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SUBCUTANEOUS CONTROLLED DELIVERY OF ESTRADIOL BY COMPUDOSE® IMPLANTS: IN VITRO AND IN VIVO EVALUATIONS

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ABSTRACT

The in vitro and in vivo releases of estradiol from the recently marketed Compudose®-200 and -400 implants were evaluated. These subdermal implants are designed for subcutaneous controlled administration of estradiol in steers for 200- or 400-day growth promotion.

Analysis of the in vitro release profiles of estradiol from Compudose implants indicated that the release of estradiol is under a matrix diffusion-controlled process and follows a linear Q vs. $t^{1/2}$ relationship.

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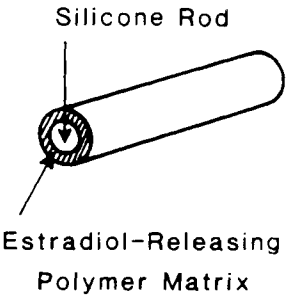
The release flux from both Compudose-200 and -400 implants was found to be dependent upon the volume fraction of polyethylene glycol 400, which acts as the solubilizer for estradiol, in the aqueous elution solution.

Subcutaneous release of estradiol from Compudose implants was also conducted in laboratory rats for a duration of up to 114 days. Results indicated that the Q vs. $t^{1/2}$ linearity is also followed. The subcutaneous rate of release was calculated to be 520 mcg/cm^2/day$^{1/2}$ for Compudose-200 and 360 mcg/cm^2/day$^{1/2}$ for Compudose-400.

INTRODUCTION

Anabolic steroids have been often used to improve the growth rate and feed conversion efficiency of finishing cattle. The anabolic steroids which have been found useful for growth promotion are estrogenic steroids, like Zeraronol (1) and estradiol-17β (2-4), or androgenic steroids, like trenbolone acetate and testosterone propionate (5-11). Conventionally, the cattle is treated with subcutaneous implantation of a compressed hormone-containing pellet formulation made from pharmaceutical excipients and binders. The formulations for the commercial products, like Finaplix, Ralgro, Synovex S or Synovex H are somewhat similar with different active ingredients. The basic problem associated with the pellet formulation is its fast decaying release rate, which follows either a first- or a second-order release kinetics (12). Therefore, in the initial stage of the implantation, overdose could occur, while at the later state underdose is very common. Consequently, the maximal duration of efficacy the implant can provide is only 60 days at most. So, re-implantation has to be done three to four times during a production cycle, resulting in a significant variation in drug absorption.



Compudose Implant

Figure 1: Diagrammatic illustration of a compudose subdermal implant (See Table I for physical dimensions).

An application of the polymer-controlled drug release technology in subcutaneous controlled administration of anabolic steriods is the development of Compudose subdermal implant (Elanco/Eli Lilly Company). It consists of a non-medicated silicone rod coated with a medicated silicone coating containing an effective dose of estradiol-17β (Fig. 1). Compudose-200 implants, which provide a release of estradiol-17β over a period of 200 days, were evaluated in suckling, growing, and finishing steer calves (13, 14) and improvements in weight gain and in feed efficiency were reported. One of the advantages for this subdermal implant is that it can provide a long-term continuous release of the active ingredient at a predetermined low dosage rate. Therefore, only one single implant is needed for each animal during the whole production cycle. Additionally, the implant remains intact throughout the course of implantation and can be removed easily, if needed.

This paper intends to report the mechanism and kinetics of release of estradiol from the Compudose implants under both *in vitro* and *in vivo* conditions. The relationship between *in vitro* and *in vivo* release will also be established.

EXPERIMENTAL

Equilibrium solubility of estradiol in polyethylene glycol 400

Excess amount of estradiol[1] was added into 10 ml of aqueous solution containing 10-50% v/v of polyethylene glycol (PEG) 400[2]. All experiments were done in triplicate. The dispersions were vortexed for 2 minutes at the maximum speed and then equilibrated in shaking waterbath[3] at 37°C for 72 hours. The dispersions were filtered through a Millipore membrance filter[2]. The clear filtrates were diluted and measured spectrophotometrically[4] at λ_{max} of 275 nm. The concentration of estradiol in the aqueous PEG 400 solutions was calculated from the calibration curve established from the standard solutions of estradiol.

In Vitro release studies

Aqueous solutions containing 20, 50 and 75% v/v of PEG 400[2] were prepared. Ten mililiter aliquots of each PEG 400 solution, as the elution solution, were pipetted into a series of 30 ml Pyrex® test tubes[2]. The first set of nine tubes were pre-equilibrated in the shaking waterbath[3] at 37°C and 40 cycles/minute overnight. Each of nine Compudose implants was secured at one end with a piece of silk string (for retrieval purpose) and immersed in the elution solution with the silk string secured by the screw cap. The tubes were then shaken in the waterbath for 24 hours at the above settings. At the same time, another set of nine test tubes containing 10 ml of PEG 400 solution was placed in the waterbath for temperature equilibration at 37°C. At the end of 24-hour drug release, the implants were transferred to the second set of elution solution. Care was taken to remove all the adhering solution from the implants by gentle scraping against the inner surface of the tube before transferring. The procedure was repeated daily over a ten-day period. Estradiol concentration in the elution medium was determined daily by spectrophotometric analysis[4]. The amount of estradiol release daily from each

Compudose implant was then determined by reference to the standard curve constructed from the standard solutions of estradiol.

The same procedure was followed for determination of the in vitro released of estradiol from both Compudose-200 and -400 implants.

In Vivo release studies

Male rats[5] (Sprague Dawley, 250 grams) were used in this study. Thirty rats were divided into six groups with five animals in each group. Proper identification was marked. The animals were weighed at the beginning of the study and also at weekly intervals throughout the study.

Compudose implants, either -200 or -400, were each cut into six equal sections and weighed prior to implantation.

Animals were anesthetized by intraperitoneal injection of Nembutal® sodium[6] (40 mg/Kg) in saline solution[7]. Following the induction of anesthesia, the abdominal area was shaved and sterilized with Betadine solution. A small midline incision was made through the abdominal skin, and a subcutaneous "pocket" was created in the vicinity of the incision by separating the connective tissue from the skin. One implant section was placed in the pocket using sterile forceps, and the incision closed with Autoclips[8]. Animals were returned to their cages for recovery and received standard care and feeding following the recovery.

At scheduled intervals, groups of animals were sacrificed. Each of the implant sections was removed, washed with distilled water, cut into small pieces, and then extracted by 100 ml of methanol with vigorous shaking for 24 hours[9] to determine the amount of drug remaining. An aliquot of extract was withdrawn and the concentration of estradiol in the sample was determined spectrophotometrically[4]. Sections of the unused Compudose implants were also extracted at the same time as the external standard to determine the yield of drug extraction.

Table I: Physical Parameters of Compudose Subdermal Implants

	Compudose Implant	
	200	400
1) Length	3 cm	3 cm
2) Surface area	4.84 cm^2	4.84 cm^2
3) Estradiol-medicated layer		
thickness	250 μm	500 μm
drug content	200 mg/Gm	200 mg/Gm
coating density	1.13 g/cm^3	1.13 g/cm^3
estradiol content	24 mg	45 mg
4) Core rod		
diameter	4.19 mm	3.76 mm
density	1.18 g/cm^3	1.18 g/cm^3

RESULTS

Calculation of the Theoretical Release Rate of Estradiol from Compudose® -200 Implants

The physical parameters of Compudose implants are outlined in Table I. Since the implant is a matrix dispersion-type drug delivery system, the release profile of estradiol is expected to follow the classical Higuchi relationship (15, 16):

$$Q = [(2A - C_p)\, C_p\, D_p t]^{1/2} \quad (1)$$

where A is the initial loading dose of estradiol (226 mg/cm^3), which can be calculated from the drug content and coating density of the estradiol-medicated layer (Table I); C_p is the solubility of estradiol in the silicone elastomer (16.3 mcg/cm^3) (17); and D_p is the diffusion coeffi-

cient of estradiol in the silicone elastomer matrix (1.93×10^{-7} cm^2/sec) (18). Since the loading dose, A, is much higher than the polymer solubility, C_p, Equation 1 can be simplified into Equation 2:

$$Q/t^{\frac{1}{2}} = [2A \cdot C_p \cdot D_p]^{\frac{1}{2}} \quad (2)$$

The theoretical value for the release flux of estradiol calculated from the literature values on C_p, D_p and A was 350.53 $mcg/cm^2 day^{\frac{1}{2}}$.

Determination of Equilibrium Solubility of Estradiol in PEG 400 Solutions

In order to maintain the appropriate sink conditions in the in vitro release studies of Compudose implants, the equilibrium solubility of estradiol in various aqueous solutions of polyethylene glycol 400, as the solubilizer for estradiol, was determined (Figure 2). As expected (19, 20), the equilibrium solubility of estradiol increases exponentially as increasing the volume fraction of PEG 400 in the aqueous solution. From this linear relationship, aqueous solution containing any given concentration of PEG 400 can be prepared to provide a sink condition required in the in vitro estradiol release studies.

In Vitro Release of Estradiol from Compudose Implants

Figure 3 shows the release profiles of estradiol from Compudose -400 in aqueous solutions containing various volume fractions of PEG 400. As expected from Equation (1), the release of estradiol from Compudose implants followed the linear Q vs. $t^{\frac{1}{2}}$ relationship. The release fluxes ($Q/t^{\frac{1}{2}}$) of estradiol in various release media are compared in Table II. Using the paired-t test, the release flux of estradiol at steady state is independent of PEG 400 concentrations. Therefore, the difference in the release profiles of estradiol from Compudose implants in various release media is due to the initial phase of drug release, particularly in the first day of release. As the volume fraction of PEG 400 in the release medium is low, the release of estradiol shows some time lag effect.

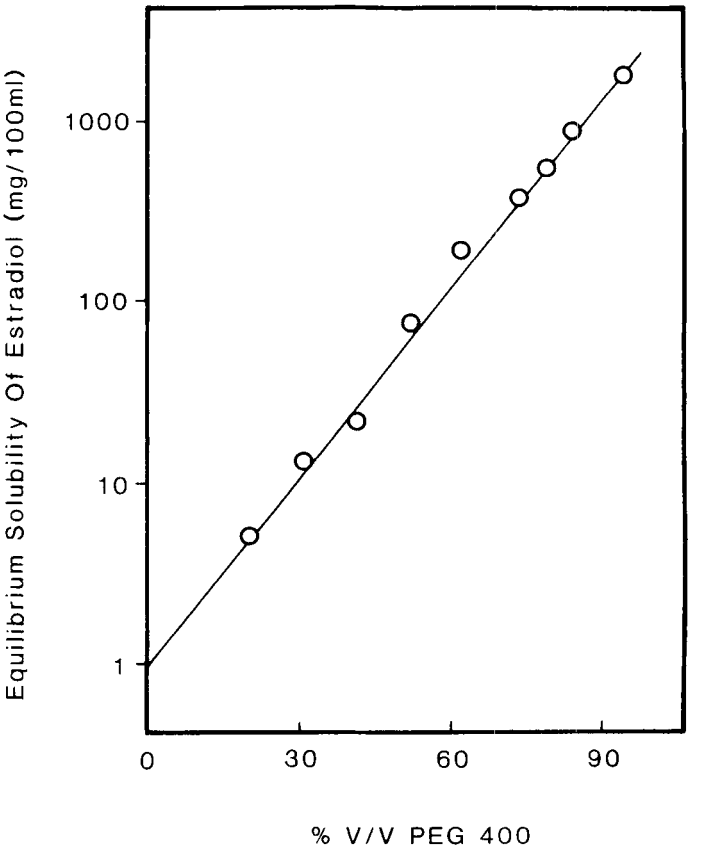


Figure 2: Equilibrium solubility of estradiol as a function of the volume fraction (% v/v) of polyethylene glycol (PEG) 400 in the aqueous solution.

As the volume fraction of PEG 400 increases, the release of estradiol shows a burst effect (Fig. 3).

Subcutaneous Release of Estradiol from Compudose Implants in Rats

The mean amount of estradiol released from Compudose-200 implants in rats was 2.002, 2.671, 3.340, 3.778 and 4.125 mg/cm^2 after 28, 49, 64, 86 and 96 days of implantation, respectively. The variation at each time point was within 10%, except the first time point being 26.5%.

Table II: Comparison in In Vitro Release Fluxes of Estradiol from Different Compudose Implants

Release Medium	Release Fluxes ($mcg/cm^2/day^{1/2}$) A[1)]	B[2)]
20% PEG 400	273	275
40% PEG 400	-	300
50% PEG 400	288	-
75% PEG 400	370	285

[1)] Compudose-200 implant

[2)] Compudose-400 implant

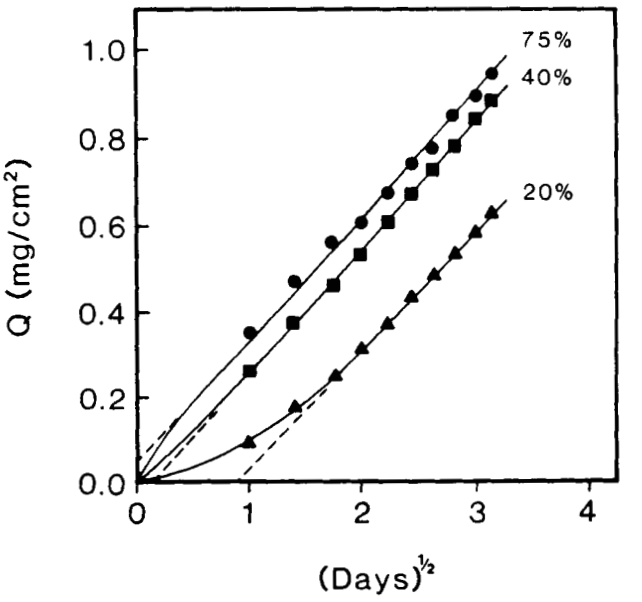


Figure 3: In Vitro release profiles of estradiol from Compudose®-400 implants. Keys (●), 75% PEG 400; (◐), 40% PEG 400; (○), 20% PEG 400.

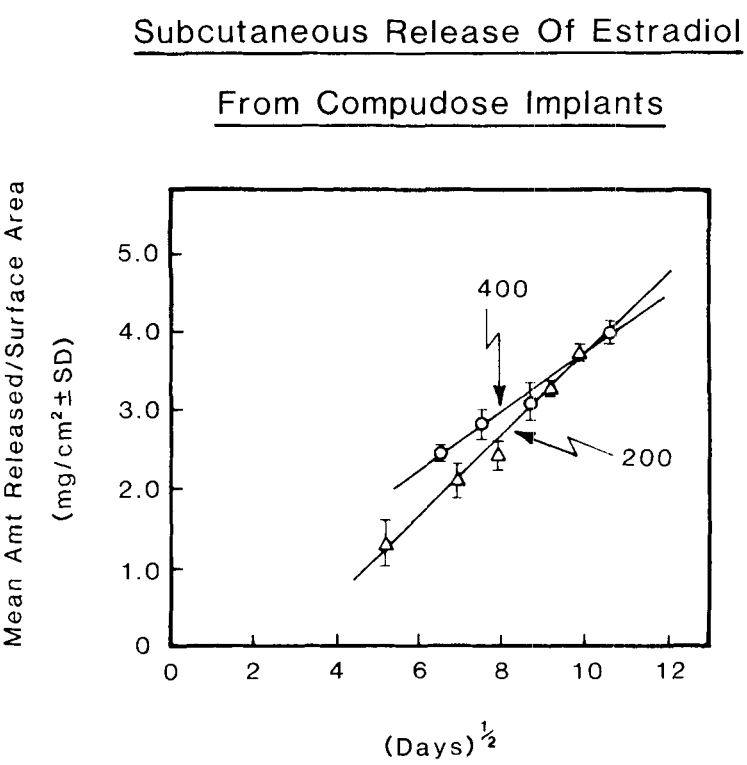


Figure 4: Subcutaneous release profiles of estradiol from Compudose implants in the 60 rats. Keys: implant (release flux)-(○), Compudose-400 (362 mcg/cm^2/day$^{1/2}$); (△), Compudose-200 (520 mcg/cm^2/day$^{1/2}$).

While, the mean amount of estradiol released from Compudose-400 implants in rats was 2.466, 2.800, 3.073, 3.958 mg/cm^2 after 44, 57, 77 and 114 days of implantation, respectively. The variation at each time point was within 10%.

Since Compudose implant is a matrix diffusion-controlled drug delivery device and in vitro drug release studies demonstrated that the release profile of estradiol from Compudose implants follow Equation (1), so, the cumulative amount of estradiol release in the subcutaneous tissue

was also plotted as a function of the square root of implantation time (Figure 4). The results demonstrated that the subcutaneous controlled release of estradiol from the Compudose implants also follows the linear relationship of Q vs. $t^{1/2}$ with Compudose-200 and -400 implants releasing estradiol at release flux of 520 and 362 mcg/cm^2/day$^{1/2}$, respectively.

DISCUSSION

There were about 115.7 million heads of cattle slaughtered in the United States in 1982 alone, over 90% of them receiving implants with some forms of hormonally active substance (21). The hormonally active products currently on the market are mostly compounded with lactose, cellulose, and binder to form tablets which are formulated to disintegrate slowly in the subcutaneous tissue. Due to its unpredictable release of active components within a short duration, three or four times of reimplantation are often required during a production cycle. The nonbiodegradable, retrievable subdermal implants fabricated from the biocompatible silicone elastomer, like Compudose, in contrast, can predictably control and significantly prolong the release of estradiol in the subcutaneous tissue for over 200 days, suggesting that it has great beneficial advantage over the conventional dosage forms in long-term growth promotion management in the cattle.

The *in vitro* release profiles of Compudose implants were conducted in an elution solution maintained under the sink conditions for 10 days which are only a fraction of 200-to 400-day implantation projected under the *in vivo* situation. The analysis of both *in vitro* and *in vivo* release profiles, by plotting the cumulative amount of estradiol release versus the square root of time, confirmed that Compudose implants are a matrix diffusion-controlled drug delivery device. The Compudose-200 and -400 implants appeared to release estradiol in the subcutaneous tissue at

release fluxes which are, respectively, 140-190% and 121-132% of those obtained by the in vitro drug release studies. The difference could be attributed to a thinner hydrodynamic diffusion layer achieved in the subcutaneous tissue as a result of good tissue/implant contact by subcutaneous implantation (15).

The instantaneous release rate of estradiol from the Compudose implants in the subcutaneous tissue can be derived by taking derivation of Equation (1):

$$\frac{dQ}{dt} = \left(\frac{AC_pD_p}{2t}\right)^{\frac{1}{2}} = (AC_pD_p)^{\frac{1}{2}}(2t)^{-\frac{1}{2}} \qquad (3)$$

since $2A >> C_p$.

From the actual amount of estradiol released from the Compudose implants over a period of subcutaneous implantation, the mean daily dose of estradiol delivered to each rat can be calculated. This mean daily dose delivered should be linearly proportional to $(2t)^{-\frac{1}{2}}$ as expected from Equation (3). The results in Figure 5 confirm that experimentally this linear relationship is followed for the subcutaneous release of estradiol from both Compudose-200 and -400 implants.

The data in Figure 4 indicate that Compudose-200 and -400 implants deliver estradiol in subcutaneous tissue at release flux of 520 and 362 mcg/cm^2/day$^{\frac{1}{2}}$, respectively. As reported in Table I, each of the Compudose -200 and -400 implants has a surface area of 4.84 cm^2 and contains, respectively, 24 and 45 mg of estradiol. Using these data, the useful life span for each Compudose implant can be estimated. The results suggested that the useful life span estimated is 91 days for Compudose-200 and 660 days for Compudose-400 (Fig. 5). In other words, Compudose-200 implants deliver estradiol at release flux of 520 mcg/cm^2/day$^{\frac{1}{2}}$ and all the 24 mg of estradiol dose incorporated in the implant are expected

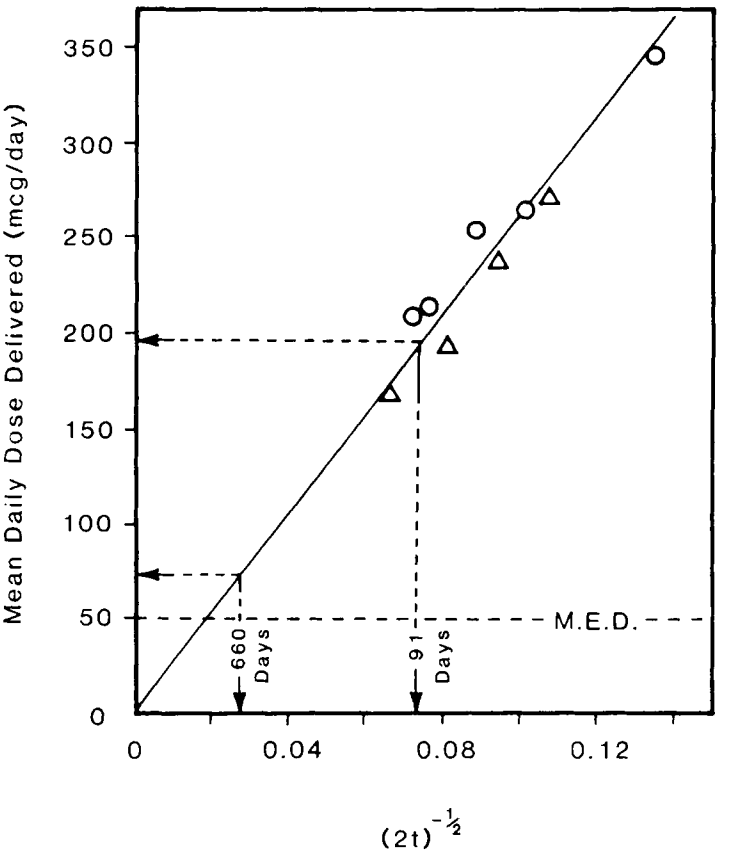


Figure 5: Linear relationship between the mean daily dose of estradiol delivered subcutaneously from the Compudose-200 (△) and -400 (◯) implants and $(2t)^{-\frac{1}{2}}$ as predicted from Equation (3). Keys: M.E.D. denotes the minimum effective daily dose required for growth promotion.

to be depleted by Day 91, at which a mean daily dose of $\geq$ 196 mcg/day of estradiol is delivered to the cattle subcutaneously (Figure 5). On the other hand, Compudose-400 implants deliver estradiol at lower release flux of 362 mcg/cm^2/day$^{\frac{1}{2}}$ and all the 45 mg of estradiol dose incorporated in the implant are predicted to be depleted by Day 660, at which a mean

daily dose of $\geq$ 73 mcg/day of estradiol is delivered to the cattle subcutaneously (Figure 5).

Unfortunately, both Compudose implants deliver excess amount of estradiol daily to the cattle (Compudose-200 implant delivers a daily dose of $\geq$ 196 mcg/day for up to a period of 91 days, while Compudose-400 implant delivers a daily dose of $\geq$ 92 mcg/day for up to 400 days). This excess amount of estradiol delivered subcutaneously may induce an abnormal sexual activity in the treated cattle. Ideally a better designed subdermal implant should release the required daily dose of estradiol (50 mcg/day) at constant (zero-order) rate, with no or minimum burst release of estradiol in the initial period of implantation, throughout the duration of treatment prescribed.

The theoretical release flux of estradiol from Compudose implants, calculated from the literature values on various physical parameters in Equation (2), was 351 mcg/cm^2/day$^{1/2}$. On one hand, the *in vitro* release fluxes obtained in this investigation were slightly lower (by 20%) than this theoretically predicted value, possibly due to the existence of hydrodynamic diffusion layer on the implant surface. On the other hand, the subcutaneous release flux of estradiol from Compudose-400 implant was found to be rather agreeable with this theoretical value (362 vs. 351 mcg/cm^2/day$^{1/2}$). However, the release flux from Compudose-200 was found higher, by almost 50%, than this theoretical value. This discrepancy cannot be explained on the basis of the experimental data obtained to date.

Acknowledgment

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FOOTNOTE

1. Roussel Uclaf, Paris, France
2. Fisher Scientific, Springfield, N. J.
3. Shaking Waterbath (Model 127), Fisher Scientific, Springfield, N. J.
4. UV/Vis spectrophotometer (Model 559), Perkin-Elmer, Norwalk, Ct.
5. Perfection Breeders, Inc., Douglassville, Pa.
6. Lot # 43-637-AF, Abbott Laboratories, North Chicago, Il.
7. Lot # CH 7837L, Cutter Co., Berkeley, Ca.
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9. Burrel Wrist Action Shaker (Model 75), Pittsburgh, Pa.

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