tion of normal slices and a significant depression of the oxygen uptake of potassium stimulated slices. This apparent interference of narcotics with respiration is not taken to indicate that the mechanism of their depressant action is an interference with oxidative processes. On the contrary, the decreased oxygen uptake may be a result of an inability of anesthetized brain cortex slices to utilize the products of cellular oxidations, especially in view of the fact that high energy phosphate has been shown to increase during the anesthetic state(12). The depression of the response to potassium stimulation then would be due to a decreased ability of narcotic-depressed tissue to respond to such stimulation rather than to a narcotic suppression of the subsequent increased respiration. In this respect it is noteworthy that Grenell *et al.* (13) were unable to find convincing evidence that the net synthesis of adenosinetriphosphate was a crucial factor in narcosis or that narcotic action was the result of a decreased oxygen uptake.

Summary. 'Valmid', a new nonbarbituric hypnotic, has been compared with 'Seconal Sodium', urethane, and a convulsant barbiturate with respect to its ability to inhibit normal and potassium-stimulated respiration of rat brain cortex slices *in vitro*. In concentrations equivalent to those obtained during anesthesia, only 'Seconal Sodium' depressed the oxygen uptake of normal tissue whereas both 'Seconal Sodium' and 'Valmid' inhibited the respiration of KCl-stimulated tissue.

1. McIlwain, H., *Biochem. J.*, 1953, v53, 403.
2. Ghosh, J. J., and Quastel, J. H., *Nature*, 1954, v174, 28.
3. Buchel, L., *Anesthésie et Analgésie*, 1953, v10, 1.
4. Gruber, C. M., Jr., *Fed. Proc.*, 1955, v14, 347.
5. Slater, I. H., Swanson, E. E., Henderson, F. G., and Anderson, R. C., *ibid.*, 386.
6. Foltz, E. L., Dracos, F., and Gruber, C. M., Jr., *Am. J. M. Sc.*, 1955, v230, 528.
7. Swanson, E. E., and Chen, K. K., *Quart. J. Pharm. and Pharmacol.*, 1939, v12, 657.
8. Domino, E. F., Fox, K. E., and Brody, T. M., *J. Pharmacol. and Exp. Therap.*, 1955, v114, 473.
9. Brody, T. M., and Bain, J. A., *J. Pharmacol. and Exp. Therap.*, 1954, v110, 148.
10. Deutsch, W. J., *J. Physiol.*, 1936, v87, 560.
11. Quastel, J. H., *Anesthesia and Analgesia*, 1952, v31, 151.
12. Stone, W. E., *J. Biol. Chem.*, 1940, v135, 43.
13. Grenell, R. G., Mendelson, J., and McElroy, W. D., *J. Cell. and Comp. Physiol.*, 1955, v46, 143.

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A Revised Method for Isolation of *Schistosoma mansoni* Eggs for Biological Experimentation.* (22612)

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Many of the pathologic, immunologic and clinical manifestations of Manson's schistosomiasis appear to be caused by the presence of the egg stage of the parasite in the host tissues(1,2,3,4,5,6,7). Consequently, studies of the egg stage and its pathogens, independently from the cercarial and adult stages, are assuming increasing importance in understanding the disease. We have developed a method of isolating *Schistosoma mansoni* eggs in adequate quantities and in pure enough state to allow for biological assays *in vitro* and *in vivo*. Our method was developed as a modification of comminution and filtration technics(3,8) with the addition of differential sedimentation through media of differing viscosities whereby gross contaminants may be separated and removed.

Materials and methods. Golden hamsters (9) are exposed to approximately 300 cercariae of *Schistosoma mansoni*. Eight weeks after exposure to infection one or 2 hamsters are sacrificed and stored at ordinary refrigerator temperature for 24 or 48 hours. All

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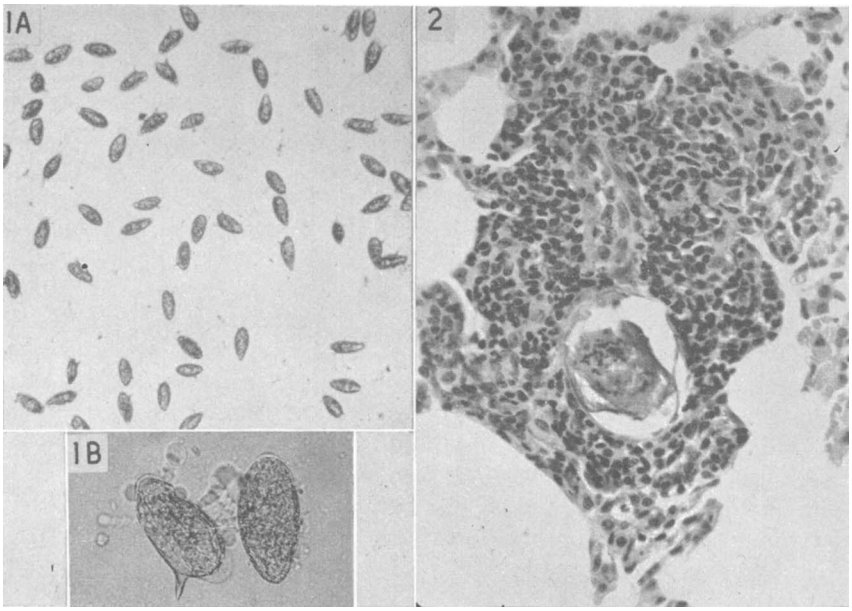

FIG. 1A. Sample of schistosome eggs isolated from hamster liver by method described in text. 40×.

FIG. 1B. Circumoval precipitates around the "purified" egg after 24-hr incubation in human immune serum. 180×.

FIG. 2. Cellular reaction around a schistosome egg in lung of an experimental mouse 30 days after injecting the mouse intravenously with purified eggs. 200×.

subsequent operations can be carried out at room temperature. The livers are removed from the animals and comminuted for 2 min. in 150 ml 1.7% NaCl aqueous solution in a household model Waring blendor. The concentration of saline used is sufficient to prevent premature hatching of the eggs without affecting them adversely(3). The homogenate is poured through 2 nesting wire mesh filters (phosphor bronze screening of 50 and 80 mesh) into a conical glass settling flask of 6 in. depth and 275 ml capacity, flushing the screens with added quantities of the saline sufficient to fill the cone. Thirty minutes later the contents of the flask are carefully decanted until about 1¼ in. remain. This remainder, containing the settled eggs and some contaminating material from the liver, is transferred to a centrifuge tube and washed 4 or 5 times in 1.7% saline with very mild 1½ min. centrifugation between washes. The resulting rough-cleaned sediment is concentrated in about 5 ml of saline, agitated with a pipette to break up adhering clumps, and then very carefully overlaid (using a fine pipette) upon a filtered solution of freshly prepared 0.5 molar sucrose in a standard 50 ml burette, the capillary tip of which has been removed. If observation of falling particles is desired, a dissection microscope is mounted in a horizontal position and focused at the lowest centimeter of the burette column.

Settled particles heavier than the eggs may be drawn off in 3-5 ml, and discarded, at the end of 15 min. At the end of one hour, 15 ml are drawn off into a small centrifuge tube. This tube contains the bulk of the eggs which are then washed free of sucrose, and concentrated, in 1.7% saline, using very light centrifugation. If further purification is desired the concentrate is carefully flushed over a small piece of 325 mesh screen to remove particles smaller than the eggs, and the eggs are then flushed off the top of the screen into a suitable container.

Results. If scrupulously cleaned containers and filtered solutions are used, and if the entry of extraneous dirt and dust is prevented, the purified eggs in the final concentrate are practically free of foreign material (Fig. 1a),

occasionally even without the final screening step. They maintain their morphologic integrity (Fig. 1b). When placed in distilled water at the end of the treatment, many of them hatch rapidly producing actively swimming miracidia. They retain their acid-fast property(10) and the normal antigenic quality of reacting with circumoval precipitins in human immune serum(3, Fig. 1b). After bringing the eggs into physiological salt solution we have injected them intravenously(11, 12) into mice, finding that the mice survived for at least 30 days and that there was a host cellular response to the injected eggs trapped in the lungs(11, Fig. 2). Eggs recovered from the upper portion of the column of sucrose, after one hour, are few in number, tend to be much smaller, immature, of bizarre structure and reduced hatchability, or else are empty.

Discussion. The technic outlined above offers a fairly rapid method of isolating *Schistosoma mansoni* eggs, in a relatively pure state, from hamster livers. The method can be adapted and refined to suit a variety of research purposes and source materials of eggs. We experimented with infected hamster livers of various degrees of freshness and found that best results were obtained from "stale" livers, that is those in which postmortem autolysis had been given an opportunity to occur through one or 2 days of storage of the killed animals at refrigerator temperature. When fresh livers were used, there was often an increased amount of tissue debris in the original homogenate and a tendency for the eggs to agglutinate or become enmeshed with tissue particles so that separation was incomplete. It may be, however, that the use of stale livers, or the passage of the eggs through glucose, may alter the eggs in their suitability for tests other than those which we used. Livers with appreciable amounts of fat were generally unsuitable.

Summary. A simple procedure has been outlined for isolating Schistosoma eggs from host liver tissue by a combination of comminution, filtration, and sedimentation through a viscous medium (sucrose). The resultant purified eggs retain certain normal qualities tested such as morphological integrity, hatchability, acid-fastness, ability to react with circumoval precipitins in immune serum, and ability to incite tissue reactions when injected into experimental animals. The procedure also provides for a degree of separation of fully mature eggs from other types, and for microscopic observation and some degree of control of the separation process.

1. Koppisch, E., *Puerto Rico J. Pub. Health and Trop. Med.*, 1937, v13, 1.

2. ———, *ibid.*, 1940-41, v16, 395.

3. Oliver-Gonzalez, J., *J. Infect. Dis.*, 1954, v95, 86.

4. Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 186.

5. Oliver-Gonzalez, J., Ramos, F. L., and Coker, C. M., *Am. J. Trop. Med. and Hyg.*, 1955, v4, 908.

6. Coker, C. M., and Oliver-Gonzalez, J., *ibid.*, 1956, v5, 385.

7. Lichtenberg, F., *Am. J. Path.*, 1955, v31, 757.

8. Flaschentrager, B., and Taha, M. H., *Hoppe-Seyler's Z. f. Physiol. Chem.*, 1953, v295, 285.

9. Stirewalt, M. A., Kuntz, R. E., and Evans, A. S., *Am. J. Trop. Med.*, 1951, v31, 57.

10. Lichtenberg, F., and Lindenberg, M., *Am. J. Trop. Med. and Hyg.*, 1954, v3, 1066.

11. Sorour, M. F., *Ann. de Parasitol.*, 1929, v7, 381.

12. Rafucci-Arce, F. L., personal communication.

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