletin No. 120, 1930, Iowa Agricultural Experiment Station, Ames.