

In a third paper, "Brain, Heart, Thyroid, Adrenals and Habitat" (*Growth*, 10, 15-23 [1946]), the power equation was applied to adrenal-body weight relations of 310 animals including tropical and subarctic rodents, carnivores, ungulates; white whales and porpoise.

In a fourth paper, "Studies in the Comparative Anatomy of the Endocrine System," the following log-log graphs of adrenal-body weights were included: Figure 1, Adrenal and thyroid—body weight relations for 112 reptiles; Figure 2, Adrenal and thyroid—body weight relations for 2709 birds; Figure 3, Adrenal and thyroid—body weight relations for 256 rodents; Figure 4, Adrenal and thyroid—body weight relations for 158 primates.

In addition to the above, G. W. Crile and D. P. Quiring published in the *Ohio Journal of Science* (40, 219-259 [1940]), "A Record of the Body Weight and Certain Organ and Gland Weights of 3,690 Animals."

D. P. QUIRING

Anatomy Department, Cleveland Clinic
Cleveland, Ohio

Manuscript received February 16, 1953.

Treatment of *Cryptococcus neoformans* in Mice with Stilbamidine¹

THE OBSERVATION of Elson (1) that certain pathogenic fungi were inhibited by low concentrations of propamidine has centered interest in the treatment of fungus diseases with the diamidines. This interest has been kindled by the discovery of the efficacy of stilbamidine (4,4'-stilbenedicarboxyamidine) in the treatment of blastomycosis (2, 3) and actinomycosis (4) and of propamidine (*p,p'*-(trimethylenedioxy) dibenzamidine) as an adjunct to ethyl vanillate (ethyl 4-hydroxy 3-methoxy benzoate) in the treatment of histoplasmosis (5). Infections due to *Cryptococcus neoformans* have remained resistant to treatment. The favorable response obtained in the treatment of other fungus diseases prompted use of stilbamidine in experimentally induced infections with *Cryptococcus neoformans* of the central nervous system in mice.

Mice were infected with *Cryptococcus neoformans* according to the method of Smith, Mosberg, Magan-icillo, and Alvarez de Choudens (3). A 48-hr broth culture of *Cryptococcus neoformans* was centrifuged and then resuspended in 1 cc of physiologic saline. After the mouse was anesthetized with ether and the head prepared sterilely, the midpoint of a line drawn between the eyes and the external auditory meatus was found. About 0.2 cm to 0.3 cm above this point a 28-gauge needle about 0.5 cm in length was inserted in a rotating fashion to pierce the skull and enter the cerebral cortex. About 0.05-0.08 cc of the suspension of *Cryptococcus neoformans* was injected, the latter being the maximal possible amount.

¹ Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Four mice so infected died between the 8th and the 15th day. Fourteen mice similarly infected were treated with 100 mg/kg stilbamidine diethionate² in 5% glucose in distilled water intraperitoneally. All the mice died between the 6th and 16th day after infection.

Stilbamidine, 50-100 mg/kg, administered intraperitoneally, has been reported as the maximum tolerated dose for mice and it is stated that 1/4 to 1/2 of this dose repeated over several days is usually well tolerated (6). The mice in this experiment received 100 mg/kg up to a period of 12 days, before death from the infection. This large dosage did not affect the course of the disease.

JOSEPH M. MILLER

GEORGE W. SMITH

WILLIAM H. HEADLEY

Surgical Service, Veterans Administration,
Fort Howard, Maryland

Department of Neurosurgery, Johns Hopkins Hospital, Baltimore, Maryland

Department of Neurosurgery, University of Maryland School of Medicine,
Baltimore

References

1. ELSON, W. O. *J. Infectious Diseases*, **76**, 193 (1945).
2. SCHOENBACH, E. B., MILLER, J. M., and LONG, P. H. *J. Am. Med. Assoc.*, **146**, 1317 (1951).
3. SMITH, G. W., et al. *Bull. School Med. Univ. Maryland* (in press).
4. MILLER, J. M., LONG, P. H., and SCHOENBACH, E. B. *J. Am. Med. Assoc.*, **150**, 35 (1952).
5. ELLIS, F. F., JR., SCOTT, R. J., and MILLER, J. M. *Antibiotics & Chemotherapy*, **2**, 347 (1952).
6. SCHOENBACH, E. B., and GREENSPAN, E. M. *Medicine*, **27**, 327 (1948).

Manuscript received December 3, 1952.

² The stilbamidine diethionate was supplied by Merck and Company, Incorporated.

A Combined Method for the Rapid Fixation and Adhesion of Ciliates and Flagellates

IN the preparation of protozoan slides, difficulty is often encountered in affixing the animals to the slide without distortion. The method described below eliminates the need of egg albumin and also the drying process. The whole technique takes only 15 seconds, and the animals are simultaneously fixed and attached to the slide. The method makes use of the fact that dispersal currents cause protozoa to adhere to the surface of a glass slide. Among the reagents that produce this effect are: Formalin, ethylene glycol, acetone, ether, chloroform, and the lower alcohols (methyl, ethyl, propyl, butyl, amyl) and some of their isomers. Though all these compounds cause adhesion to the slide, tertiary butyl alcohol yields best results. Ethyl and methyl alcohols may be substituted for tertiary butyl alcohol, but they seem to cause more nuclear distortion.

A mixture of the reagents given below will affix almost all the animals in a droplet of culture. No cellular distortion occurs and cilia, cirri, cytoplasmic