with a helium gas flow rate of 55 ml./min.) and equipped with a flame detector, gave a single peak with an R_t of 34 min.

The specific activity was determined[4] to be 1.61 mc./mmole (4.90 mc./g.).

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Effect of Formulation of Anagestone Acetate on Progestational Proliferation of Rabbit Uterus after Oral Administration

JAWAHAR S. SAWARDEKER and JOHN McSHEFFERTY

Abstract □ A method is presented for the determination of *in vitro* dissolution rates for anagestone acetate, which yields results representative of *in vivo* bioavailability in animal studies. This procedure appeared to be satisfactory in preliminary screening of tablet formulations of anagestone acetate.

Keyphrases □ Anagestone acetate—formulation effects of progestational proliferation, rabbit uterus, oral administration □ Dissolution rates, *in vitro*, anagestone acetate—correlated to *in vivo* bioavailability, rabbits □ Formulation, effect on progestational proliferation, rabbit uterus—preliminary tablet screening of anagestone acetate

Even though tablet disintegration time is widely used, it has inherent defects which limit its usefulness in screening tablet formulations. Various reviews (1, 2) emphasized that the length of time required for a tablet to disintegrate *in vitro* cannot be taken as a direct indication of the time required for *in vivo* availability. Much of the literature suggests that better correlations are obtained using *in vitro* dissolution-rate studies; however, no single dissolution test can be applied to all drugs. The possibility that a single dissolution test can be applied to drugs having similar physicochemical properties remains to be established.

In an effort to develop tablet formulations containing anagestone acetate[1] that would allow ease of formulation and yet ensure maximum bioavailability, a correlation has been utilized between dissolution-rate studies and a physiological test in rabbits depending upon the drug's presence *in vivo* at its site of action. This paper reports the results of these studies for tablets prepared by varying the manner in which the drug was added to the tablet formulations.

EXPERIMENTAL

Test Products—Three different formulations were studied initially. Formulation A was prepared by incorporating anagestone acetate into the tablet formulation as a solution in methylene chloride. Formulation B was prepared by adding micronized anagestone acetate to the tablet formulation in the dry form. Formulation C was prepared by incorporating anagestone acetate powder (30 mesh) into the tablet formulation in the dry form.

Two additional test formulations, D and E, were prepared in the same manner as that described for Formulation A. Formulation D was identical to Formulation A in all respects. Formulation E differed from Formulation D in that it contained 10% starch, whereas Formulation D contained 22% starch.

All the tablet formulations contained the same amount of anagestone acetate (2 mg.), and each tablet weighed 100 mg.

The weight, hardness, and thickness of these formulations were essentially the same, and the disintegration times (with disks) ranged from 4 to 6 min. for all five formulations.

Dissolution Apparatus—An assembly similar to that described by Levy and Hayes (3) was used in the dissolution-rate studies. The apparatus was modified slightly by placing a coarse screen[2] 2.54 cm. (1 in.) above the bottom of the 400-ml. Griffen beaker. A four-blade 5-cm. diameter metal stirrer, attached to a stirring motor affording precise speed control, was used. Due to the very low aqueous solubility of anagestone acetate, a dissolution medium of 25% *tert*-butanol in water was employed to improve the "sink" conditions of the system. Three hundred milliliters of this dissolution medium was placed in the beaker, and the system was equilibrated at 37°. The stirrer was immersed in the dissolution medium to

[1] Anagestone acetate, a progestational agent, is 6α-methyl-4-pregnen-17-ol-20-one acetate, supplied by the Organic Chemistry Division of Ortho Research Foundation.

[2] Ten mesh, No. 23 (0.025 in.) W & N gauge woven stainless steel.

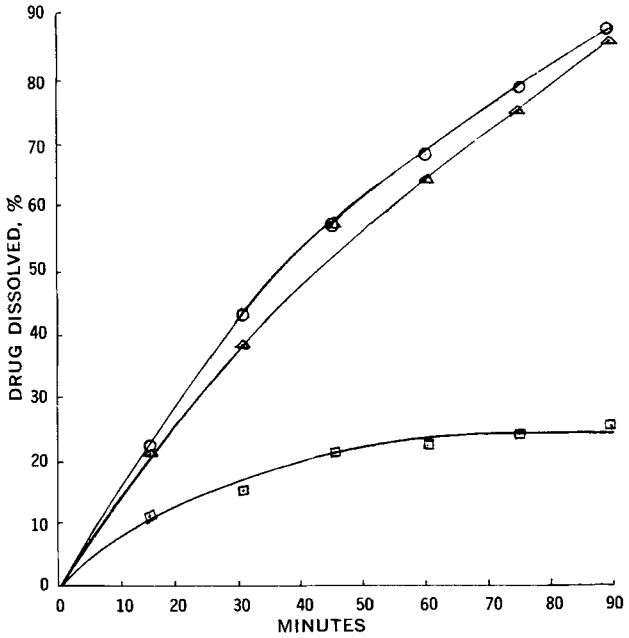

Figure 1—*Dissolution of anagestone acetate from tablets. Key:* ○, *Formulation A;* △, *Formulation B; and* □, *Formulation C.*

a depth of 25 mm., carefully centered, and rotated at 45 r.p.m. Six tablets of the test drug were placed in the beaker; 1 ml. of the fluid was withdrawn at specified intervals for analysis, using a pipet wound with glass wool at the tip, and replaced with 1 ml. of fresh fluid preheated to 37°.

Method of Drug Analysis—The dissolution samples were analyzed by GC (4)[3]. A 0.9-m. (3-ft.) column with 3% diethylene glycol succinate was used isothermally at 215°. Dissolution aliquots were injected directly into the chromatograph, and measurements were made using an internal standard technique.

Test of *In Vivo* Availability in Rabbits—A physiological test specific for the detection of *in vivo* progestational activity of the administered steroid was utilized to estimate the bioavailability of anagestone acetate when administered orally in the test formulations. The progestational steroids caused proliferation of the uterine endometrium; the bioassay, using the McPhail method (5), involving the induction of uterine endometrial changes in estrogen-primed immature rabbits, was used to evaluate relative potency of various formulations. This test is the most satisfactory and meaningful technique for evaluation of progestational activity, and can be used for quantitative comparisons (6). The standard error of this procedure is about ±20%.

The drug was administered over 5 days to nonfasting rabbits. Five animals were treated at each of three dose levels. The animals were sacrificed at the end of the test, and the histological examination was performed on the uterus. The uterine sections were graded for the degree of progestational proliferation according to McPhail (5). These data were not subjected to statistical analysis.

RESULTS AND DISCUSSION

Figure 1 illustrates that the dissolution rate of Formulation C was slower than that of the other formulations and that Formulations A and B had virtually identical dissolution rates in the system employed. From these data, it can be concluded that the particle size of the drug affected dissolution rate. Since both Formulations A and B exhibited similar dissolution profiles and Formulation A had the easier manufacturing process, it was tentatively decided that the addition of drug to the tablet formulation as a solution in methylene chloride (Method A) was the procedure of choice.

The three formulations studied eventually were judged unsuitable for manufacture, since they tended to produce a soft tablet. It was found that reducing the starch concentration in Formulation A from 22 to 10% (Formulation E) produced a tablet of the desired hardness and yet the dissolution profile remained identical to the one shown for Formulation A.

The five test formulations were subjected to *in vivo* evaluation in rabbits. Table I summarizes the endometrial response (McPhail index) for the test products at three dosage levels. The four formulations (A, B, D, and E) that exhibited similar rapid dissolution rates also exhibited similar *in vivo* activity, whereas virtually no activity was observed when the slower dissolving Formulation C was administered. This finding correlates well with the observed rate of dissolution for this formulation.

The observed correlation of dissolution patterns wth biologic (endometrial) response (in rabbits) seems to suggest that dissolution rates determined in a 25% *tert*-butanol–water system might be used as a guide in the development of tablet formulations for anagestone acetate. Additional work is in progress to determine the applicability of the procedure for related steroidal compounds.

Table I—Progestational Proliferation of Rabbit Uterus after Oral Administration

Formulation	Dose Level, mg.	Endometrial Response, McPhail index (5)
A	0.05	0.6
	0.10	1.8
	0.20	2.1
B	0.05	0.3
	0.10	1.1
	0.20	2.6
C	0.05	0
	0.10	0.1
D	0.05	0.6
	0.10	2.7
	0.20	3.5
E	0.05	0.0
	0.10	2.7
	0.20	3.0

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[3] An F&M model 5750, equipped with dual-flame detector system, was utilized.