Implant Pellets I: Effects of Compression Pressure on *In Vivo* Dissolution of Delmadinone Acetate Pellets

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Abstract □ A formulation containing 95% delmadinone acetate was compressed at three different pressures. These pressures resulted in a pellet density difference of 19%. *In vivo* dissolution profiles were determined for five lots of pellets. The pellets were implanted subcutaneously in rats, removed periodically, and assayed chemically for remaining steroid. The resulting data were fit, using the computer program NONLIN, to a dissolution model. The dissolution rate for the lot with the lowest density made at the lowest compression was statistically ($p < 0.05$) different from the four other lots. A possible explanation for this increased dissolution rate could be that channeling occurs within the pellet, thereby increasing the effective dissolving surface. The results also indicate that equivalent dissolution rates between lots are reached at a certain compression and density.

Keyphrases □ Implants—delmadinone acetate pellets, effect of compression pressure on *in vivo* dissolution profile, rats □ Delmadinone acetate—pellet implants, *in vivo* dissolution profile, rats □ Dissolution—delmadinone acetate pellet implants, effect of compression pressure, rats

Steroid pellet implants were suggested many years ago (1) as a method for continuously delivering a steroid drug for a long period. This principle of drug delivery has been used frequently, and an excellent review of the subject has appeared (2).

In manufacturing a pellet implant, it is necessary to examine factors that may affect the *in vivo* dissolution of the pellet, including (*a*) the particle-size distribution of the steroid, (*b*) the pressure load used in pellet compression (hence, pellet density), (*c*) the granulation procedure, and (*d*) polymorphism.

Previous investigators (3, 4) demonstrated *in vivo* that the variation in compression (pellet density) had no effect on pellet dissolution. Cowie and Foley (4) examined a fivefold range in compression of hexosterol pellets, but only a 5% variation in pellet densities resulted. The *in vivo* dissolution result, not too surprisingly, demonstrated no difference in dissolution rate between groups. Other investigators (3) varied the compression pressure fourfold (a reported 15,000–60,000 psi) in making desoxycorticosterone acetate pellets (radius = 0.32 cm) and found no change in the pellet dissolution rate.

The compression or density effect on pellet dissolution was tested *in vitro* (5) over a 25% variation in pellet densities. The results indicated no difference in the *in vitro* dissolution rate over the density range and at the particular stirring rate studied.

The purpose of this preliminary investigation was to determine if varying the compression (or density) in the manufacturing of delmadinone acetate pellets would affect the *in vivo* pellet dissolution. The results of this study should be very helpful in setting production and quality control specifications for the pellets.

Delmadinone acetate (6-chloro-17-hydroxypregna-1,4,6-triene-3,20-dione acetate), the major component of the pellets studied, is a potent progestin with antiestrogenic and strong antiandrogenic properties (6, 7). Delmadinone acetate was studied for reproduction control in the cat (8), for which the pellet product would have application.

Table I—Delmadinone Acetate Pellet Implant Characteristics

Group	Compression Characteristic	Initial Pellet Weight, mg (*SD*)	Initial Pellet Height, cm	Density (ρ), g/ml
1	Low	29.48 (0.67)	0.393	0.944
2	Standard	27.55 (0.70)	0.298	1.16
3	High	28.38 (0.81)	0.307	1.16
4	Standard	27.36 (0.82)	0.295	1.17
5	Standard	26.52 (0.64)	0.286	1.17

EXPERIMENTAL

Pellet Formulation—The steroid pellets were composed of 95% delmadinone acetate and 5% inert ingredients. The pellet granulations were compressed on a single-punch tablet machine[1], using 0.318-cm (0.125-in.) flat-face beveled-edge punches and die.

The groups of pellets are identified in Table I. Groups 1–3 were manufactured from the same granulation. Each group, however, was compressed under a different compression load. The compression used is indicated as low, standard, or high. At the time of pellet manufacture, the tablet press was not instrumented so arbitrary terms were assigned. Since then the press has been instrumented with strain gauges so precise load values can be obtained. By using the results from pellet compression experiments with the instrumented press, approximate load values can be given to the arbitrary terms used here. Low compression corresponds to about 45.4–90.8-kg (100–200-lb) load, standard to about 227-kg (500-lb) load, and high to about 272.4-kg (600-lb) load.

Analytical Method—The pellets were analyzed for delmadinone acetate initially and after removal from implantation. The pellet was dissolved in chloroform, and the delmadinone acetate was isolated by TLC, using a silica gel plate with benzene–ethyl acetate (70:30) as the solvent. Final determination was by 4-aminoantipyrine hydrochloride color development, with absorbance measured at 420 nm.

Animals and Design—Twenty-four male Sprague–Dawley-derived albino rats, 200–220 g, were acclimatized to laboratory conditions for 1 week. The animals then were identified by a standard ear punch code, and six rats were randomly assigned to four groups.

On Day 0, each rat was implanted subcutaneously with six pellets, three in each flank. Each pellet was implanted separately so that each could be individually identified.

The animals were housed two to a standard rat cage with food and water *ad libitum*. One pellet from alternate sites was removed from each animal 28, 63, 119, 154, 182, and 238 days after subcutaneous implantation.

The implantation procedure for Group 5 was somewhat different than for Groups 1–4. Eighteen animals were implanted subcutaneously with two pellets, one in the right and one in the left flank areas. On Days 29, 59, 90, 120, 151, and 179 after implanta-

[1] Stokes model E.

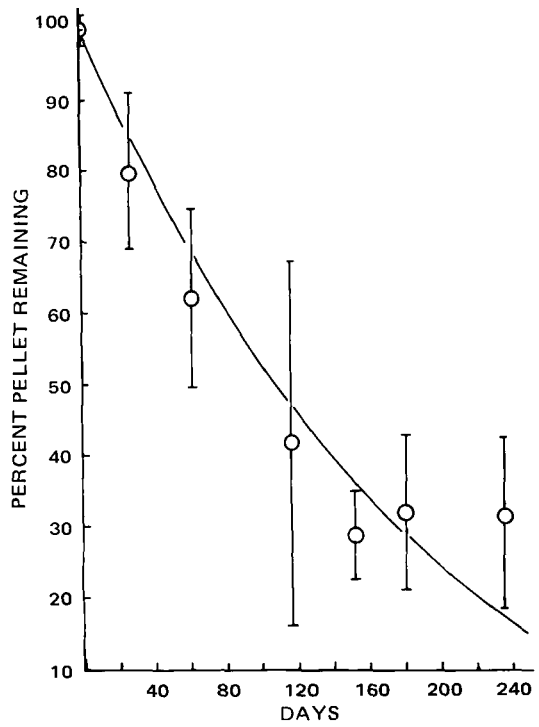

Figure 1—*Dissolution profile of delmadinone acetate pellets, Group 1, implanted subcutaneously in rats. Key: O, mean of observations with standard deviation as solid bar; curved solid line is computer fit of data to dissolution model.*

tion, groups of three rats were sacrificed and the pellets were removed for chemical analysis.

Data Treatment—The dissolution of a pellet implant is dependent on many factors, such as composition, surface area, and solubility. The weight of a dissolving pellet at any time, t, may be described as a function of the product of the pellet density and pellet volume (2). The dissolution equation assumes that the shape does not change during dissolution, nor does the dissolution rate per unit surface area change as the result of a change in diffusional distance or surface irregularities. The equations for a cylindrical pellet (2), such as the one under study, are:

$$W_t = (\pi)(\rho)(R_0 - kt)^2(H_0 - 2kt) \qquad \text{(Eq. 1)}$$

Table II—Percent of Delmadinone Acetate Pellet Remaining after Removal from Implantation at Specified Times[a]

	Days					
Group	28	63	119	154	182	238
1	79.6 (10.7), $n = 4$	62.4 (12.6), $n = 6$	41.3 (25.6), $n = 3$	28.5 (6.1), $n = 3$	32.2 (11.1), $n = 3$	30.1 (12.0), $n = 3$
2	87.7 (3.0), $n = 5$	74.2 (6.2), $n = 4$	64.4 (13.4), $n = 3$	51.6 (10.6), $n = 5$	58.6 (11.1), $n = 4$	35.1 (5.5), $n = 4$
3	87.1 (5.9), $n = 9$	75.6 (6.5), $n = 5$	69.5 (8.7), $n = 5$	61.1 (17.1), $n = 3$	65.4 (15.1), $n = 4$	35.9 (9.7), $n = 5$
4	90.6 (3.2), $n = 5$	76.5 (5.4), $n = 6$	66.6 (7.2), $n = 6$	45.3 (5.7), $n = 5$	47.5 (5.3), $n = 3$	37.5 (7.5), $n = 6$
	Days					
	28	59	90	120	151	179
5	88.2 (3.2), $n = 6$	80.7 (2.6), $n = 6$	67.5 (7.9), $n = 6$	76.4 (9.8), $n = 5$	54.7 (14.1), $n = 6$	50.8 (18.1), $n = 5$

[a] Numbers in parentheses are standard deviations; n = number of samples.

$$W_t = (\pi)(\rho)[-2k^3t^3 + R_0(4 + Q)k^2t^2 - 2R_0^2(1 + Q)kt + R_0^3Q] \qquad \text{(Eq. 2)}$$

where:

W_t = weight in milligrams of the pellet t days after initiation of dissolution
R_0 = initial radius of the pellet in centimeters
H_0 = initial height of the pellet in centimeters
$Q = H_0/R_0$
$\rho = W_0/\pi R_0^2 H_0$, pellet density
k = dissolution rate constant with units of centimeters per day

The constant k describes the rate at which the pellet radius decreases. The value for k was estimated using the NONLIN computer program (9). This program provides an estimate for k plus statistics such as the standard error about k, the 95% confidence limits, and the correlation coefficient. In addition, the program calculates the pellet weights from the equation, the pellet dissolution rate, the dissolution rate of active steroid, and the pellet surface area, all at each measured time point. The equations used follow:

$$\text{dissolution rate at time } t \text{ per pellet} = \frac{-dw}{dt} = 2\pi(\rho)k[(R_0 - kt)^2 + (R_0 - kt)(R_0Q - 2kt)] \qquad \text{(Eq. 3)}$$

$$\text{surface area at time } t \text{ per pellet} = 2\pi(R_0 - kt)^2 + 2\pi(R_0 - kt)(R_0Q - 2kt) \qquad \text{(Eq. 4)}$$

$$\text{dissolution rate per pellet} = \frac{-dw}{dt} = (\rho)(k)(\text{surface area}) \qquad \text{(Eq. 5)}$$

$$\text{active dissolution rate per pellet} = (\text{fraction active})(\text{pellet dissolution rate}) \qquad \text{(Eq. 6)}$$

RESULTS AND DISCUSSION

The results are presented in Table II and Figs. 1–5. The shape of the dissolution profiles conforms to the expected result, as indicated by the computer fit. The variation, as indicated by the standard deviation, was occasionally higher compared to earlier and later

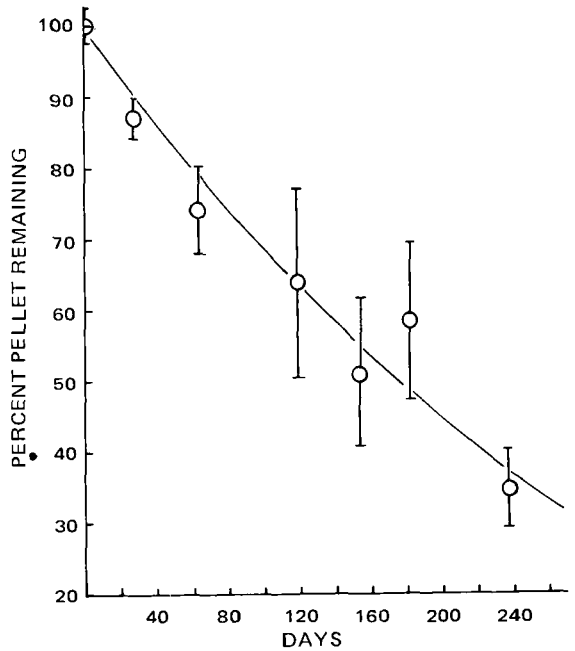

Figure 2—*Dissolution profile of delmadinone acetate pellets, Group 2, implanted subcutaneously in rats. Key: O, mean of observations with standard deviation as solid bar; curved solid line is computer fit of data to dissolution model.*

Table III—Summary of Output from Computer Fit of Dissolution Data

Group	Tablet Press Compression Setting	$k \times 10^4$ cm/day (*SE*)	95% Confidence Limits (Support Plane)	Correlation Coefficient	Dissolution Rate ($k \times \rho$), $\mu g/cm^2$ day: Mean (*SE*)	Dissolution Rate ($k \times \rho$), $\mu g/cm^2$ day: 95% Confidence Limits (Support Plane)
1	Low	3.24 (0.27)	2.31–4.17	0.972	306 (25.5)	218–394
2	Standard	1.80 (0.12)	1.39–2.21	0.974	209 (13.9)	161–256
3	High	1.59 (0.15)	1.09–2.09	0.948	184 (17.4)	126–242
4	Standard	1.94 (0.093)	1.62–2.26	0.987	227 (10.9)	190–264
5	Standard	1.69 (0.12)	1.26–2.11	0.961	198 (14.0)	147–247

experiments with this pellet formulation. This finding implies more variability in the present experiment

In the present experiment with Groups 1–4, it was discovered that the rat was difficult to keep alive through the duration of the experiment due to the stress produced with pellet removal. Therefore, the number of animals varies at each time point, and this fact may be in part the reason for some of the variability observed. Procedures similar to those for Group 5 were subsequently followed.

The use of a dissolution model provides a method to compare each set of data using parameters that have physical meaning. The NONLIN program coupled to the equations of the model provides an efficient and accurate method for determining the parameters of the model. The correlation coefficient of the mean data fit to the dissolution model is found in Table III; the data fit to the model is quite good.

The calculated k value for each group, with standard error and 95% confidence limits, is also found in Table III. If all pellet groups were of the same density, the k value could be used to compare the dissolution properties of each group. To make this comparison on an equivalent basis, density must be included. By derivation, the product of k and density is equivalent to the dissolution rate per unit surface area. The mean dissolution rates per unit surface area of the five groups, with standard error and 95% confidence limits, are found in Table III.

To compare the dissolution rates of each group with each other, a t test was performed. The mean dissolution rates of Groups 2–5 were not significantly different and that of Group 1 was significantly different ($p < 0.01$) compared to those of Groups 2–5.

There is a possible explanation for this significant increase in dissolution with the low density lot of pellets. For the observed dissolution rate to increase, one or combinations of the following need to occur:

1. An increase in drug solubility.
2. An increase in fluid dynamics around the pellet, thereby reducing diffusional distance.
3. An increase in diffusivity of the drug away from the pellet.
4. An increase in surface area due to channeling within the pellet and/or existence of a porous, uneven surface.

There is no reason to believe that items 1, 2, and 3 would be any different than the values for the other groups, since nothing changed in the experiment that would affect these factors. However, the decrease in density could affect the actual available surface, increasing it as mentioned and causing the observed increase in dissolution.

Possible reasons why previous investigators (3, 4) did not observe this effect were that the pellet densities were not as low as the one tested in this study and the pellets in the previous studies were 100% steroid.

The present results do not contradict those results observed earlier but suggest the importance of very low pellet densities which may result in increased surface areas after implantation. After a certain pellet density is achieved, however, the dissolution rate does not appear to change.

The *in vitro* dissolution situation is much different than the *in vivo*. Mainly, the hydrodynamics are increased, resulting in a decreased diffusional distance and, hence, an increased dissolution rate. Under these conditions, an increase in the dissolution rate due to an increase in surface due to channeling in a low density pellet would not be observed. This effect was observed in an *in vitro* dissolution study comparing Groups 1–4 where the dissolu-

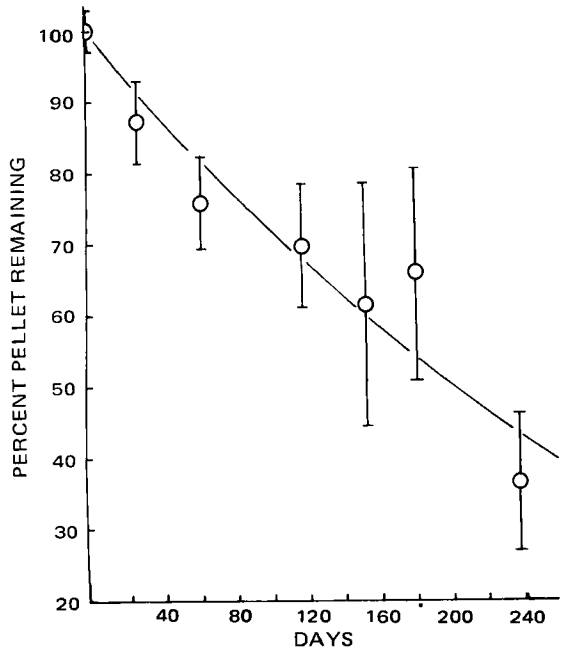

Figure 3—*Dissolution profile of delmadinone acetate pellets, Group 3, implanted subcutaneously in rats. Key: O, mean of observations with standard deviation as solid bar; curved solid line is computer fit of data to dissolution model.*

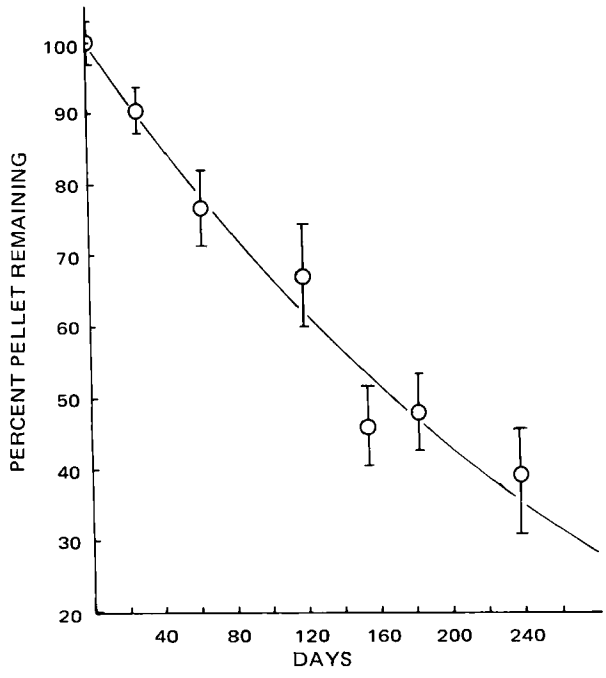

Figure 4—*Dissolution profile of delmadinone acetate pellets, Group 4, implanted subcutaneously in rats. Key: O, mean of observations with standard deviation as solid bar; curved solid line is computer fit of data to dissolution model.*

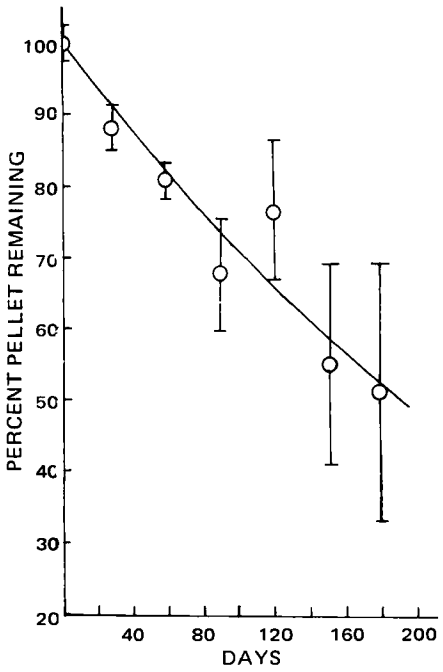

Figure 5—*Dissolution profile of delmadinone acetate pellets, Group 5, implanted subcutaneously in rats. Key: O, mean of observations with standard deviation as solid bar; curved solid line is computer fit of data to dissolution model.*

tion medium was ethanol–water (30:70). This test did not show significant differences between groups. A similar result was reported previously (5).

This finding indicates that an *in vivo* dissolution experiment is required to ascertain which physical parameters are important in pellet manufacture that cannot be immediately determined by an *in vitro* dissolution test. In the present study, the low compression group dissolved at a significantly greater rate than those pellets produced at a higher compression load. This result indicates that pellets with the desired dissolution rate could be produced by controlling the compression load (pellet density) without the necessity of an *in vitro* dissolution test.

Future studies will examine the *in vivo* dissolution rate of batches of pellets manufactured under known compression loads in the "standard compression" range and will examine the influence of steroid particle-size distribution on pellet manufacturability and *in vivo* dissolution.

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Neurogenic Influences of Bilateral Adrenalectomy on Monoamine Oxidase

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Abstract □ Bilateral adrenalectomy (10 days) increased the monoamine oxidase activity of the rat heart, vas deferens, spleen, superior cervical ganglion, and hypothalamus but not that of the rest of the brain, kidney, and liver. Experiments were made to determine whether the increased activity was due to neurogenic influences and whether the enhanced activity of monoamine oxidase was intra- or extraneuronally located. Ganglionic blockade with chlorisondamine failed to alter the rise in cardiac monoamine oxidase. Likewise, superior cervical ganglion monoamine oxidase was unaffected by surgical denervation. 6-Hydroxydopamine abolished the increase in monoamine oxidase activity of the vas deferens, spleen, and superior cervical ganglion but failed to alter that of the kidney, hypothalamus, and the rest of the brain. Cardiac monoamine oxidase was reduced markedly by 6-hydroxydopamine, but the remaining activity was still significantly elevated over the respective control values. The data suggest that the increase in organ monoamine oxidase is predominantly of neuronal origin and that this increase is not due to transsynaptic induction.

Keyphrases □ Monoamine oxidase—activity, effect of bilateral adrenalectomy, 6-hydroxydopamine, chlorisondamine, rat organs □ 6-Hydroxydopamine—effect on monoamine oxidase activity after bilateral adrenalectomy, rat organs □ Chlorisondamine—effect on monoamine oxidase activity after bilateral adrenalectomy, rat organs □ Adrenalectomy, bilateral—effect on monoamine oxidase activity, rat organs

Bilateral adrenalectomy increases the monoamine oxidase [monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4] activity of the rat heart (1–3) and of certain other organs (2). However, the mechanism(s) whereby steroids or steroid insufficiency influences monoamine oxidase remains speculative (4).