spores are formed, but because of its small size, *Laboulbenia formicarum* would not appear to lend itself too well to such a study. The developmental morphology, however, can be followed readily.

The discovery in this species of separate male plants which produce spermatia, and female plants which give rise to perithecia, constitutes the first demonstration of dioecism in any of the 400-odd species of the genus *Laboulbenia*. In the order Laboulbeniales, dioecism has previously been shown to occur in two subfamilies of the Laboulbeniaceae, the Amorphomyceteae and the Herpomyceteae, and in the Dimorphomyceteae of the family Peyritschielliaceae. Most species of the Laboulbeniales are monoecious.

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A Method for Concentration and Segregation of Malignant Cells from Bloody, Pleural, and Peritoneal Fluids

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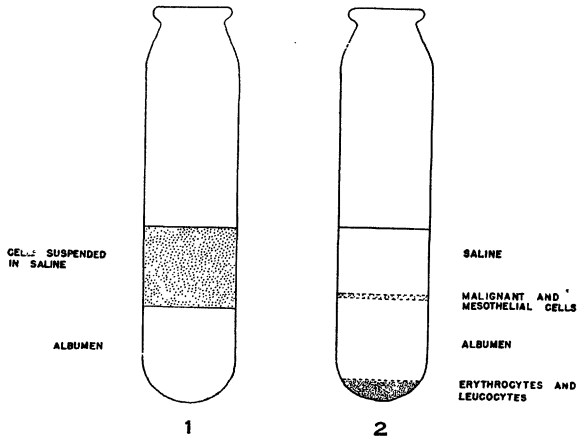
The separation of parasitized erythrocytes (*Plasmodium vivax*) from normal red blood cells (*1*), and of leukocytes from whole blood (*2*), by means of flotation on isosmotic solutions of bovine serum albumin has been described. The method presented here adapts the underlying principle of these procedures to the concentration of malignant cells and their segregation from other cellular elements and debris in pleural and abdominal fluids obtained from cancer patients. This is a preliminary report on work in progress.

The physical chemistry of the albumin solution[3] has been discussed in a previous paper (*2*). Pleural and peritoneal fluids submitted to the laboratory for routine cytological diagnosis were used in the study.

Pilot experiments are carried out to determine the optimal range of specific density for the separation desired. For this purpose the 35% albumin solution, having a specific density of approximately 1.10, is diluted with normal saline to result in specific densities of about 1.05 to 1.07. Solutions covering this density range are prepared by diluting 3.2, 3.4, 3.6, and 3.8 ml of the stock albumin up to 5 ml with saline in each of four 50-ml round-bottom Pyrex bottles. The 35% albumin is viscous and difficult to pipette. It has been found convenient, therefore, to use a 5-ml hypodermic syringe with a 4-in., 18-gage needle to aspirate the albumin from its rubber-capped bottle. The desired amount is extracted and saline is then drawn into the same syringe up to the 5-ml mark. The contents of the syringe are gently mixed and delivered to the bottom of the 50-ml centrifuge bottle.



FIGS. 1. and 2. 1—Diagram showing the saline cell suspension layered onto the albumin before centrifugation. 2—The appearance after centrifugation. Four layers are now discernible.

Four 50-ml aliquots of the original serosanguinous fluid are centrifuged in 50-ml Pyrex bottles at 2000 rpm for 10 min. The clear supernatant is decanted and the residues are pooled and resuspended in 20 ml of isotonic saline, thus accomplishing a 10-fold concentration of the cells. Depending on the cellular content of the fluid being studied, the degree of concentration may be increased or decreased. Five ml of the resuspended material is carefully layered onto the albumin dilutions in each of the four tubes (Fig. 1). The bottles are centrifuged for 5 min at 500 rpm and then for 30 min at 3000 rpm. At the end of this period the material in the tubes is stratified in four layers (Fig. 2). These are pipetted off into separate 15-ml conical centrifuge tubes and spun down. The cellular elements of each are then identified by histological examination of stained smears. The layers, in our experience, have consisted of: cell-free saline; malignant cells with or without mesothelial cells, layered at the saline-albumin interface; albumin, more or less cell-free; erythrocytes, leukocytes, and debris. The relative composition of these layers varies with the specific density of the albumin used.

We have studied so far three pleural and two abdominal fluids which, by conventional techniques of histological examination, were found to contain malignant cells. The diagnoses were adenocarcinoma with pulmonary metastases, primary site unknown (two fluids), primary carcinoma of the lung, and colloid carcinoma of the stomach and adenocarcinoma of the stomach, both with peritoneal metastases. Pilot experiments were also car-

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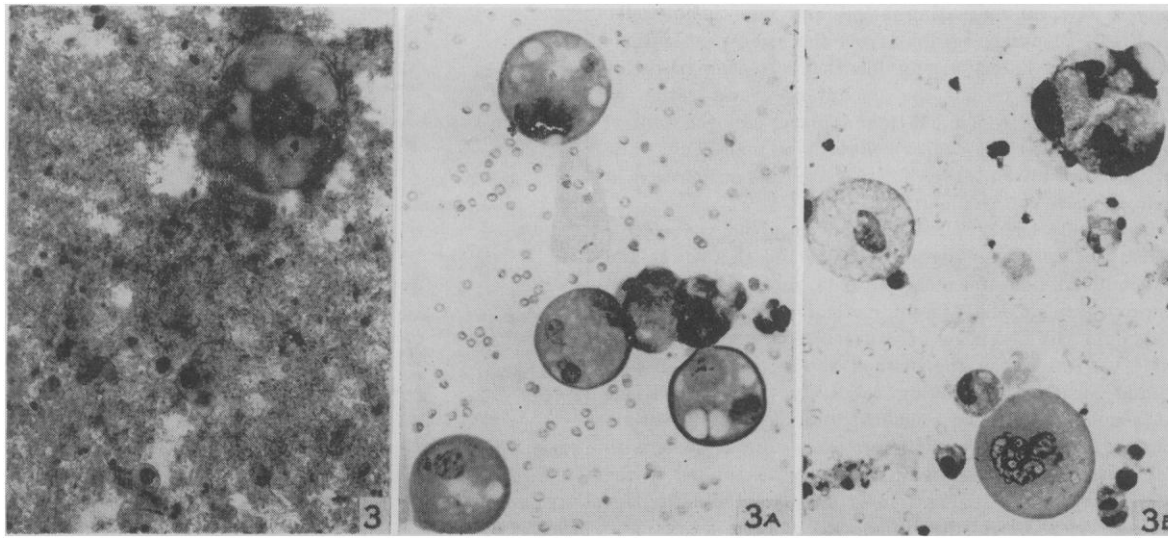
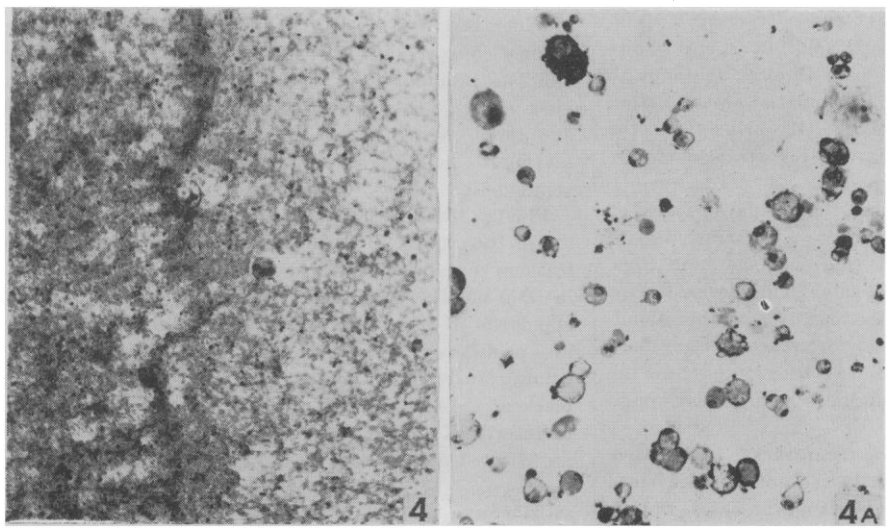
[3] Crystallized bovine plasma albumin, 35% solution, was made available to us through the generosity of Dr. J. B. Lesh, Armour Laboratories, Chicago, Illinois.



FIGS. 3, 3A, and 3B. 3—Photomicrograph of a smear prepared in the conventional manner from the pleural fluid of a patient having adenocarcinoma with pulmonary metastases. One large malignant cell can be made out against a confusing background of erythrocytes, leukocytes, mesothelial cells, and cellular debris. Papanicalaou stain. Magnification ×200. 3A and 3B—Photomicrographs from a smear prepared from the same pleural fluid after flotation of the malignant and mesothelial cells on albumin. Several large cancer cells now stand out clearly in each field. A few erythrocytes remain which failed to separate. Wright's stain. Magnification × 200.

ried out on two pleural fluids containing mesothelial and blood cells but devoid of malignant cells. The best concentration of malignant cells was achieved with albumin dilutions of 3.2 ml and 3.4 ml made up to 5 ml, corresponding to specific densities of about 1.05 and 1.06. It has been shown that normal erythrocytes have a specific density of about 1.09, and leukocytes vary from 1.07 to 1.08 (*2*). These cells would be expected to sink through the albumin to the bottom of the tubes, and this is, indeed, the case. Relatively few erythrocytes and leukocytes are found at the interface. Mesothelial cells and histiocytis are regularly found with the malignant cells. Although there have been varying degrees of segregation of these two cell types, we have not yet determined the point of maximal separation of these cells from cancer cells.

An admixture of mesothelial cells and histiocytis is not particularly objectionable when the method is used simply as an aid to the cytological diagnosis of cancer from bloody fluids. It is, however, a great advantage to be able to eliminate largely the numerous blood cells which often seriously interfere with detailed examination of malignant cells. Fig. 3a shows the sediment of one of the pleural fluids prepared by the conventional procedure. One large malignant cell is seen more or less obscured by an abundance of intact and hemolyzed red blood cells, leukocytes, and mesothelial cells. Figs. 3a and 3b show the material separated from the same fluid by albumin flotation. A number of malignant cells are now clearly visible with



FIGS. 4 and 4A. 4—Photomicrograph of the control smear presented in Fig. 3 but at half the magnification. Three cancer cells can be seen in this microscopic field. Papanicalaou stain. Magnification × 100. 4A—A microscopic field of the same area from a smear made after albumin flotation. Many malignant cells are visible. Wright's stain Magnification × 100.

only a few contaminating blood and mesothelial cells. In Fig. 4 photomicrographs from the same preparations are shown at half the magnification. A comparison of Fig. 4 with 4a reveals the amount of concentration of malignant cells which has taken place. In the control smear, Fig. 4, three cancer cells can be made out, while in the same area of Fig. 4a, prepared after flotation, a score or more can be identified.

This study has revealed information about the specific density of certain malignant cells found in pleural and abdominal fluids of cancer patients, making possible an improvement in the present method of examining such fluids for the diagnosis of cancer. Apart from its possible clinical value, the method shows promise of providing means of obtaining relatively pure populations of neoplastic cells for chemical and physical investigations. Experiments now in progress suggest that, with certain modifications, the method may be applied to the concentration and segregation of desquamated malignant cells in vaginal fluids by elimination of certain other cell types from the total population.

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A Multiple-Standard, Vertical-View Comparator for Microdeterminations

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The author developed the apparatus to be described in response to needs of the Department of Bacteriology of the Johns Hopkins University School of Medicine. When used for pH determinations, this device, based on the same colorimetric principle as that of the horizontal-view comparator of Gillespie (1920), employs three glass cups arranged in a vertical series to form a standard against which is compared a single test cup containing a specimen sample with 1 ml of indicator solution. Light passes upward from the light-source base through the cups to the observer. Fig. 1, a diagrammatic cross section through the comparator, summarizes the principle of the cup and comparator design in a working arrangement using Gillespie "drop-ratio" standards.

The light-source base, a box, the inside surfaces of which are painted dull white, has a sloping frosted glass window across the entire back side (not shown in Figs. 2 and 3) and a sloping front side. This design serves better to receive the light of the usually available illumination and distribute it uniformly over the reflecting floor of the base. A sliding panel may be grooved into the floor so that painted standards may be used for rough work.

The base supports two trays, "cup racks," one above the other. These may be seen in the unassembled view, Fig. 2. The lower rack has been left on the base. Dowels

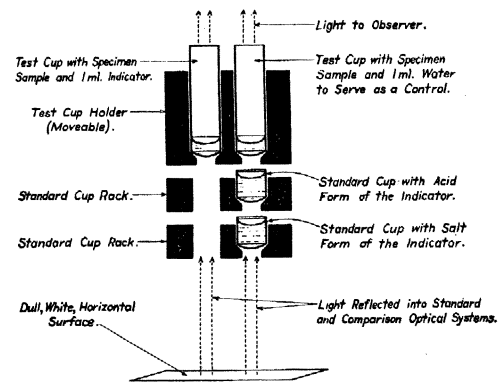

FIG. 1.

keep the racks in proper alignment. In the comparator shown provision has been made for the simultaneous use of two indicators, that is, each rack has two rows of "cup holes" separated by a middle row of simple "optical holes." Each row has thirteen holes and the cup racks

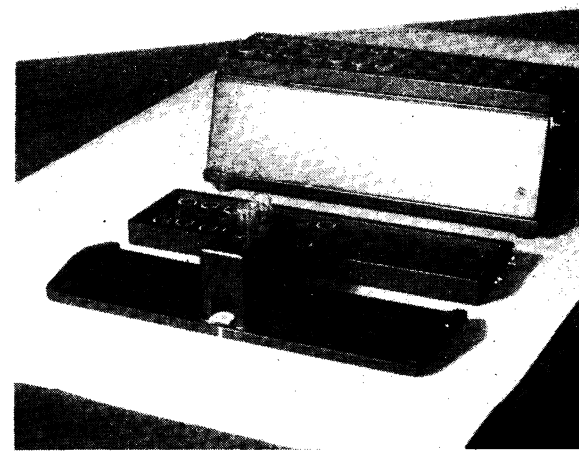

FIG. 2.

are identical with respect to their plan views. In Fig. 2 some cups are left out of the cup holes of the front and back standard row better to expose these holes to view. Metal hooks are attached at the ends of the base and these engage pins in the upper cup rack, thereby serving to hold the cup racks and base together as a single unit.

Finally, there is the "test cup holder" which slides on top of the upper cup rack. This has three cup holes. A test cup containing indicator and specimen may be placed in the middle hole and conveniently compared with standards of the same indicator by moving the holder into positions over the various standards in the cup racks below. Just below the test cups there is a slot in the block of the test cup holder into which color filters[1] may be inserted. All surfaces, except the inside of the base, are painted dull black.

[1] Wratten filters, obtainable at Eastman Kodak stores, are satisfactory.