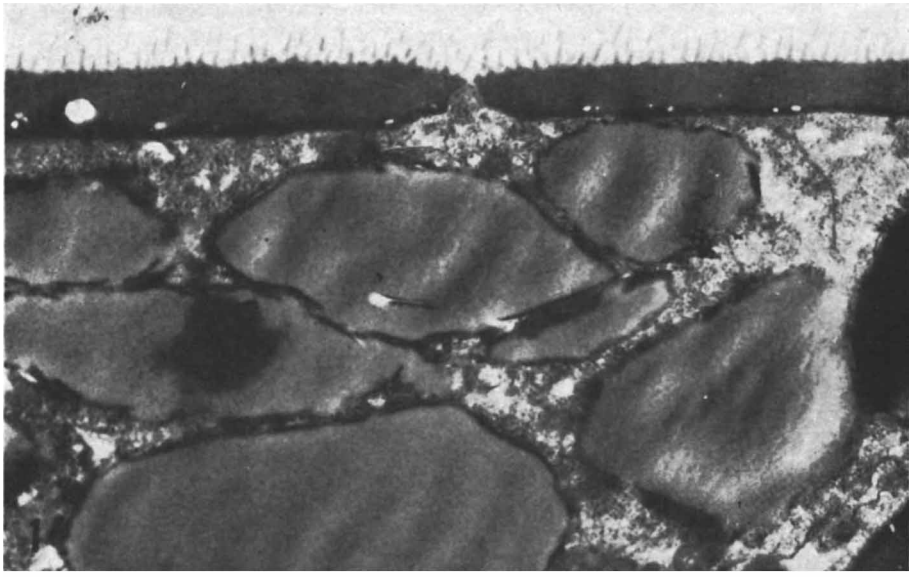
Schistosoma mansoni Eggs: An Electron Microscopic Study of Shell Pores and Microbarbs (33706)

G. J. RACE, R. M. MICHAELS, J. H. MARTIN, J. E. LARSH, JR., AND J. L. MATTHEWS

Department of Pathology, Baylor University Medical Center, Dallas, Texas; the Department of Medicine, Cornell University Medical Center, New York, New York; the Department of Parasitology, University of North Carolina School of Public Health, Chapel Hill, North Carolina; and the Department of Anatomy, Baylor University College of Dentistry, Dallas, Texas 75246



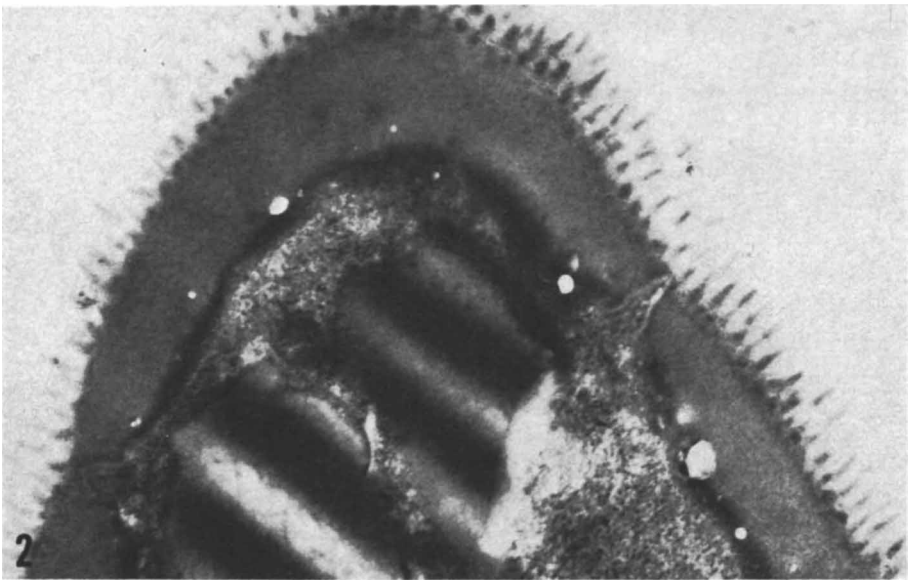


FIG. 1. Section through the anterior end of a schistosome egg. Microbarbs and membrane-lined pore are visible; ×17,500.

FIG. 2. Anterior end of schistosome egg showing two membrane-lined pores; ×36,000.

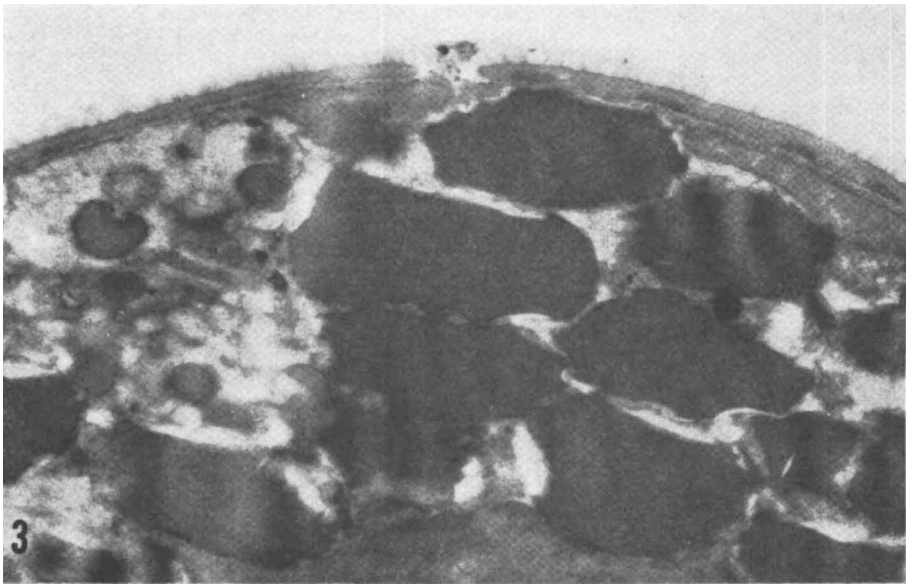


FIG. 3. Section through the anterior end of a schistosome egg. A distinct membrane-lined pore is visible; ×18,000.

An electron microscope study of the shell of living schistosome eggs was made in an effort to elucidate further the mechanism of their nutrition and excretion. Stenger *et al.* (1) studied the fine structure of murine hepatosplenic *Schistosomiasis mansoni* and also studied the fine structure of the schistosome egg. These authors described a fenestrated shell and also observed microspicules. Extracellular degradation of these shells in host tissues was observed. They also credit J. H. Smith and F. Von Lichtenberg for observing the fenestra and microspicules independently. The published photographs by Stenger *et al.* show their fenestrations to be irregular in size, of random distribution, and to be unlined by a plasma or unit membrane. They suggested that these fenestrations might facilitate diffusion of antigenic material from *S. mansoni* eggs, since Andrade *et al.* (2) and Lichtenberg (3) demonstrated schistosomal antigens in granuloma tissue.

In our study, living eggs grown *in vitro* by the method of Lee and Michaels (4) were isolated, fixed in collidine buffered paraformaldehyde, and embedded in epon according to the method of Lynn *et al.* (5). Thin sections were made on an ultramicrotome, stained with lead citrate (Reynolds, 6) and uranyl acetate (Watson, 7), and viewed with an RCA EMU 3. A shell with microbarbs was observed (Fig. 1). The shell also had discrete pores that traversed the shell in a direct manner. These pores were located principally in the anterior portion of the eggs. No pores could be demonstrated in the spurs, suggesting that the function of the spur is essentially mechanical. Each pore was lined by a trilaminar unit membrane, in contradistinction to the unlined, randomly oriented, fenestrated spaces described in the presumed degenerating eggs of other studies. In our studies, the pores seen in Figs. 2 and 3, which are lined by a trilaminar unit membrane, cannot be interpreted as the possible result of a fixation or degradative artifact. The present findings do not settle the question of whether these pores are the only existing shell fenestrations of these eggs, or whether such pores are limited to an early maturation stage during extrauterine development that requires about 6 days. In any case, the presence of such pores offers a possible explanation for the route of exchange of metabolites, antigenic materials, and might also function as the route for exit of the enzymatic secretions that are thought to be responsible for the eruption of the eggs from veins

into the tissues. The microbarbs probably allow the eggs to cling to the vascular endothelial lining and facilitate their penetration into adjacent tissues. The microbarbs could not be removed by incubation in thioglycollic acid or pepsin.

1. Stenger, R. J., Warren, K. S., and Johnson, E. A., Exptl. Mol. Pathol. 7, 116 (1967).
2. Andrade, Z. A., Paronetto, F., and Popper, H., Am. J. Pathol. 39, 589 (1961).
3. Lichtenberg, F., Am. J. Pathol. 31, 757 (1955).
4. Lee, H. G. and Michaels, R. M., Exptl. Parasitol. 22, 256 (1968).
5. Lynn, J. A., Martin, J. H., and Race, G. J., Am. J. Clin. Pathol. 45, 704 (1966).
6. Reynolds, E. S., J. Cell Biol. 17, 208 (1963).
7. Watson, M. L., J. Biophys. Biochem. Cytol. 4, 475 (1958).

Received Sept. 30, 1968. P.S.E.B.M., 1969, Vol. 130.

Reversal of Streptozotocin Diabetes with Nicotinamide (33707)

W. E. DULIN AND B. M. WYSE
Diabetes Research, The Upjohn Company, Kalamazoo, Michigan

Streptozotocin (*N*-nitroso derivative of glucosamine) (1) can be produced by *Streptomyces achromogens* and has been reported to have both antitumor (2), and tumor inducing activities (7), antibacterial (3, 4)and diabetogenic activities (2, 5, 6, 8). The diabetogenic effect of streptozotocin is caused by irreversible damage to the beta cells of the pancreas (6, 8). Nictoinamide treatment will prevent the diabetogenic action of streptozotocin but not its antitumor activity (9). The present studies showed that nicotinamide will prevent "streptozotocin diabetes" also when given as long as 2 hr following the diabetogenic compound.

Methods. Streptozocin (Upjohn lot no. 6742-DEG-30-5) was injected as a single rapid i.v. dose in citrate buffer (pH 4.5) within 5 min after it was in solution. The volume was adjusted so that each animal received the equivalent of 0.5 ml/150 g of body weight. Animals were intact males weighing 135–175 from the pathogen-free colony of The Upjohn Company. Drug injections were given following an overnight fast. Nicotinamide was given intraperitoneally in 0.5 ml of saline 15 min before and 30, 60, 120, and 240 min following streptozotocin. NAD[1] and nicotinic acid were given i.p. in saline, 15 min before streptozotocin. In one study, the pH of NAD solution was adjusted to 6.0 with 1 *M* NaOH. Blood sugars were determined by a microferricyanide method utilizing the AutoAnalyzer on blood obtained from the orbital sinus of animals 1 week after treatment. The animals were not fasted prior to bleeding.

[1] Nicotinamide adenine dinucleotide or DPN.

Results. Nicotinamide, at doses of 250 and 500 mg/kg, completely inhibited the diabetogenic activity of 50 mg/kg of streptozoic when injected 15 min before or 30, 60, and 120 min after the streptozotocin (Table I). Nicotinamide was ineffective in blocking the diabetogenic action of streptozotocin when it was given 240 min following the diabetogenic compound. Nicotinic acid (250 mg/kg) and NAD (1,500 mg/kg) were both ineffective when injected 15 min prior to streptozotocin (Table II).

Discussion. Nicotinamide was an effective inhibitor of streptozotocin diabetes since it blocked streptozotocin activity when given as late at 2 hr following streptozotocin injection. Results showing that nicotinamide was effective when given 15 min prior to streptozotocin agree with the results of Schein *et al.* (9). Results show that changes observed 1–2 hr following streptozotocin injection (6) are reversible. It is also noteworthy that nicotinamide and nicotinic acid both inhibit alloxan diabetes if given prior to alloxan, but not if given following alloxan injection (11, 15). The fact that streptozotocin diabetes