some pathogenic fungi in the range of 1–10 μg/ml. Examples are given in Table 1.

TABLE 1

INHIBITION RANGE OF FRADICIN AGAINST SEVERAL PATHOGENIC FUNGI

Organism	Effective inhibition range* (μg/ml)
Candida albicans	2–4
Cryptococcus neoformans	2–4
Microsporium canis	1–3
Trichophyton mentagrophytes	3–5
Microsporium gypseum	3–10
Histoplasma capsulatum (yeast phase)	1–3

* Readings were made after 9 days' incubation.

Acute toxicity tests in mice have shown that by intraperitoneal injection, the LD_{50} was approximately 4 mg/kg. Acute oral mouse toxicity by single dose was in this same range. Some kidney ischemia was observed. Skin tests employing rabbits (*7*) have shown that, in a hydrophilic ointment, irritation was considerable at 500 μg/g, moderate at 100 μg/g, and slight at 50 μg/g.

References

1. SWART, E. A., HUTCHISON, D., and WAKSMAN, S. A. *Arch. Biochem.*, **24**, 92 (1949).
2. SWART, E. S., ROMANO, A. H., and WAKSMAN, S. A. *Proc. Soc. Exptl. Biol. Med.*, **73**, 376 (1950).
3. KORNFELD, E. C., and JONES, R. G. *Science*, **108**, 437 (1948).
4. MARKUNAS, P. C., and RIDDICK, J. A. Paper No. 22, Abs., Am. Chem. Soc. Meeting, Houston, April 1950.
5. FRITZ, J. S. *Anal. Chem.*, **22**, 578 (1950).
6. CAMPBELL, C. C. *J. Bact.*, **54**, 263 (1947).
7. DRAIZE, J. H., WOODARD, G., and CALVERY, H. O. *J. Pharmacol. Exptl. Therap.*, **82**, 377 (1944).

Electron Microscopy of Thin-sectioned Spirostomum

Harold E. Finley

Department of Zoology,
Howard University, Washington, D. C.

A great deal of attention is being devoted to the study of ultramicroscopic structures in several kinds of organisms. The author's interest in the morphology of protozoa suggested the possibility of applying electron microscopy to the heterotrichous ciliate *Spirostomum ambiguum*. Accordingly, the microsectioning method developed at the National Bureau of Standards by Newman, Borysko, and Swerdlow (*1*) was modified for *Spirostomum*. Specimens were prepared for study as follows: Live animals were concentrated in centrifuge tubes, fixed in Bouin's or Navashin's fluid, dehydrated through a dioxan series, infiltrated, embedded, sectioned according to the method cited (*1*), and studied with the 50-kv, RCA-type EMU electron microscope. Some sections were shadowed with chromium. Figs. 1 and 2 illustrate typical results obtained when specimens were prepared in the manner described.

In Fig. 1 some of the numerous particles in the

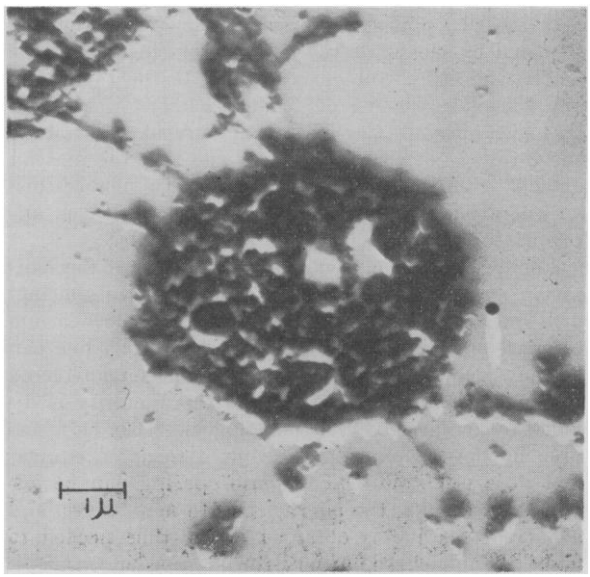

FIG. 1. Macronucleus. Chromium-shadowed (4 : 1), × 7800.

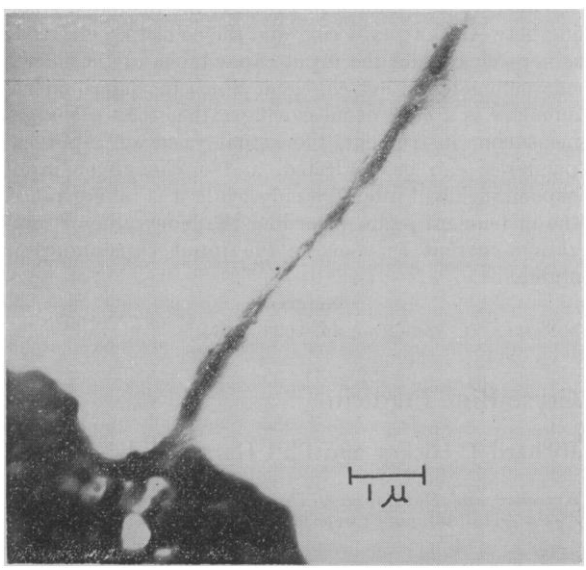

FIG. 2. Chromium-shadowed cilium, × 9000.

macronucleus are oval and others are globular in shape. Their diameters may be estimated in comparison with the shadowed particle of polystyrene latex[1] that appears in the right-central region of the figure. In making this comparison it should be remembered that the polystyrene particle was superimposed above the collodion substrate, whereas the macronuclear particles were partially imbedded in the substrate. In the macronucleus of *Spirostomum*, by suitable methods, one may discern "granular" material which gives a positive response to the Feulgen reaction; this may be a clue to the nature of the oval and globular particles shown in Fig. 1.

Fig. 2 reveals that locomotory cilia are composed

[1] Dow Chemical Co., Lot 580 G, diam approx 2600 A.

of compact bundles of fibrils. This finding is comparable to the observation reported by Jakus and Hall (*2*), who found "ultramicroscopic fibrils" in the cilia of *Paramecium*. The cilia of *Paramecium* and *Spirostomum* may be regarded as having a complex type of organization, perhaps approaching that which characterizes contractile elements of more highly specialized organisms.

The accompanying figures and numerous original micrographs indicate that the methods mentioned above may be applied to the study of protozoa. Fine detail which has not been resolved by ordinary light microscopy has been photographed from sections prepared as indicated here. A more detailed account of this work will be published elsewhere.

References

1. NEWMAN, S. B., BORYSKO, E., and SWERDLOW, M. *Science*, **110**, 66 (1949).
2. JAKUS, M. A., and HALL, C. E. *Biol. Bull.*, **91**, 141 (1946).

A Comparison of the Number of Circulating Blood Cells in Different Parts of the Circulatory System[1]

Daniel L. Azarnoff, Thomas V. Batty, Paul G. Roofe, and Marguerite Maffet

Department of Anatomy, School of Medicine, University of Kansas, Lawrence

Conflicting statements have appeared in recent literature concerning the differences in the number of circulating blood elements in heart and peripheral blood, Roofe *et al.* (*1*), Quimby, Saxon, and Goff (*2*), and Law and Heston (*3*) have shown what they consider to be significant differences in the blood count of different parts of the circulatory system of guinea pigs, rats, and albino mice, respectively. Nevertheless, Nichols and Miller (*4*) reported no significant differences in either erythrocyte or leucocyte counts done on heart and peripheral blood of rats.

Several investigators have found that anesthesia exerts an influence upon the number of circulating blood cells. Hahn, Bale, and Bonner (*5*) reported that 30% of the normal amount of circulating cells of dogs are found in the spleen after sodium pentobarbital (Nembutal) anesthesia, and that spleens removed under Nembutal anesthesia were four times the size of those removed under ether. Similarly, Hausner, Essex, and Mann (*6*) found that anesthesia with barbituric acid derivatives produces an enlargement of the spleen, with a subsequent decrease in the number of circulating erythrocytes. Ether anesthesia has an opposite effect, producing constriction of the spleen and an increase in the number of erythrocytes found in the blood stream. In male cats, Nembutal causes a 10% increase in plasma volume (*7*). The animals of Nichols and Miller (*4*) were either anesthetized with ether or stunned by a blow on the head in order to obtain heart blood. One author (*1*) administered anesthesia after taking the peripheral counts and before doing the heart counts.

Because of the contradictory reports of these several authors, and because various techniques were followed, some of which have been shown to influence the number of erythrocytes in central and peripheral parts of the vascular system, we judged it profitable to review the problem, avoiding the use of anesthesia. It is the purpose of this paper to report our studies on the number of erythrocytes and leucocytes in heart, venous, and peripheral blood in unanesthetized dogs.

Twenty female and 12 male mongrel dogs were used in this study. All animals were fasted for 12 hr before blood was drawn. Without anesthesia and with a minimum amount of struggle the dogs were fastened in a supine position to an animal board, and blood was drawn in the following manner: (1) Peripheral blood was obtained by carefully removing the hair from an area of the ear and then nicking the ear with a sharp razor blade. Counts were made in the standard manner, using Thoma pipettes certified by the National Bureau of Standards and hemocytometers with improved Neubauer rulings. (2) Venous blood was obtained by inserting a 20-gauge needle, with as little trauma as possible, into the large vein just above the lateral malleolus. The vein was distended by pressure upon the femoral vessels. The needles were previously coated with a solution composed of 2 parts of xylol and 1 part Dri-film 9987.[2] Thoma pipettes were filled with blood as it flowed freely from the hub of the needle. Smears for differential counts were also made from this freely flowing blood. Some of the blood was collected in oxalated tubes and placed in Wintrobe hematocrit tubes. Hematocrit determinations were made by contrifuging these tubes at 2,500 rpm for 30 min. (3) Heart blood was obtained during heart puncture by inserting an 18-gauge needle, also treated with xylol and Dri-film, into the thorax through the tenth intercostal space just to the left of the mid-line. In a similar manner to the method used for venous blood, blood was obtained from, presumably, the left ventricle. It was assumed that vigorously spurting blood indicated that the needle was in the ventricle.

Erythrocyte counts made upon peripheral blood ranged from 5,320,000 to 7,840,000 cells/mm^3, with the exception of one animal that had a count of 3,540,000 cells/mm^3. This animal appeared to be in excellent condition, and the anemia could not be anticipated from its external physical appearance. Although the counts of this animal will not be included in the following ranges for erythrocyte counts, it will be included in the statistical analysis reported in Tables 1 and 2. Venous blood erythrocyte content ranged from 5,100,000 cells/mm^3 to 8,330,000 cells/mm^3. That of the heart blood ranged from 5,170,000 to 8,040,000 cells/mm^3.

[1] Done in accordance with a contract between the Office of the Surgeon General, U. S. Army, and the University of Kansas.

[2] The Dri-film was obtained from the General Electric Co., Schenectady, N. Y.