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Production of Hypertension with Desoxycorticosterone Acetate-Impregnated Silicone Rubber Implants

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Abstract □ A simple method for the production of drug-impregnated silicone rubber implants for sustained-release drug administration is described. This method involves incorporating the drug in unpolymerized silicone rubber, adding catalyst, and casting the drug–silicone rubber mixture in a hemicylindrical shape in a methacrylate mold. The utility of this method was investigated with desoxycorticosterone acetate. Desoxycorticosterone acetate-impregnated silicone rubber implants were inserted subcutaneously in rats, unilateral nephrectomy was performed, and the rats were maintained on 1% sodium chloride solution in place of drinking water. Systolic blood pressure was determined by an indirect tail cuff method. Rats receiving doses of desoxycorticosterone acetate, ranging from 50 to 500 mg./kg., developed hypertension within 3 weeks. Desoxycorticosterone acetate in a dose of 100 mg./kg. appeared to be most suitable for inducing sustained hypertension. The advantages of this method for the production of experimental hypertension and the general applicability of this method for sustained-release drug administration are discussed.

Keyphrases □ Desoxycorticosterone acetate-impregnated silicone rubber implants—preparation, used to induce hypertension, rats □ Silicone rubber implants, desoxycorticosterone acetate impregnated—preparation, used to induce hypertension, rats □ Hypertension—induced by desoxycorticosterone-impregnated silicone rubber implants, rats □ Implants, desoxycorticosterone acetate-impregnated silicone rubber—preparation, used to induce hypertension, rats

Dimethylpolysiloxane has been shown to be inert in the presence of many chemicals and to be well tolerated by biological tissues following intradermal or subcutaneous administration (1). For these reasons, dimethylpolysiloxane and other types of silicone rubber have found wide application as tissue prostheses and, more recently, as an experimental means of sustained-release drug administration. In 1965, Bass *et al.* (2) demonstrated a prolonged pharmacological response following the subcutaneous administration of atropine encapsulated in segments of silicone rubber tubing. Dziuk and Cook (3) reported that subcutaneous administration of melengestrol acetate encapsulated in silicone rubber tubing or sheeting was a suitable method for the inhibition of estrous in ewes. Numerous *in vitro* studies (3–5) demonstrated that a variety of steroids are able to diffuse through silicone rubber barriers.

Chang and Kincl (6) compared the effectiveness of megestrol acetate as a function of the route of administration. They observed that subcutaneous implantation of segments of silicone rubber tubing containing megestrol acetate was the most efficacious mode of administration; from 6 to 25 times less steroid was needed to produce comparable biological effects. Later, Chang and Kincl (7) presented further evidence indicating that the effectiveness of subcutaneous administration of steroids encapsulated in silicone rubber is much greater than conventional subcutaneous administration of steroids in oil.

A review of this literature suggested that a modification of these procedures might be a convenient method of drug administration for the production of experimental hypertension in rats. Studies are currently in progress in this laboratory to evaluate the antihypertensive activity of a series of grayanotoxin analogs. To evaluate antihypertensive activity properly, it is necessary to conduct the studies in hypertensive animals. Hypertension may be produced in rats by a variety of procedures. One convenient procedure involves unilateral nephrectomy, chronic administration of desoxycorticosterone acetate, and maintenance of

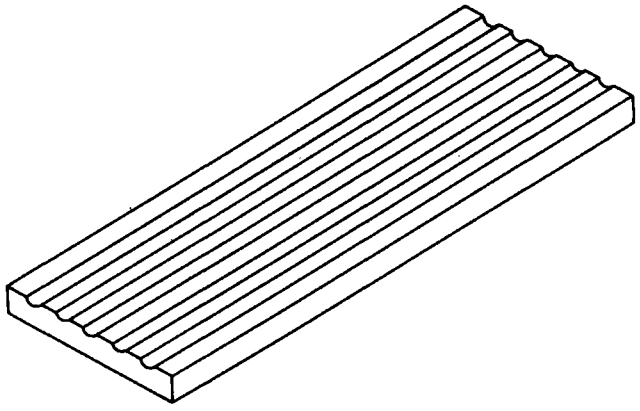

Figure 1—*Plexiglas mold used to cast silicone rubber implants. The mold is constructed of methacrylate plastic, with five hemicylindrical grooves routed in it. The number of grooves and the dimensions of the mold are not critical.*

the rats on 1% sodium chloride solution in place of drinking water. A significant degree of hypertension usually develops within 4–8 weeks. Daily administration of desoxycorticosterone acetate over this period becomes quite time consuming. For this reason, a variety of sustained-release preparations of desoxycorticosterone acetate have been evaluated, but these preparations have the disadvantage of being time consuming to prepare and/or are expensive.

This study describes a simple and convenient method for the preparation of drug-impregnated silicone rubber implants. This preparation was used to produce hypertension in rats.

EXPERIMENTAL

Construction of Mold—A silicone rubber implant of constant geometry and constant drug content was prepared by curing a silicone rubber fluid in a suitable mold. The mold was constructed from a 1.27-cm. thick sheet of methacrylate[1] plastic, 10.32 cm. wide and 31.00 cm. long (0.5 × 4.0 × 12.0 in.). The dimensions of this mold are not critical (Fig. 1). The plastic sheet was routed lengthwise to form five hemicylindrical grooves with a radius of 0.322 cm. each. The interior surfaces of these grooves were smoothly polished to facilitate removal of the cured silicone rubber. Sticking was further decreased by coating the mold with a thin film of silicone mold releasing agent[2] before each use.

Preparation of Implants—Preliminary experiments had suggested that a convenient concentration of desoxycorticosterone acetate[3] was approximately 10–100 mg./implant. Five doses of desoxycorticosterone acetate were tested, and a multiple of each dose was carefully weighed and added to a quantity of uncured silicone rubber[4] sufficient to produce a desired number of implants. The drug was thoroughly incorporated into the silicone rubber in geometric dilution by stirring with a spatula on a watch glass (15-cm. diameter).

After the drug was incorporated into the silicone rubber, the catalyst, stannous octoate[5], was added and thoroughly mixed. The addition of 48 drops of catalyst/454 g. of drug–silicone mixture provided an adequate working time of about 10 min. The mixture was then transferred into the grooves of the mold. The edge of a spatula or a glass microscope slide was used to smooth the fluid mixture into the grooves and ensure that the surface of the hemicylinder being formed was even with the surface of the mold. The silicone was allowed to cure at room temperature for 24 hr. After curing, the silicone rubber hemicylinder was stripped from the mold. The appropriate dosage of desoxycorticosterone acetate could then be easily obtained by laying the silicone rubber hemicylinder beside a ruler and cutting off a given length with a razor blade.

Table I—Desoxycorticosterone Acetate Treatment Schedule

Treatment Group	Number of Animals Treated	Number of Animals Alive at Week 11	Dose, mg./kg.
Control	7	5	0
1	9	8	1
2	11	10	10
3	8	7	50
4	10	8	100
5	8	0	500

Production of Hypertension—Male Rolfsmeyer rats, with an average weight of 144 g., were assigned to six treatment groups of a completely random design with unequal replication. Systolic blood pressure of each rat was indirectly determined at weekly intervals using a tail cuff plethysmograph apparatus[6]. After control blood pressure parameters were established, the rats were anesthetized with methoxyflurane and unilaterally nephrectomized using aseptic technique. A 1-cm. transverse incision was made through the skin just anterior to the scapulae, and a 5-cm. silicone rubber implant was inserted subcutaneously. The implant was positioned with its flat surface parallel to the spine on the dorsal musculature of the rat, and the incisions were closed with wound clips. The treatment schedule is presented in Table I.

The rats were randomly housed in individual cages, with free access to commercial rat food and to 1% sodium chloride solution in place of drinking water. It was necessary to add tetracycline (5 g./l.) to the sodium chloride solution at periodic intervals to prevent the development of chronic respiratory disease. The rats received the tetracycline–sodium chloride solution during Weeks 2, 4, 6, and 8. During Weeks 0, 1, 3, 5, 7, 9, and 10, the rats received sodium chloride drinking water only.

The data were analyzed by an analysis of variance for a completely random design with unequal replication (8). Statistical significance was determined using Waller–Duncan's (9) multiple-comparison procedure for a "k" ratio of 100:1, which corresponds to the conventional 5% level of significance. This method was used to test the data within each week.

Rats with systolic blood pressures greater than 160 mm. of mercury were considered to be hypertensive.

RESULTS

Figure 1 illustrates a convenient mold design for producing silicone implants of the type used in this study. The results of various desoxycorticosterone acetate implant treatments are presented graphically in Fig. 2. Figure 2 presents the mean systolic blood pressure of the various treatment groups as a function of time in weeks. The major findings illustrated by Fig. 2 are:

1. Doses of desoxycorticosterone acetate ranging from 50 to 500 mg./kg. produced significant ($p < 0.05$) hypertension by Week 3 (2 weeks postimplantation).
2. Doses of desoxycorticosterone acetate of 1 and 10 mg./kg. did not produce any significant ($p > 0.05$) change in systolic blood pressure.
3. The initial rate of development of hypertension was similar after desoxycorticosterone acetate implants of 50, 100, and 500 mg./kg.

A desoxycorticosterone acetate–silicone implant containing a 100-mg./kg. dose of steroid appears to be most suitable for inducing hypertension. This dose (100 mg./kg.) produced hypertension significantly greater ($p < 0.05$) than any other dose after Week 3, except for the 500-mg./kg. dose. The hypertension produced by 100 mg./kg. desoxycorticosterone acetate was sustained throughout the entire study. The 500-mg./kg. dose level of desoxycorticosterone acetate produced hypertension which was not significantly different

[1] Plexiglas.
[2] IMS silicone spray parting agent.
[3] 21-Hydroxypregn-4-ene-3,20-dione acetate.
[4] Dow Corning Silastic 382.
[5] Dow Corning Catalyst M.
[6] Narco Biosystems, Inc.

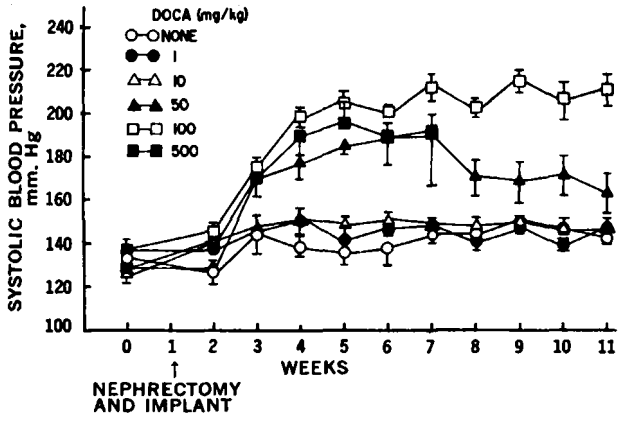

Figure 2—*Production of desoxycorticosterone acetate (DOCA)–sodium chloride hypertension in rats. The systolic blood pressure was determined indirectly at weekly intervals by tail cuff plethysmography. At Week 1, all animals were unilaterally nephrectomized and a 5-cm. silicone rubber implant was inserted subcutaneously. Five of the six treatment groups received implants containing various doses of desoxycorticosterone acetate. All animals were maintained on 1% NaCl solution in place of drinking water. The data are expressed as the mean systolic blood pressure in millimeters of mercury* ± SEM versus *time in weeks.*

($p > 0.05$) from that produced by the 100-mg./kg. dose; however, all of the rats that received the 500-mg./kg. dose were dead by Week 8 of the study.

DISCUSSION

The subcutaneous insertion of desoxycorticosterone acetate-impregnated silicone rubber implants combined with unilateral nephrectomy and maintenance on 1% sodium chloride solution is an effective means of producing hypertension in the rat. Desoxycorticosterone acetate in doses of 50-, 100-, and 500-mg./kg. produced statistically significant elevations of systolic blood pressure. Apparently, the dose of 50 mg./kg. is approximately the threshold dose for producing sustained hypertension, since some animals within this treatment group remained hypertensive while others returned to control blood pressure levels. The 100-mg./kg. dose appears to be the most suitable, because apparent metacorticoid hypertension was produced with this dose of desoxycorticosterone acetate. The highest dose was clearly unsuitable, since all of the animals treated with desoxycorticosterone acetate in doses of 500 mg./kg. died within 8 weeks. The total dose of desoxycorticosterone acetate necessary to produce a statistically significant degree of hypertension is much less than that employed in other desoxycorticosterone acetate–sodium chloride methods (10, 11).

The method described here appears to have several advantages over previously described methods of inducing desoxycorticosterone acetate–salt hypertension. The desoxycorticosterone acetate-impregnated silicone rubber implants take only minutes to prepare, are ready for implantation within 24 hr., and do not require any specialized, expensive equipment for their preparation. The molds may be made easily and inexpensively in practically any desired size or shape. Dosage adjustment is easy and reproducible.

The results presented here suggest that desoxycorticosterone acetate slowly diffuses from the silicone rubber implant to produce sustained blood levels of desoxycorticosterone acetate. The kinetics of this release are currently under investigation. The possibility of altering the kinetics of release with various adsorbents and changes in implant geometry and drug concentration are also being studied.

It is apparent that this method may have general applicability for the chronic administration of a variety of drugs. Some possibilities include the chronic administration of histamine or nicotine to induce gastric ulceration, the administration of antibiotics, use in chronic toxicity studies, and the induction of experimental drug dependence.

SUMMARY

1. A simple and inexpensive method of producing drug-impregnated silicone rubber implants was described.

2. The implants were used to produce hypertension in the rat. The method involves unilateral nephrectomy, subcutaneous insertion of a desoxycorticosterone acetate-impregnated silicone rubber implant, and maintenance of the rats on 1% sodium chloride solution in place of drinking water.

3. The general utility, advantages, and potential uses of this method were discussed.

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