PRO EXPERIMENTIS

Method for long term delivery of soluble agents to the chick chorioallantoic membrane

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Summary. Osmotic minipumps were used to administer thyroxine to the chick embryo. This technique proved to be a convenient and efficacious method for the delivery of soluble agents to the chorioallantoic membrane. The thyroxine induced the precocious appearance of extensive cell sloughing by the corneal epithelium.

The chick embryo is often used to study the effects of various agents upon developing systems. It is often desirable to administer the agent over a long period of time rather than as a single injection into or onto the chorioallantoic vessels. With certain limitations, osmotic minipumps provide a convenient means for long-term delivery of agents to the chorioallantoic membrane. In a previous study of the effects of thyroxine on the developing chick corneal epithelium, thyroxine was administered as a single injection onto the chorioallantoic membrane[2]. That study demonstrated that the proliferation of microvilli by the developing corneal epithelial surface cells could be enhanced by exogenous thyroxine. In the present study osmotic minipumps have been used to deliver thyroxine to the chick embryo chorioallantoic membrane. This induces the precocious appearance of extensive cell sloughing by the chick embryo cornea, a phenomenon that normally occurs at 20 days of incubation[3].

Materials and methods. Fertile chicken eggs were incubated at 38 °C and 85% relative humidity in a circulating air incubator. At 9 days of incubation, windows were made through the shell and shell membranes and the eggs were connected to alzet minipumps (Alza Corp.). The apparatus was assembled as follows: 1. The minipumps were filled with L-thyroxine (prepared as previously described[2]; 0.2 µg/µl) according to instructions provided with the pumps and the pumps were temporarily set aside. 2. The point of a 20-gauge hypodermic needle was inserted into the end of a 15-cm-length of PE 60 intramedic tubing. 3. Into the other end of the tubing a flow moderator with catheter fitting (provided with pump) was inserted. 4. The needle was connected to a syringe containing the thyroxine solution and the needle and catheter were filled. 5. While

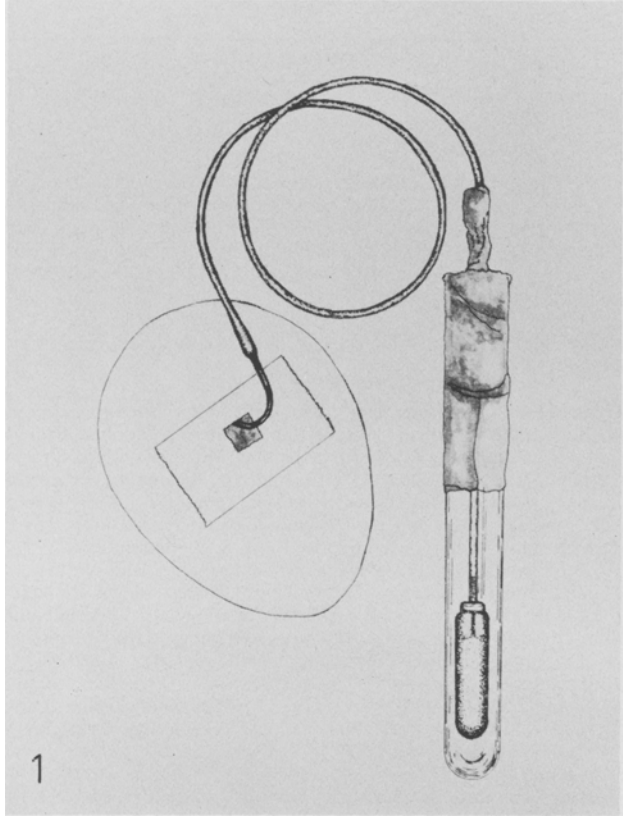

Fig. 1. This sketch illustrates the use of osmotic minipumps for the delivery of thyroxine to the chorioallantoic membrane of the chick embryo. See text for details.

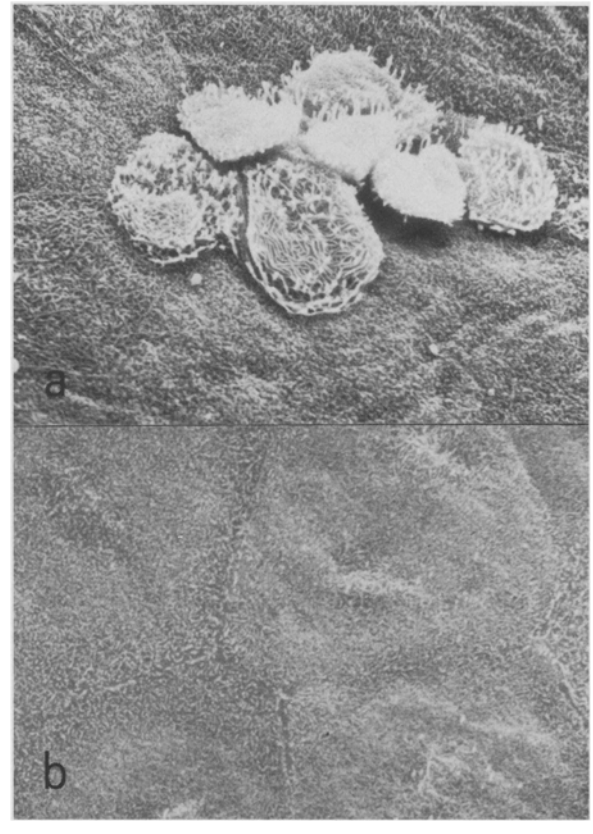

Fig. 2. Scanning electron micrographs comparing experimental corneal surface with control corneal surface. *a* Experimental corneal surface nine days after initiation of thyroxine treatment (18 days of incubation). A group of sloughing cells is present on the surface. × 2000. *b* Control corneal surface after 18 days of incubation. Sloughing cells are not present and were never observed this early on control cornea. × 2500.

maintaining a slight pressure on the syringe plunger (this is necessary to avoid introducing air into the system) the flow moderator was inserted into the minipump. 6. The needle was severed near its attachment to the syringe and bent at a 90° angle about 5 mm from the cut end. 7. The minipump was placed into a small test tube, the tube filled with distilled water and the top of the tube sealed with parafilm. 8. The bent end of the needle was hooked over the edge of the egg window and cellophane tape was used to secure the needle and cover the window. 9. The entire apparatus (figure 1) was returned to the incubator for further incubation of the eggs.

Embryos were collected at various intervals after operation and the corneas were prepared for scanning electron microscopy of the epithelial surface as previously described[3]. Control embryos were either treated with the drug vehicle or remained untreated.

Results. The survival rate of chick embryos treated with thyroxine in this study was 81% as opposed to 50% in previous studies (unpublished data) when 1.0 µg of thyroxine was administered as a single injection[2]. In the present study the chick embryo received 0.5 µg thyroxine per day for a total maximum dosage of 3.5 µg (the minipumps had a pumping rate of 1 µl/h and a lifetime of 7 days).

Figure 2 illustrates that the appearance of sloughing cells can be accelerated by the administration of thyroxine. Figure 2a shows sloughing cells on a corneal surface taken from an embryo that was sacrificed 9 days after the egg was connected to the minipump (18 days of incubation). By contrast, figure 2b illustrates the corneal surface of a control embryo that was sacrificed 9 days after operation. Sloughing cells were never observed on control corneas until 20 days of incubation.

Discussion. These results indicate that the use of osmotic minipumps is a very practical way of administering drugs to the chick embryo. This method has the disadvantage of a limited pumping rate (0.5 or 1 µl/h). Therefore, one could be limited in the dosage range available by the solubility of the agent to be delivered. However, this can be overcome to some degree by connecting more than one pump to the same egg. This system is much simpler and less cumbersome than previously described systems[3] and should have wide applicability for embryological and teratological workers who use the chicken embryo as an experimental animal.

The accelerated appearance of sloughing cells by the experimental animals indicated not only the efficacy of osmotic minipumps but that this is another parameter of chick corneal development that can be affected by exogenous thyroxine[2,5-7].

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