pharmacokinetics of desmethyldiazepam following the administration of clorazepate can be used to describe the blood concentration–time profiles of diazepam and desmethyldiazepam following both single-dose and chronic administration of the drug, suggesting that there is no enzyme induction or inhibition of diazepam metabolism following multiple-dose administration. This model has also been adapted to predict the concentrations of diazepam and desmethyldiazepam in CSF following the administration of diazepam.

REFERENCES

(1) L. O. Randall, G. A. Heise, W. Schallek, R. E. Bagdon, R. Banziger, A. Boris, R. A. Moe, and W. B. Abrams, *Curr. Ther. Res. Clin. Exp.*, **3**, 405 (1961).

(2) F. P. Pignataro, *Curr. Ther. Res. Clin. Exp.*, **4**, 389 (1963).

(3) A. Di Francesco, *Am. J. Psychiat.*, **119**, 989 (1963).

(4) H. M. Beerman, *Am. J. Psychiat.*, **120**, 870 (1964).

(5) R. A. Katz, J. H. Aldes, and M. Rector, *J. Neuropsychiat.*, Suppl. 3, S91 (1962).

(6) S. A. Kaplan, M. L. Jack, K. Alexander, and R. E. Weinfeld, *J. Pharm. Sci.*, **62**, 1789 (1973).

(7) C. W. Abruzzo, T. Macasieb, R. E. Weinfeld, J. A. Rider, and S. A. Kaplan, *J. Pharmacokinet. Biopharm.*, **5**, 377 (1977).

(8) D. J. Greenblatt, *J. Pharm. Sci.*, **67**, 427 (1978).

(9) F. Moolenaar, S. Bakker, J. Visser, and T. Huizinga, *Int. J. Pharm.*, **5**, 127 (1980).

(10) A. J. Wilensky, R. H. Levy, A. S. Troupin, L. Noretti-Ojemann, and P. Friel, *Clin. Pharmacol. Ther.*, **24**, 22 (1978).

(11) P. J. Carrigan, G. C. Chao, W. M. Barker, D. J. Hoffman, and A. H. C. Chun, *J. Clin. Pharmacol.*, **17**, 18 (1977).

(12) A. H. C. Chun, P. J. Carrigan, D. J. Hoffman, R. P. Kershner, and J. D. Stuart, *Clin. Pharmacol. Ther.*, **22**, 329 (1977).

(13) J. A. F. de Silva and C. V. Puglisi, *Annal. Chem.*, **42**, 1725 (1970).

(14) S. A. Kaplan, M. Lewis, M. A. Schwartz, E. Postma, S. Cotler, C. W. Abruzzo, T. L. Lee, and R. E. Weinfeld, *J. Pharm. Sci.*, **59**, 1569 (1970).

(15) M. A. Brooks, M. R. Hackman, R. E. Weinfeld, and T. Macasieb, *J. Chromatogr.*, **135**, 123 (1977).

(16) C. M. Metzler, G. L. Elfring, and A. J. Mc Ewen, *Biometrics*, **30**, 562 (1974).

(17) H. M. Dasberg, *Psychopharmacologia*, **43**, 191 (1975).

(18) S. Cotler, J. Gustafson, and W. A. Colburn, *J. Pharm. Sci.*, in press.

(19) D. J. Greenblatt, H. R. Ochs, and B. L. Lloyd, *Psychopharmacology*, **70**, 89 (1980).

(20) C. Hallstrom, M. H. Lader, and S. H. Curry, *Br. J. Clin. Pharmacol.*, **9**, 333 (1980).

(21) J. Kanto, L. Kangas, and T. Siirtola, *Acta. Pharmacol. Toxicol.*, **36**, 328 (1975).

(22) J. Hendel, *Acta Pharmacol. Toxicol.*, **37**, 17 (1975).

(23) D. J. Greenblatt, T. P. Laughren, M. D. Allen, J. S. Harmatz, and R. I. Shader, *Br. J. Clin. Pharmacol.*, **11**, 35 (1981).

(24) M. D. Allen and D. J. Greenblatt, *J. Clin. Pharmacol.*, **20**, 639 (1980).

In Vivo Release of Norethindrone Coupled to a Biodegradable Poly(α-amino acid) Drug Delivery System

M. A. ZUPON *, S. M. FANG ˣ, J. M. CHRISTENSEN ‡, and R. V. PETERSEN

Received November 23, 1981, from the *Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84112.* Accepted for publication October 5, 1982. Present addresses: *Division of Product Development, Schering-Plough Corp., Bloomfield, NJ 07003 and ‡School of Pharmacy, Oregon State University, Corvallis, OR 97331.

Abstract □ The *in vivo* release of norethindrone from a biodegradable steroid–polymer conjugate was studied in rats. The drug–polymer conjugate, consisting of [^{3}H]norethindrone coupled *via* a 17-carbonate bond to poly-N^5-(3-hydroxypropyl)-L-glutamine was administered to female rats by subcutaneous injection. The *in vivo* release of steroid, determined by measuring the daily radioactivity output in urine and feces, was fairly constant though it showed a gradual decrease during the 9-month study period. The data indicate that this biodegradable norethindrone–polymer conjugate is a potential candidate for the controlled delivery of norethindrone to effect long-term contraception.

Keyphrases □ Norethindrone—sustained release, biodegradable steroid–polymer conjugate, *in vivo* release rate □ Delivery systems—sustained release of norethindrone, biodegradable steroid–polymer conjugate, *in vivo* release rate □ Contraceptives—norethindrone, sustained release, biodegradable steroid–polymer conjugate, *in vivo* release rate

Although controlled release of drugs at uniform predictable rates from various drug dosage forms has been an object of considerable research for many years, the use of synthetic polymeric materials in the design of controlled-release devices is of recent origin. Of major interest has been the incorporation of contraceptive progestins into various polymers to form controlled, sustained-release drug delivery systems. The use of monolithic devices of poly(dimethylsiloxane) with progestins has been the subject of numerous reports (1–8). These devices, however, have the disadvantage of requiring implantation and removal. Furthermore, significant foreign-body reactions were observed in tissues surrounding these implants, which may be the cause of difficulty in achieving prolonged, constant release of progestins from these devices. Alternatively, the "Uterine Progesterone System" has been the subject of several studies (9–11). The safety and effectiveness of this device has been well established (9, 10, 12). Hydrogel polymers, *e.g.*, poly(hydroxyethyl methacrylate), have also been used to prepare sustained-release devices for progestins (13, 14) with favorable results.

Recently, biodegradable polymers as carriers for controlled, sustained release of various drugs have been evaluated. These polymers are synthesized from monomers which are composed of normal body constituents or which exhibit good compatibility with the body physiology. Various biodegradable polymers have been prepared for the controlled release of contraceptive steroids (15–19). The materials employed for these studies are poly(lactic acid) (15, 16), glutamic acid–leucine copolymer (17), and polyesters of homo- or copolymers of glycolide, DL-lactide, ϵ-caprolactone, or DL-ϵ-decalactone (18). Studies on the *in vitro* and *in vivo* hydrolysis of homopolymers of δ-benzyl-L-glutamate have been reported (20, 21). The

0022-3549/83/1100-1323$01.00/0

© 1983, American Pharmaceutical Association

Table I—Characteristics of Norethindrone–Polymer Conjugate

	Batch A	Batch B
Degree of Norethindrone Coupling	39%	60%
Particle Size	N.D.[a]	100 ± 80 μm
Molecular Weight[b]	N.D.[a]	230,000

[a] Not Determined. [b] The molecular weight of poly-N^5-(3-hydroxypropyl)-L-glutamine. The norethindrone–polymer conjugate does not have enough solubility to render a determination of the molecular weight.

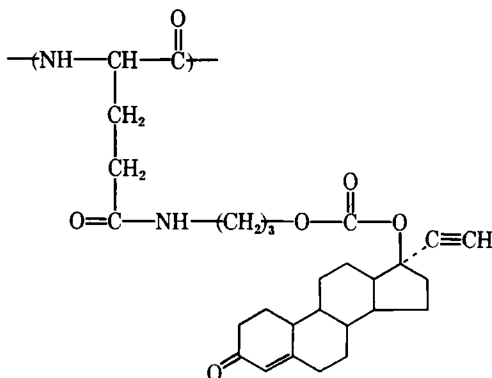
results indicated they could be favorable materials for the sustained release of contraceptive steroids.

In this laboratory, a drug delivery system consisting of norethindrone covalently coupled to poly-N^5-(3-hydroxypropyl)-L-glutamine was recently prepared (22). This drug delivery system should be ideally suited for the controlled release of norethindrone. The polymeric device is biogradable leaving no empty shell for removal at the end of the drug therapy. The hydrolytic product, L-glutamic acid, is a normal body constituent and should show compatibility with the body tissues. Furthermore, the steroid, which is covalently bonded to the biodegradable polymer by a labile bond, may be released in concert with the gradual erosion of the polymer backbone around the surface of the polymer particle. A sustained release of steroid may thus be achieved without the involvement of any diffusion process.

EXPERIMENTAL

Reagents—Tritium-labeled norethindrone and [^{14}C]testosterone obtained for a commercial source[1] were purified by TLC before use. The stability of the tritium atoms in [^{3}H]norethindrone was evaluated by repetitively placing the labeled drug in an ethanol-water (1:1) solution at 25° for 24 hr followed by vacuum distillation of the solution. The extent of exchange between the tritium atoms of the steroid and the hydrogen atoms of the medium was assessed by measuring the radioactivity associated with the distillates. The tritium atoms in [^{3}H]norethindrone were found to be quite stable. Only a negligible amount of radioactivity was found in the distillates: 0.15 and 0.012% in the first and second distillates, respectively.

Polymeric Drug Delivery System—The norethindrone–polymer conjugate consists of norethindrone covalently coupled to poly-N^5-(3-hydroxypropyl)-L-glutamine through the 17-carbonate bond (22):



Two batches of the compound (A and B) were obtained (Table I). Batch A was administered to two rats (A_1 and A_2) at doses of 4.9 and 10 mg, respectively, while 10 mg of batch B was administered to each of five rats.

Biological Half-Life of Norethindrone in Rats—Female Sprague–Dawley rats (200–240 g) were anesthetized with sodium pentobarbital (0.3 mg/g of body weight). An ~3-cm incision was made on the abdominal wall to expose the inferior vena cava. A catheter was placed into the inferior vena cava to inject and withdraw samples. Tritium-labeled norethindrone solution (1.88 μg/25 μCi/0.1 ml Ringer's solution) was injected into the vein, and 0.3 ml of blood was withdrawn at 30-min intervals with a syringe previously rinsed with heparin. Each 0.3-ml blood sample was replaced with 0.3 ml of Ringer's solution to restore blood volume. A portion (0.1 ml) of each 0.3-ml blood sample was pipetted into a conical centrifuge tube containing 5 μl each of nonlabeled norethindrone (1×10^{-3} M) and testosterone (1×10^{-3} M) and a known amount of [^{14}C]testosterone, which served as the internal standard. The blood sample was centrifuged, and the plasma was transferred to another tube. The cellular fraction was extracted four times with 0.3-ml portions of Ringer's solution, which was then combined with the plasma fraction. The above procedures were carried out quickly at 0–4° to prevent any metabolism of the internal standard. The combined plasma fraction was immediately extracted four times with 2-ml aliquots of methylene chloride. The methylene chloride fractions were pooled and evaporated to dryness under a nitrogen stream. The residue, after being dissolved in 100 μl of methanol, was spotted on a silica gel TLC plate. The plate was eluted with a benzene–ethyl acetate solution (4:1), and the spots corresponding to the parent drug and internal standard were isolated and placed into scintillation vials. To each fraction was added 12 ml of a scintillation fluor cocktail[2], and the radioactivity was measured in a scintillation counter[3]. The efficiency of recovery for norethindrone was normalized according to the amount of recovery of the internal standard, [^{14}C]testosterone, from the TLC plate.

The biological half-life of the total radioactivity, which represents the parent drug and all metabolites, was determined by measuring the radioactivity in 0.1-ml aliquots of the blood sample after treatment with 2 ml of a tissue solubilizer[4] and 0.5 ml of benzoyl peroxide solution (12% in toluene).

***In Vivo* Steroid Release Rate Determination**—The tritiated norethindrone–polymer conjugate (10 mg as a fine powder) was placed in 5 ml of sterile Ringer's solution and let stand overnight. The next day, the supernatant was decanted off, and the precipitate was rinsed several times with sterile Ringer's solution. A small amount of the resulting suspension was plated onto sheep's blood and trypticase soy-base agar plates and incubated for 48 hr at 37° for sterility testing. No colonies were found on the agar plates.

Mature female Sprague–Dawley rats, weighing 210–225 g, were each injected subcutaneously with 0.5 ml of the suspension into the dorsal side just below the neck. The rats were then placed in individual metabolism cages, and their feces and urine were collected at 24-hr intervals for at least 14 days following implantation. Thereafter, the biological samples were collected three times a week, 2 days apart.

The total volume of urine was measured, and a 1-ml aliquot of urine was sampled. To the urine sample, 0.6 ml of water and 12 ml of a scintillation fluor cocktail were added, and the radioactivity in each sample was counted in a scintillation counter. The total amount of fecal matter collected from each metabolism cage was weighed and put in an oven at 60° for ~12 hr until dry. The dry mass was then weighed and pulverized to an homogenous mixture of fine powders in a blender. Two aliquots of the dried fecal matter (0.2 g/sample) were accurately weighed and placed in separate glass scintillation vials. To each vial was added 0.3 ml of 70% perchloric acid and 0.6 ml of 30% hydrogen peroxide to effect complete digestion. The vials were then tightly sealed and incubated for 12 hr at 60° in a shaking water bath until all the fecal matter was completely solubilized. After the digestion, the vials were cooled for 30 min at room temperature before adding 12 ml of a scintillation fluor cocktail[5]. The vials were then kept in a dark place for at least 24 hr before they were counted in a scintillation counter. This procedure effectively minimized chemiluminescence. With this digestion procedure, no appreciable loss of radioactivity due to the processing was observed. These data were used to calculate the daily and cumulative output of radioactivity in the urine and feces.

RESULTS AND DISCUSSION

If the release of [^{3}H]norethindrone from the steroid–polymer conjugate approximates a zero-order kinetic process, the amount of total radioactivity in the body would gradually build up to an eventual steady-state value as shown in the following equation:

$$Q_b = (k_0/k_{el})(1 - e^{-k_{el}t}) \quad \text{(Eq. 1)}$$

where Q_b is the amount of the total radioactivity in the body, k_0 is the

[1] New England Nuclear Corp., Boston, Mass.

[2] Formula 950A; New England Nuclear Corp., Boston, Mass.
[3] Model LS9000; Beckman Instruments, Inc., Irvine, Calif.
[4] Protosol; New England Nuclear Corp., Boston, Mass.
[5] Biofluor; New England Nuclear Corp., Boston, Mass.

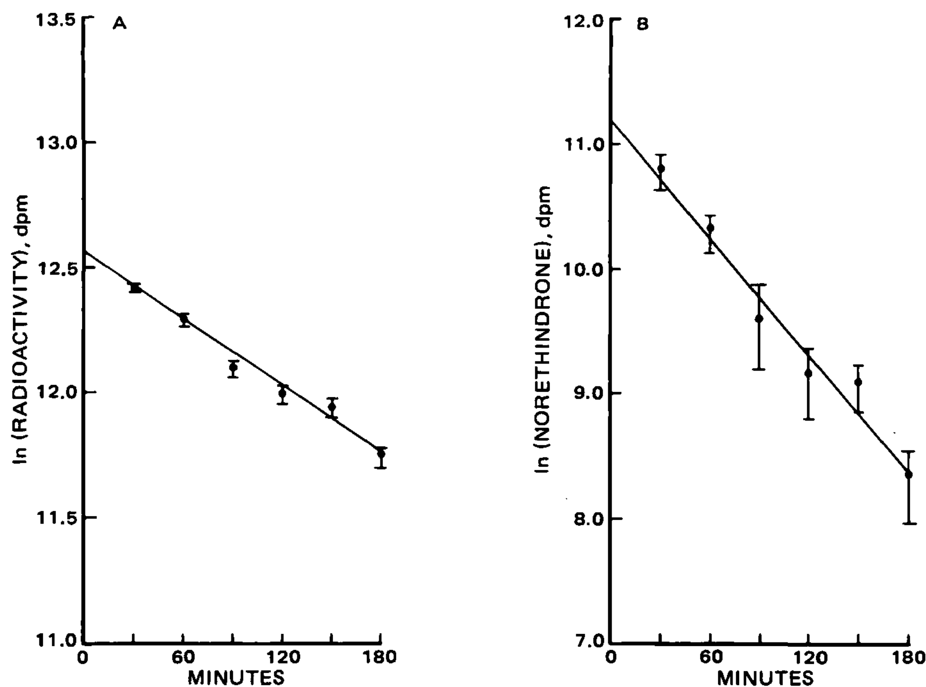


Figure 1—*Semilogarithmic plot of the elimination of total radioactivity (A) or [^{3}H]norethindrone (B) in female Sprague-Dawley rats. Values were determined after intravenous injection of [^{3}H]norethindrone (mean* ± SD, n = *6).*

steroid release rate from the steroid–polymer conjugate, and k_{el} is the elimination rate of the total radioactivity. Since the amount of the total radioactivity excreted in the urine and feces (Q_{ex}) equals $k_{el}{\cdot}Q_b$, then:

$$Q_{ex} = k_0(1 - e^{-k_{el}t}) \quad \text{(Eq. 2)}$$

and at steady state ($t \rightarrow \infty$):

$$Q_{ex} = k_0 \quad \text{(Eq. 3)}$$

Equation 3 states that at steady state the amount of the total radioactivity excreted from the body is the same as that of the total radioactivity released from the steroid–polymer conjugate. The rate of the daily output of radioactivity in urine and feces at steady state is, therefore, a valid indication of the daily release rate of steroid from the biodegradable drug delivery system.

Since the above argument is valid only under steady-state conditions, it is necessary to know exactly when steady state is achieved in female rats. From Eq. 1, it can be shown that the time required to reach 95% of steady state is:

$$t_{0.95} = 4.3 \times t_{1/2} \quad \text{(Eq. 4)}$$

The biological half-life of norethindrone in female rats was found to be 44 min (Fig. 1B), which agrees with that reported earlier by Back *et al.* (23). The elimination of the total radioactivity from rats after the intravenous injection of [^{3}H]norethindrone, however, was slower than that for parent [^{3}H]norethindrone. The half-life for the total radioactivity elimination was 161 min (Fig. 1A). This means that if the norethindrone–polymer conjugate releases norethindrone at a constant zero-order rate after its injection, it will take ~12 hr before the *in vivo* release rate in the urine and feces would reach a constant steady-state value.

Following intravenous and subcutaneous injection of [^{3}H]norethin-

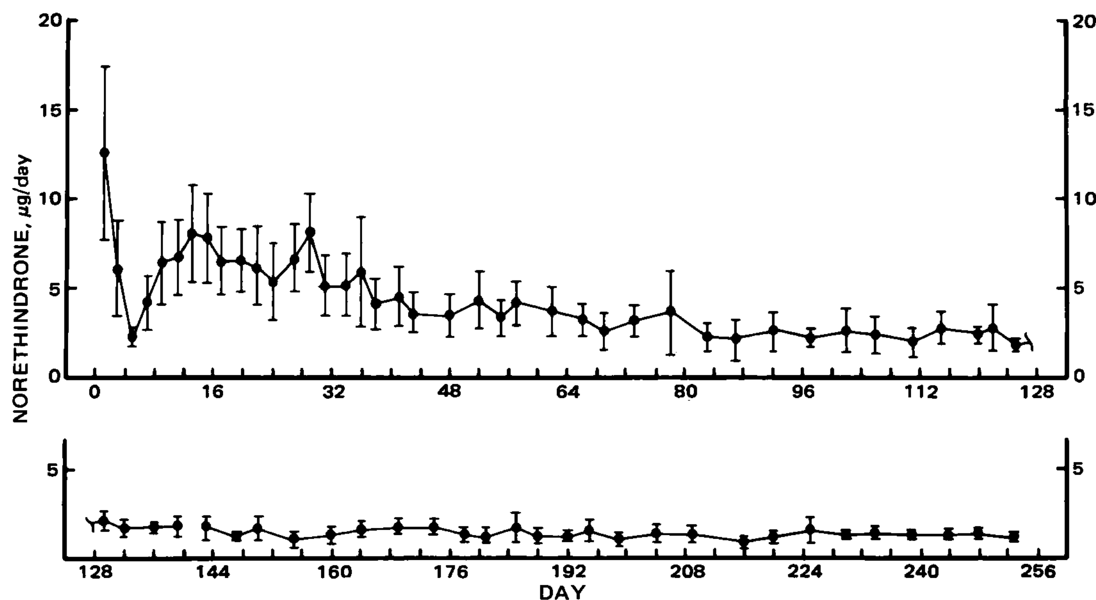


Figure 2—*Daily excretion of radioactivity in urine and feces. Norethindrone–polymer conjugate, batch B (10 mg), containing [^{3}H]norethindrone was injected subcutaneously into female Sprague-Dawley rats. The radioactivity excreted in the urine and feces was measured at various times after the administration (mean* ± SD, n = *5).*

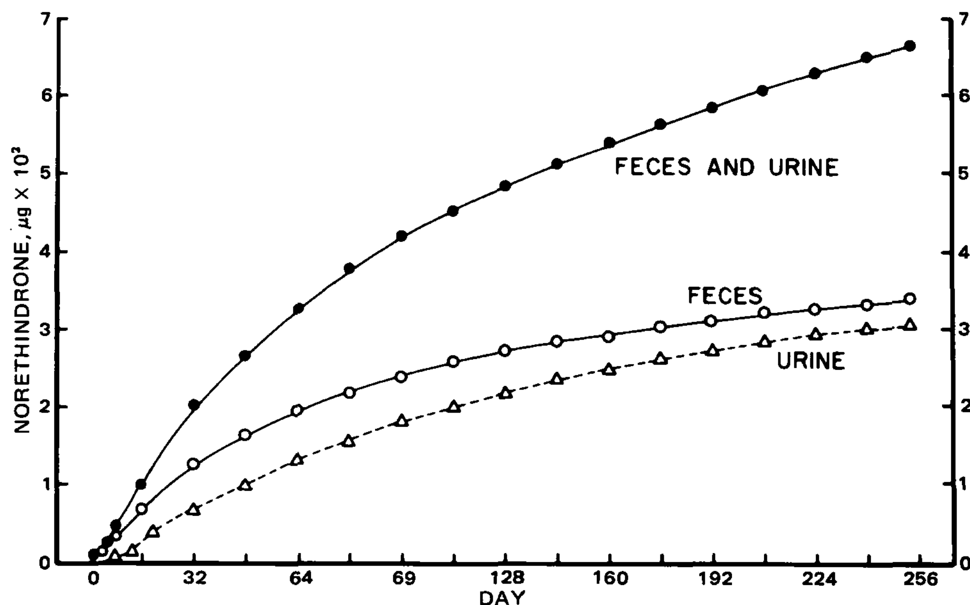


Figure 3—*Cumulative excretion of radioactivity in urine and feces. Norethindrone–polymer conjugate, batch B (10 mg), containing [³H]norethindrone was injected subcutaneously into female Sprague–Dawley rats. The total radioactivity excreted in the urine and feces at various times after the administration is presented (mean, n = 5).*

drone to female Sprague–Dawley rats, it was observed that ~50–60% of the total radioactivity was excreted in the feces. A similar pattern was observed in rats administered the norethindrone–polymer conjugate.

Figures 2 and 3 show the *in vivo* steroid release rate profile for batch B; similar results were observed with batch A. In all animals administered [³H]norethindrone–polymer conjugate (either batch A or B), the release rate profile was characterized by a slight burst of radioactivity release immediately after the injection, followed by a fairly constant release rate for a period up to 10 months. However, a gradual decrease in the rate of steroid release over this long period was evident.

The average *in vivo* release rate of steroid for batch B from day 17 through day 253 was 0.37 μg of norethindrone/mg of norethindrone–polymer conjugate/day. For batch A, the *in vivo* steroid release rate from day 22 through day 292, expressed as μg of norethindrone/mg of norethindrone–polymer conjugate/day, was 0.27 and 0.26 for rats A_1 and A_2, respectively. The daily steroid release from these two animals administered batch A at two different doses were essentially equivalent. The daily steroid release rates from the five animals administered batch B were also very similar, as evidenced from the relatively small standard deviation (Fig. 2). Additionally, both batches A and B appeared to show similar long-term steroid release profiles throughout the study period.

The work measured the *in vivo* release of norethindrone which was covalently coupled to a biodegradable polyglutamate polymer. The system showed a fairly constant release of steroid for a time period in excess of 9 months. A slight decrease in the amount of norethindrone release was evident, however, over this long period.

The data showed that the norethindrone–polymer conjugate is a potential candidate for the controlled delivery of norethindrone to effect long-term contraception. Variation of parameters (*e.g.*, molecular weight, particle size, degree of norethindrone coupling, and the length of spacer group of the steroid–polymer conjugate) may result in products capable of achieving a specified *in vivo* duration and rate of steroid release.

REFERENCES

(1) L. L. Dogle and T. H. Clew, *Am. J. Obstet. Gynecol.*, **101**, 564 (1968).

(2) G. Benaginano, M. Ermini, C. C. Chang, K. Sundaram, and F. A. Kincl, *Acta Endocrinol.*, **63**, 29 (1970).

(3) A. Scommegna, A. Theresita, M. Luna, R. Rao, and W. P. Dmowski, *Obstet. Gynecol.*, **43**, 769 (1974).

(4) G. Benaginao, M. Ermini, L. Carenza, and G. Rotfini, *Acta Endocrinol.*, **73**, 335 (1973).

(5) S. El-Mahgoub, *Am. J. Obstet. Gynecol.*, **123**, 133 (1975).

(6) C. G. Nilsson and T. Luukkainen, *Contraception*, **15**, 295 (1977).

(7) D. R. Mishell, D. E. Moore, S. Roy, P. F. Brenner, and M. A. Page, *Am. J. Obstet. Gynecol.*, **130**, 55 (1978).

(8) F. G. Burton, W. E. Skiens, N. R. Gordon, J. T. Veal, D. R. Kalkwarf, and G. W. Duncan, *Contraception*, **17**, 221 (1978).

(9) A. Rosado, J. J. Hicks, R. Aznar, and E. Mercado, *Contraception*, **9**, 39 (1974).

(10) J. Martinez-Manautou, *J. Steroid Biochem.*, **6**, 889 (1975).

(11) K. Hagenfeldt and B. M. Landgren, *J. Steroid Biochem.*, **6**, 895 (1975).

(12) P. F. Brenner, D. L. Cooper, and D. R. Mishell, *Am. J. Obstet. Gynecol.*, **121**, 704 (1975).

(13) Y. W. Chein and E. P. K. Lau, *J. Pharm. Sci.*, **65**, 488 (1976).

(14) S. Z. Song, J. R. Cardinal, S. H. Kim, and S. W. Kim, *J. Pharm. Sci.*, **70**, 216 (1981).

(15) T. M. Jackanicz, H. A. Nash, D. L. Wise, and J. B. Gregory, *Contraception*, **8**, 227 (1973).

(16) C. G. Nilsson, F. D. B. Johansson, T. M. Jackanicz, and T. Luukkainen, *Am. J. Obstet. Gynecol.*, **122**, 90 (1975).

(17) K. R. Sidman, W. D. Steber, and A. W. Burg, in "Drug Delivery Systems," H. L. Gabelnick, Ed., U.S. Department of Health, Education and Welfare, National Institutes of Health, Bethesda, Md., 1976, pp. 120–141.

(18) C. Pitt, D. Christensen, R. Jeffcoat, G. L. Kimnol, A. Schindler, M. E. Wall, and R. A. Zweidinger, in "Drug Delivery Systems," H. L. Gabelnick, Ed., U.S. Department of Health, Education and Welfare, National Institutes of Health, Bethesda, Md., 1976, pp. 141–193.

(19) H. Gabelnick, "Annual Report Summary," NICHD Contract Summary, U.S. Department of Health, Education and Welfare, National Institutes of Health, Bethesda, Md., 1977.

(20) J. M. Anderson, A. Hiltner, K. Schodt, and R. Woods, *J. Biomed. Mater. Res. Symp.*, **3**, 25 (1972).

(21) J. M. Anderson, D. F. Gibbons, R. L. Martin, A. Hiltner, and R. Woods, *J. Biomed. Mater. Res. Symp.*, **5**, 197 (1974).

(22) J. Feijen, D. Gregonis, C. Anderson, R. V. Petersen, and J. Anderson, *J. Pharm. Sci.*, **69**, 871 (1980).

(23) J. D. Back, A. M. Breckinridge, F. E. Crawford, M. L. Orme, P. H. Rowe, and E. Smith, *J. Pharmacol. Exp. Ther.*, **207**, 555 (1978).

ACKNOWLEDGMENTS

This study was supported by NICHD-Contract No. N01-HD-7-2823.

The authors gratefully acknowledge Dr. D. E. Gregonis, Dr. J. Feijen, Dr. S. W. Kim, and Dr. J. M. Anderson for providing the compounds used in this study and their helpful discussion in the preparation of this manuscript. The authors also acknoweldge the technical assistance of Mr. Steve Himebaugh.