in the absence of statistical or experimental control of other variables (*4*).

Pollak (*6*) has reported an experiment indicating increasing severity of lesions with increasing age and body weight. Although in freely fed animals, not fully grown, a general parallel between age and body weight may be expected, we are unable to estimate the contribution of age in our results, since the exact ages of our animals were unknown.

The high net correlation found between relative weight gain and severity of experimental lesions is not only consistent with the findings in man (*9, 10*), but has an important application to the study of experimental atherosclerosis. In the evaluation of agents suspected of influencing experimental atherosclerosis in the rabbit, and probably in other species, there must be statistical or experimental control of changes in body weight. The low incidence of experimental atherosclerosis in rabbits rendered diabetic with alloxan prior to cholesterol administration (*2, 5*) is probably associated with the characteristic emaciation of these animals, rather than with a specific effect of the diabetes.

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Discovery of Dioecism in *Laboulbenia formicarum*

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Laboulbenia formicarum Thaxter is a very minute parasitic ascomycetous fungus that can be found very commonly on all exposed parts of the body of small red ants belonging to the genus *Lasius*. The species was originally described by Thaxter (*1*) from Cambridge, Massachusetts, and later shown in figures in Part II of his classical series of monographs on the Laboulbeniaceae (*2*). The two figures which represent the only ones Thaxter published of this species are both what appear to be mature female plants. Since no reference was made to antheridia of this species, either in the technical descriptions or in the brief notes, it seems reasonable to assume that antheridia and the plants on which they are formed were not observed by Prof. Thaxter and that he did not suspect the species of being dioecious.

During the fall of 1948, while collections were being made of parasitized individuals from a colony of red ants in Champaign, Illinois, a large number of males were obtained with wings still attached that were heavily infected with *Laboulbenia formicarum*. Plants were growing in great abundance on the wings (Fig. 1), and since these clear easily, plants in different stages of development, from the first stages in the germination of spores to mature plants, were readily observed. One of the first things to attract our attention when we started to examine this material was the way two plants were almost invariably associated (Fig. 2). A careful examination of such pairs where the perithecia of one were mature, as pictured by Thaxter, revealed that the smaller one which lacked a perithecium was not an immature or aborted individual but rather a mature male plant with well-defined antheridia. No antheridia were found on female plants and they were exactly as pictured by Thaxter. A study of younger plants has shown also that male and female plants are very much alike in early stages of their development, but even in early stages, after one has become familiar with the developmental sequence, males can be distinguished from females. The details of developmental morphology as observed in this species, accompanied by a series of figures, are being published elsewhere. The ascospores which give rise to male and female plants are discharged in pairs and cling together as they develop. A cytological study has never been made in any of the dioecious species of the Laboulbeniales of the phenomena involved in segregation of sex factors as

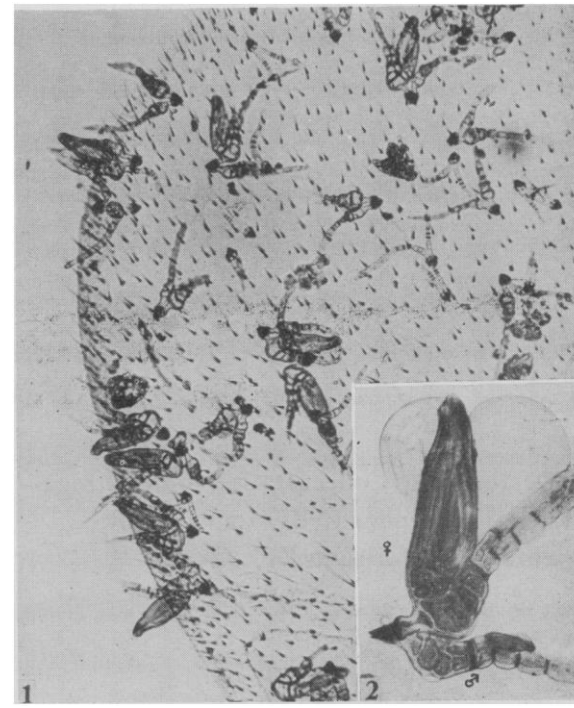

FIG. 1. Portion of the wing of a male ant heavily infected with *Laboulbenia formicarum*. Magnification × 150.

FIG. 2. A pair of mature individuals removed from the host. Magnification × 420.

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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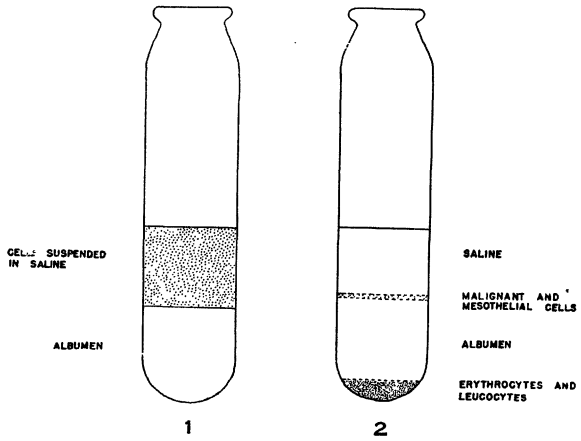
The separation of parasitized erythrocytes (*Plasmodium vivax*) from normal red blood cells (*1*), and of leukocytes from whole blood (*2*), by means of flotation on isosmotic solutions of bovine serum albumin has been described. The method presented here adapts the underlying principle of these procedures 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-

[1] John and Mary R. Markle Scholar in Medical Science.

[2] Senior Fellow of the Committee on Growth of the National Research Council. Supported by the American Cancer Society.

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