ANALYTICAL STUDIES OF SOME AGENTS FOR FERTILITY REGULATION

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Ana Belenguer

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FEMALE FERTILITY REGULATION

PART B: FEMALE FERTILITY REGULATION

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CHAPTER 6

FEMALE REPRODUCTION

6.1 Introduction

By the fifth month of human pregnancy, the fetal ovary reaches a peak of about 7 million oogonia cells. Through meiotic division the oogonia cells are transformed into primary oocytes before birth. The human ovary, in the newborn contains about 2 million oocytes, of which approximately 300,000 will persist until puberty. Throughout the reproductive years a woman might ovulate about 400 oocytes.

In women, ovulation takes place every 28 days or so, from puberty until menopause. This cycle is called a menstrual cycle because there is visible bleeding (or menstruation) at the end of it.

The hypothalamus functions as an integration centre controlling reproduction; appropriate hormonal responses are directed via the anterior pituitary controlling the cyclicity of female reproduction. The hypothalamus controls the pituitary with gonadotrophin releasing hormones (Gn-RH), activating the release of the gonadotrophic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), to the target tissues, i.e. ovaries (Figure 6-1).

In common with other protein hormones, FSH and LH interact with their targets by binding to specific receptors present on cell surfaces.

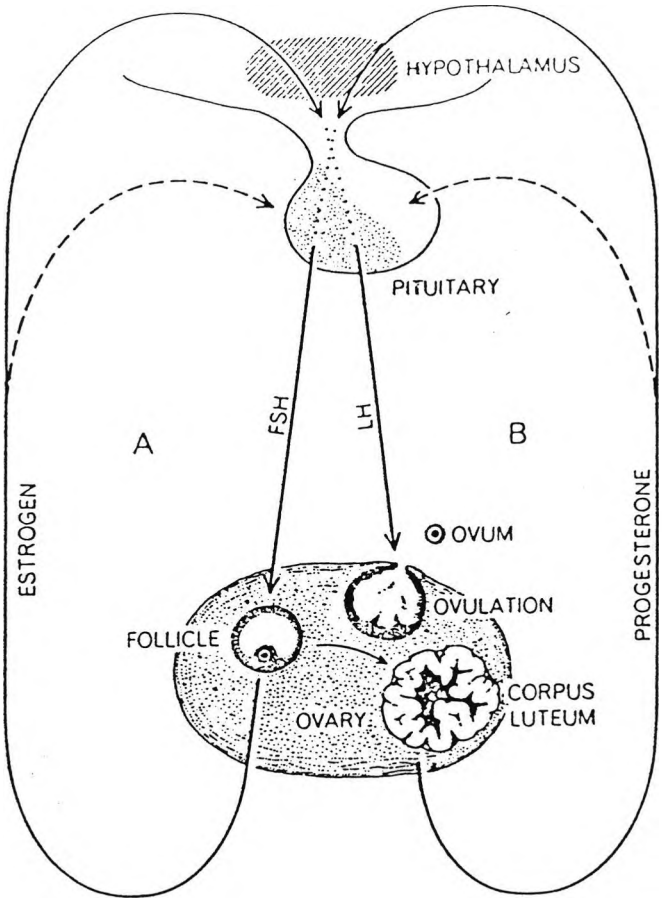


Figure 6-1: Interplay of female sex hormone

The early stages of follicle growth, which take place during the the first half of the cycle (follicular phase), involves stimulation by FSH; primary follicles do not contain LH receptors, but their granulosa cells possess FSH receptors. FSH is responsible for recruiting follicles from the ovarian pool and for stimulating their growth. Once the follicle has reached a "medium" size do receptors for LH develop within. At this stage the thecal layers have developed around the basement membrane of the follicle, where the LH receptors are located (Figure 6-2).

Thecal cells, furnished with blood supply, can synthesize steroid, and in response to LH they produce androgens. Maturation of the granulosa cells (which contain no blood supply), promoted by FSH, induces the aromatizing reaction, transforming the androgens generated by the thecal

cells to estradiol. A rising FSH level during the first half of the cycle stimulates follicular development, causing the ovaries to secrete an increasing volume of follicular fluid containing estrogen (primarily estradiol), in which the oocyte is bathed. Once menstruation is over, estrogen promotes the thickening and vascularization of the endometrium in the uterus and also changes the viscosity and spinnbarkeit of the cervical mucus to allow passage of the sperm at the time of ovulation (Figure 6-3).

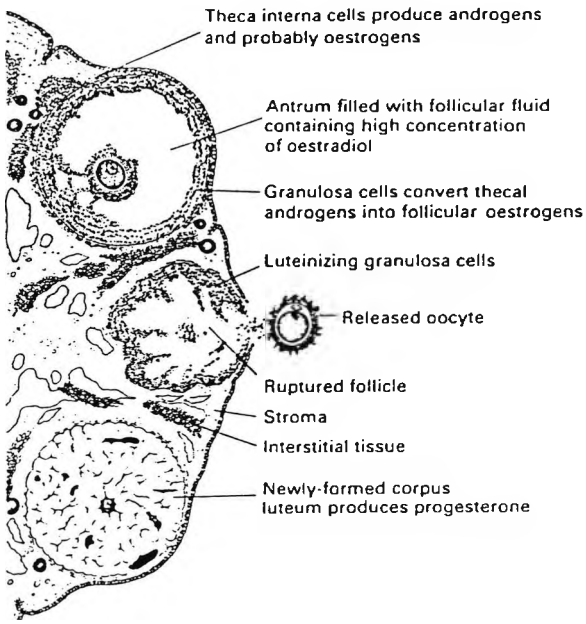


Figure 6-2: The ovary

Around the middle of the cycle, estradiol reaches it's secretion peak and it then acts by a negative feedback response on the hypothalamus, inhibiting the further release of FSH by the pituitary and stimulating the release of luteinizing hormone (LH) instead, which halts all further development of follicles. A surge in LH occurring around the 14th day of the cycle triggers ovulation: a mature egg is released from the developed follicle and the burst follicle is then converted into a corpus luteum. The corpus luteum now secretes the steroid progesterone, which further prepares the

endometrium for implantation. Progesterone is rapidly metabolized and its half life in the blood is only 4 minutes. Its metabolite, pregnanediol, causes an increase in basal metabolic rate and is responsible for the rise in basal body temperature that occurs in the second half of the ovulatory cycle. Ten to twenty per cent of ovarian progesterone appears in the urine as pregnanediol.

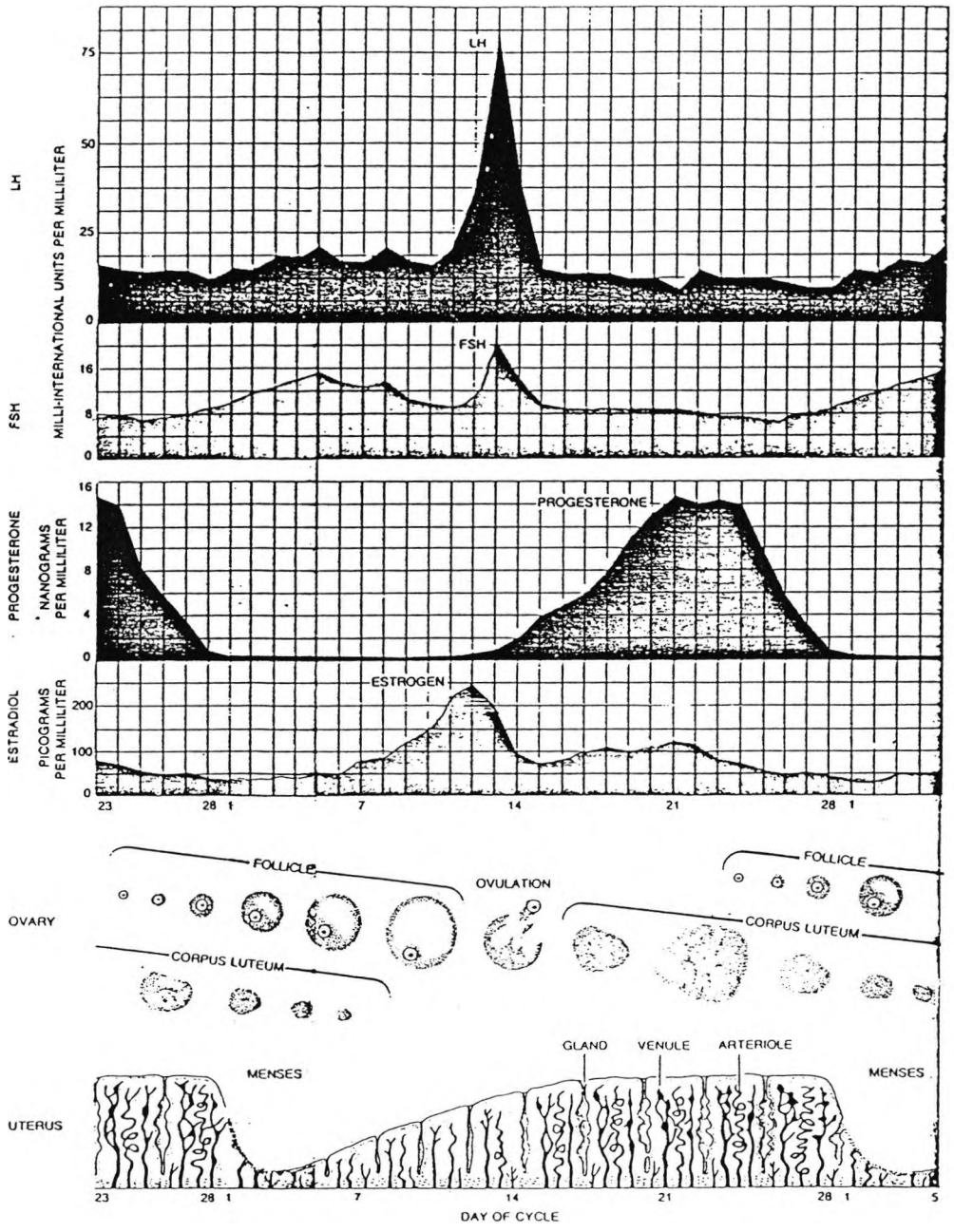


Figure 6-3: Ovulatory and menstrual cycle

After ovulation the oocyte is picked up by the fimbria of the fallopian tube. The lifetime of the oocyte is approximately 24 hours. During oocyte migration through the fallopian tube, fertilization of the oocyte by spermatozoa can occur generating a blastocyst; otherwise there is no implantation. The secretion of progesterone by the corpus luteum after ovulation also alters the fern type distribution in the cervical mucus thus preventing the sperm from crossing the cervix.

During the following 2 to 3 days, the blastocyst travels down the fallopian tube into the uterine cavity where implantation takes place in the endometrium (Figure 6-4).

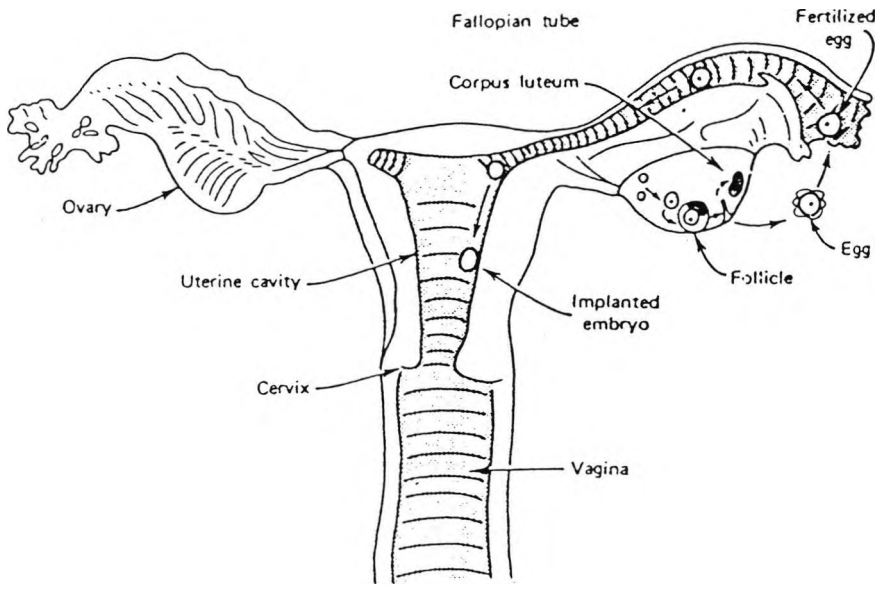


Figure 6-4: Female reproductive tract

On reaching the hypothalamus during the second half of the cycle, progesterone inhibits further pituitary release of LH, thereby completing the cycle. In the absence of fertilization, however, progesterone production falls and the endometrium is sloughed off with menstruation (Figure 6-3).

6.2 Steroid hormones in female contraception

The development of steroidal contraception is based on the observation that the secretion of progesterone inhibits the development of follicles and hence ovulation by a negative feedback effect on the hypothalamus, this in turn inhibiting pituitary secretion of the gonadotrophins FSH and LH.

Contraception in the female can be achieved by administering doses of steroids high enough to interfere with ovulation, as is achieved with oral contraceptives ("the Pill"). While this method can avoid pregnancy very efficiently it presents side effects: the use of progestogens only, produces irregular bleeding profiles; the combined use of progestogens and estrogens improves considerably the bleeding profile, but an increasing risk of heart disease has been connected with the use of estrogen. Another approach is the use of steroids in a dose not high enough to interfere with ovulation but just enough to have a local action on the viscosity of the cervical mucus, making it impenetrable to spermatozoa. This method is the basis of the minipill; although it is not as efficient as the former, it shows much fewer side effects.

Artificial steroids can be taken orally, inserted subcutaneously, given parenterally, absorbed through the vagina or even administered as an aerosol into the nasal cavity. When given orally, they must pass through the liver before reaching the general circulation, which both exposes the liver to high levels of steroid and exposes the steroid to metabolic changes in the liver.

Vaginal devices releasing constant amounts of steroid appear to represent an attractive approach to contraception, since they are self-administered, long-acting, their presence in the vagina seems to cause little, if any, inconvenience, and the exposure to the contraceptive steroid can be rapidly discontinued at any time by easy self-removal. In addition, vaginal administration of steroid results in an excellent absorption and avoids the "first passage effect" through the liver.

This project has been funded by the World Health Organization to investigate the vaginal rings needed to release progestogens at nearly zero order rate, continuously for 90 days. The rings are intended to be used for clinical trials in women. The progestogens investigated are: progesterone, specially indicated as contraceptive for lactating women and levonorgestrel, a widely used synthetic contraceptive. Progesterone, a natural progestogen, was chosen because it avoids the harmful effects connected with synthetic steroids in the infant's milk. A 5 mg/day dose has been proven to have antifertility action. The levonorgestrel rings were programmed to release steroid to the vaginal mucosa at the rate of about 20 µg per day, resembling the action of the minipill.

CHAPTER 7

CONTRACEPTIVE VAGINAL RINGS: LITERATURE SURVEY

7.1 Introduction: General background

It has been long known that many drugs, poisons and dyes are efficiently absorbed through the vaginal mucosa.[1,2] Several investigators have demonstrated that penicillin and other antibiotics placed in the vagina are absorbed into the blood stream so well that therapeutic blood levels can be achieved.[3] Greenblatt[4] in 1954 and Southam[5] in 1958 published studies about the effectiveness of vaginal absorption of progesterone suppositories.

Hartman, in 1959, proved that the transfer of substances from the vagina into the blood stream was mainly a unidirectional type of transmission with no such transmission in the reverse direction.[6]

In the treatment of an illness with drugs, it is usually desirable to maintain a drug concentration in the target tissue, which is both constant and within the therapeutically effective dose range. For a long time researchers have sought to find an ideal drug delivery system capable of minimizing the "sawtooth" fluctuations of drug levels that accompany periodic dosing.

Initially this approach concentrated in dispersing the drugs in a physiological fluid-insoluble carrier, such as beeswax. However, this drug delivery system achieved only limited success since the rate of drug release from such preparations was frequently unpredictable and unreproducible and, in most cases, still so rapid that frequent administration was found necessary. Furthermore, the carrier agents were sometimes unstable due to environmental effects,

or produced undesirable side effects, such as foreign-body reaction at the site of administration (induced by impurity or a degradation product).

A dense tablet with a controlled portion of its surface exposed to the dissolution action of the digestive tract fluids and the remainder of the surface covered with a protective, insoluble coating material proved to be an improvement in the long lasting pharmaceutical preparation, though the absorption of the drug could not always be controlled or predicted.

A different approach for long-acting steroidal preparations has been obtained by appropriate chemical manipulation of the steroid molecule. Ester formation is an example; a long chain fatty acid ester, injected subcutaneously or intramuscularly, can provide an effect lasting several months, presumably due to slow diffusion of the steroid from the oil phase into body fluids and, possibly, due to the rate of hydrolysis. It is doubtful, however, if such preparations give a truly constant release.

Silicone rubber has been found to be useful in chronic implants because it does not cause foreign body reaction, even after prolonged periods.[7] A pioneer step in the development of a long-acting drug delivery system was made by Folkman and Long[8,9] in the early 1960's. They showed that certain dyes and some other materials would pass through the walls of silicone rubber capsules. This observation was later confirmed by an experiment showing that a lipid-soluble dye, such as Sudan IV, would easily diffuse through a silicone membrane, while a water soluble dye, like methylene blue, would not. This suggested that perhaps steroids would also pass through silicone rubber.[7] Folkman and Long[8,9] carried

out extensive studies on the feasibility of using silicone polymer capsules as carriers for prolonged, continuous, subcutaneous administration of cardioregulatory drugs. These investigations demonstrated the concept of controlled drug delivery by confining a depot of pharmacologically active agent within a biocompatible polymeric capsule; only a microdose of the agent diffused through the capsule wall at a controlled release rate, thus achieving a therapeutic dose level for a prolonged period of time.

Dzuik and Cook[7] realised the potential of capsules of silicone rubber for providing a means of chronic administration of constant amounts of steroid for long periods of time with definite onset and cutoff points. They reported in 1965 that steroids will diffuse through silicone rubber and be released into the surrounding medium at fairly constant rates. Dzuik and Cook also succeeded in reducing significantly the incidence of heats during the breeding season in ewes which had a silastic tubing containing a steroid implanted subcutaneously. There was also evidence of some synchrony of heat after removal of the implants. In 1968 Chang and Kincl[10] compared steroid administration using subcutaneous injection, gavage and a silastic polymer implant. The implant provided the most efficacious way to administer the steroid: between 6 to 25 times less steroid was needed to produce comparable biological effects.

In 1970, Mishell and coworkers[11] described the first study in women on vaginally inserted rings of medical-grade silastic polymer (polydimethylsiloxane) containing a synthetic progestin. Sufficiently high plasma levels of the progestin were attained to inhibit ovulation and thereby achieve a contraceptive effect similar to the Pill. The rings

were generally left in situ for 3 weeks and removed for 1 week to induce breakthrough bleeding. This approach was followed by many others,[11-24] experimenting with different types of delivery systems which released various contraceptive steroids in high, ovulation-inhibiting doses.

Vaginal rings administration has some advantages over oral dosing. These advantages included patient compliance, self-insertion, more constant blood levels and possible avoidance of hepatic "first-pass" elimination, which in turn would lead to a lower steroid dose. The possible disadvantages of vaginal rings when compared to oral contraceptives are the degree of acceptability of a method requiring the long term placement of a foreign body within the vagina, possible discomfort, local irritation, increased risk of infection, and possible interference with intercourse. Vaginal rings have been extensively reviewed.[25-31]

In a study of rings releasing MPA in 1970, the absorption of progestin by the vaginal mucosa caused a sustained rise in the basal body temperature (BBT) throughout the 3 weeks of the treatment cycle, and exerted a progestional effect on the endometrium and the vaginal mucosa. The inhibition of ovulation was generally detected by the suppression of the midcycle surge of luteinizing hormone and the absence of elevation of pregnanediol in the urine during the luteal phase.[11] A high incidence of breakthrough bleeding (BTB) and breakthrough spotting (BTS) was also encountered during the clinical trial, which could affect the acceptability as a contraceptive method in some communities.[32]

A second approach was developed to improve the bleeding profile while still inhibiting ovulation; a combination of progestogen and estrogen was used.[33-45] Though this approach greatly improved the bleeding profile, it introduced the serious adverse side effects implicated with the use of estrogens.

A third approach has been followed since 1972 by the WHO Special Programme of Research in Human Reproduction; these studies aim at developing delivery systems that release progestogens at a constant (zero order) rate in low quantities sufficient for contraception, without invariably inhibiting ovulation. These systems can be left in the vagina for prolonged periods of time (3-6 months or longer) and it is not necessary to remove them in order to introduce a menstrual-like bleeding. Segal[46] investigated such an approach in 1966 on rats. He implanted silicone tubing containing progestin subcutaneously into rats. He achieved a daily absorption of progestin which was sufficient to prevent estrus in the rat, without interfering with ovulation. This method resembles the action of the mini pill.[47-55]

The vaginal rings described in the literature were 50-58 mm in outer diameter and had a thickness of 7-10 mm. Dependent on the construction of the ring and the type of progestogen, the release varied between 20 and 2000 µg/day.

7.2 Ring design

Preliminary studies on Silastic polymer devices containing steroids were carried out by Dzuik and Cook.[7] They performed "in vitro" studies into the rate of passage of steroids through various different silicone rubber devices. For example, tubes (sealed at both ends), packets, and disks. An isotonic saline solution at 37°C was used as the dissolution medium. They observed that when thin-walled packets and disks containing steroid were incubated, a visible accumulation appeared on the flasks, which was not seen with tubes. They also observed that once the saline solution became saturated with steroid, passage of steroid stopped from tubes. This was not the case for discs or thin walled packets. Throughout the experiments the daily passage of steroids through the silicone rubber device was seen to be reasonably reproducible. No detectable relationship could be found between the rate of release and the amount of steroid contained in the device. The rate of passage was not reduced significantly in successive incubations. No liquid was ever observed inside any of the silicone rubber devices.[7]

The data obtained from the steroid release tests with rubber tubes of different lengths suggested a direct relationship between the outer wall surface area and the rate of release. Thin wall silicone tubes released much more steroid than thick wall tubing. This implied that the rate of passage not only depends on the surface area of the tubing, but also has an inverse relationship to the thickness of the rubber tube.[7] This relationship was later confirmed in 1968 by Kincl et al:[56] maintaining the membrane thickness of silicone tubing containing steroid,

the release of the steroid was directly proportional to the surface area i.e. length of the tubing, whereas when the surface area of the tubing was maintained, the release of steroid was inversely proportional to the membrane thickness. No change in the release was found if the implants were autoclaved or if the steroid was put into the tubes as dry crystals or as an ethanol suspension.[7]

Kincl et al[56] also observed that when a small amount of dissolution medium was used, the solubility of the steroid in the medium became the rate limiting factor. When saline was used instead of water, the diffusion of steroid was decreased by about 15%.

In 1969 Pharriss and Hendrix[57] reported that the "in vitro" release from vaginal rings containing an homogeneous admixture of steroid under sink conditions in water showed an initial rapid rate, which decreased until it reached an asymptote after 2 to 3 weeks. They suggested that the initial rapid release probably reflected the presence of surface steroid and the more steady release occurs only after the outer silastic is depleted of steroid and a finite distance is established for the diffusion of the steroid. They also found that the release from rings containing different concentration of steroid led to concentration-dependant release rates, though no linear relationship was found between drug concentration and release at these concentrations.

Homogeneous rings inserted in rabbits proved to be as effective as subdermal silastic implant at 3 times lower dose as assessed by the lack of endometrial proliferation.[57]

In the mid 1970's Chien, Lambert et al[58-62] published a series of papers on mathematical models of the release of

steroids from polymeric delivery devices, including vaginal rings. When studying homogeneous rings they found that the cumulative release rate(Q) was linearly dependent on the partition coefficient(K) of the drug from polymer towards solution;[61-63] therefore when the solubility in the elution medium was low, the release process was partition-controlled and an accumulated release-time Q-t (zero order) relationship was observed (equation 7-1). A good correlation between "in vivo" and "in vitro" data was found.[58]

$$Q = \frac{C_p K D_s D_p}{K D_s h_p + D_p h_d} t \qquad \text{Equation 7-1}$$

Where C_p = Solubility of drug in polymer
K = partition coefficient of drug between polymeric device and the surrounding tissue fluid
D_s = diffusion coefficient of drug in the surrounding tissue fluid
D_p = diffusion coefficient of drug in polymer
h_p = thickness of the polymeric membrane
h_d = thickness of the hydrodynamic diffusion layer
Q = cumulative amount of drug released
t = release time

When maximum net release rate was maintained by working under sink conditions, the drug release pattern followed a $Q\text{-}t^{1/2}$ relationship and the process was "matrix controlled", (i.e. by the rate of steroid diffusion through the outer sheath of silicone rubber).[61,62] Sink conditions (in which the drug concentration is maintained at a level less than 10% of drug solubility in the elution medium) can be achieved by either using a sufficiently large volume of an aqueous solvent or by enhancing the low aqueous solubility of the steroid several hundredfold with the addition of water miscible co-solvent. Under this matrix-controlled process, the drug release profiles were independent of the variation in partition coefficient magnitude and insensitive to the change in solvent

solubility parameters (Equation 7-2).[61] However, it is interesting to note that both types of drug release profiles (Equation 7-1 and Equation 7-2) are determined by the magnitude of C_p (polymer solubility of the drug) and D_p (diffussion coefficient of the drug in polymer phase).

$$Q = [(2A - C_p)C_pD_p]^{1/2} t^{1/2} \qquad \text{Equation 7-2}$$

Where A = The initial amount of drug incorporated in a unit volume of polymeric device
C_p = The solubility of drug in the polymer
D_p = diffusion coefficient of drug in polymer
Q = cumulative amount of drug released
t = release time

The first type of vaginal ring used in women consisted of silastic polymer uniformly mixed with a progestin and moulded into a doughnut shape with an outside diameter of 70-80 mm. and a thickness of 10 mm.[11] Later, an attempt was made to design a ring capable of providing tension to avoid slippage or expulsion. This was a ring measuring 75 mm outside diameter with a 7 mm thickness, moulded around a stiff flat metal spring core.[12] This device caused a high incidence of erosion of the vaginal wall and was abandoned. A comparison of rings with either thicker (9 mm) or thinner (7 mm) outside diameters, showed that the thinner rings caused more ulceration and also slipped more frequently.[13] The thicker rings exerted their outwards force against a larger area of mucosa and the force per unit area was thus reduced in comparison to the thinner rings. It was thought likely that the incidence of ulceration with small thick rings would be minimal. Consequently there has been a convergence on the use of unsprung polymer rings with dimensions of 50-60 mm O.D. and c.a. 9 mm thickness. These rings seem to be well tolerated, rarely expelled and cause no discomfort or irritation in most women.[14-16,47]

The initial studies of vaginal rings were carried out on a ring design containing an homogeneous steroid dispersion (Figure 7-2a).[11,13-15,21,22,24] Release rate data of these rings indicates that there is generally a very high initial release rate which declines exponentially to a low, final value over a long period of time (Figure 7-1). Near to the steady level the release rate is roughly proportional to the surface area of the ring and the content of the steroid. The initial burst effect was strongly correlated with the irregular bleeding profile.

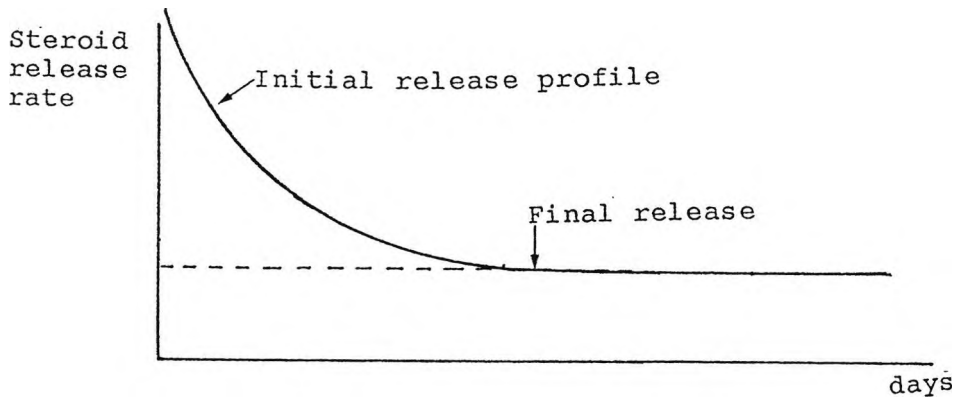


Figure 7-1

In order to try to achieve a flatter release rate profile three alternative types of ring design have been investigated: shell, core, and collagen band.

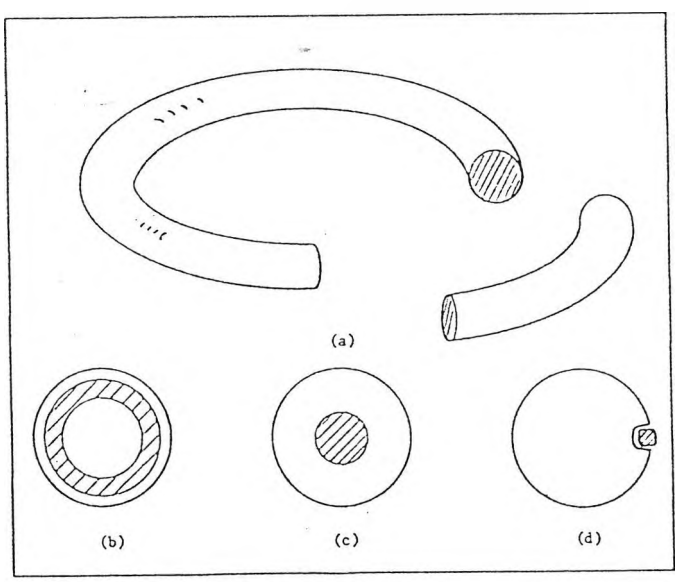


Figure 7-2: Vaginal ring designs

The shell type of vaginal ring (Figure 7-2b) was initially developed by Mishell et al[47] in 1973 and was later extensively used in clinical trials by other investigators.[16,17,33-35,37-39,42-44,48,49,55,64-66] These rings involve coating an inert inner core of polymer with a thin layer of polymer containing the dispersed steroid which is in turn coated with an outer inert layer. The intention with this design is to ensure a constant path length for the steroid diffusion to the outer surface during the whole lifetime of the device. In practice, this concept has been successful with certain steroids, releasing at a zero order rate.[33,47,49,67] The inert inner core of polymer serves to avoid the use of unnecessarily large amounts of the medicated admixture of silicone polymer and steroid which would not have any direct effect of the drug release.

An alternative to the shell idea was the development of the simpler "core design" (Figure 7-2c) which involves coating a steroid-containing polymer core with an outer sheath of polymer.[18,48,49] As with the shell design an outer sheath acts as a diffusion layer through which the steroid must pass, the release rate then being inversely related to the thickness of the outer layer.[18] A "burst" effect which was evident with shell designs is also present in core design rings with some steroids.[47,49] Since the steroid tends to saturate the outer layer during storage, the release rate only becomes diffusion controlled, from the core or shell, after the initial "burst" effect has caused depletion of the outer layer. However, it was observed with some progestins that once the progestin loss from the ring

approaches 40%, the release rate begins to fall off showing a clear depletion effect.[49] Core design vaginal rings have been extensively used in many clinical trials, releasing steroids at nearly zero order rates,[51-54,68-71]

Chien et al[59,60] and Rosenman and Higuchi[63] elaborated a mathematical model for core and shell design vaginal rings: under sink conditions a zero order relationship between accumulated release rate and time was found. The release rate was controlled by a membrane limiting process through a mathematical expression directly relating release rate with the solubility and the diffusion coefficient of the steroid in the polymeric membrane and inversely relating it with the thickness of the polymeric membrane (Equation 7-3).

$$\frac{dQ}{dt} = \frac{A\ C_p D_p}{h_p} \qquad \text{Equation 7-3}$$

Where A = Constant
C_p = Solubility of drug in polymer
D_p = diffusion coefficient of drug in polymer
h_p = diffusion distance of the polymeric membrane
dQ/dt = release rate of drug

The third approach involved the incorporation of steroid into a Dacron supported collagen band inserted into a groove in the outer edge of the silastic ring (Fig. 7-2c). This system would potentially use less steroid and would be easier to fabricate. This method was abandoned because the collagen bands frequently broke or were displaced and unpredictable blood levels of the steroid occurred.[72] Further, the release profile from this device proved to be no better than that of the shell or core design rings.

The development of devices releasing a progestogen and an estrogen simultaneously was started since it was found that irregular bleeding was severe using "progestogen-only" devices. These problems could not be solved with a combination of progestogen and estrogen in a collagen band ring concept.[72] The application of core or shell design for the release of the combination steroids possesses serious disadvantages. First of all it is not possible to adjust the ratio of the amount of each steroid released, because the same rate-controlling membrane is used for both steroids. A second disadvantage of the shell ring is that considerable steroid loss will occur due to migration of the drugs to the central core upon storage. For the shell rings the ratio of progestogen to estradiol release rate was found to be approximately 1.7 to 1 for levonorgestrel, 4.3 to 1 norethisterone and 9.5 to 1 megestrol acetate.[73] For 3-keto-desogestrel and ethynylestradiol the ratio was approximately 1 to 1.[45]

In 1986 de Leede et al[45] published the development study of a new vaginal ring system, the multicompartment vaginal ring (Figure 7-3), suitable for simultaneous zero-order release of combined progestogen/estrogen steroids. The multicompartment vaginal ring consists of two or more drug-containing silastic tubes of different lengths connected with specially shaped glass stoppers to obtain a ring of the required size. The glass stoppers prevent migration of the steroids from one compartment to the other and guarantee optimal release characteristics of both steroids even after long term storage. An additional advantage of glass is the good adherence to Silastic, enabling construction of systems with sufficient tensile

strength. The advantage of this ring design is that the release of both drugs can be programmed independently by changing the thickness of the outer membrane and the length of the steroid containing silastic tube. Since silastic medical grade tubes are only available in standard sizes, it was found sometimes necessary to incorporate a drug free compartment in the ring to obtain the most optimal outer diameter of 60 mm. Furthermore, the initial high release of estrogen from the core/shell combination vaginal rings, which would lead to bleeding irregularities due to rapid declining estrogen levels, could be prevented.

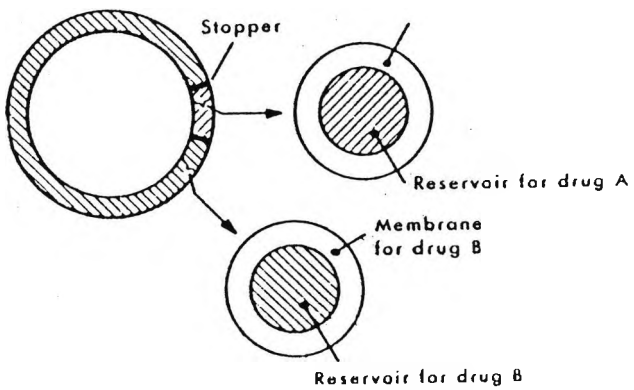
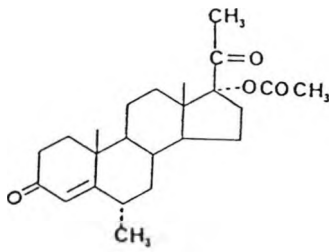


Figure 7-3 Multicompartment vaginal ring

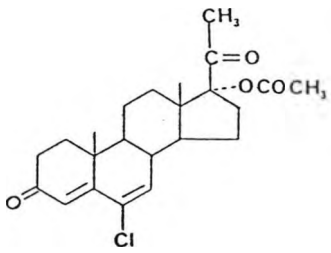
7.3 Steroids

The contraceptives progestogens used in vaginal rings are derived from the 21-carbon pregnane nucleus or are related to 19-carbon androgens termed 19-nortestosterone derivatives and their trivial names and systematic names are indicated in Table 7-1.

PREGNANE DERIVATIVES



MPA(1)



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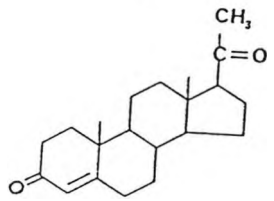
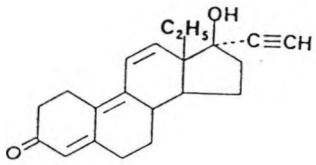


Progesterone(7)

19-NORTESTOSTERONE DERIVATIVES



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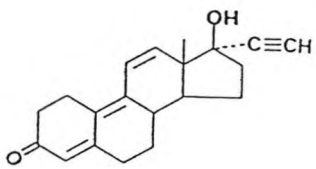

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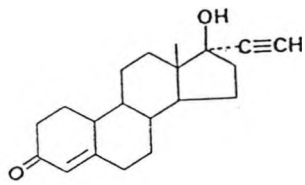
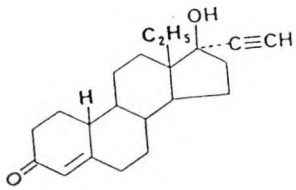
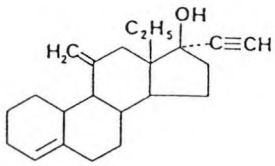


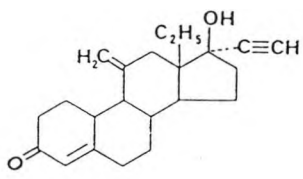
NET(5)



NOG(6)



Desogestrel(11)



3-Keto-desogestrel(12)

Trivial name	Abbreviation	Systematic name
Progesterone	P	4-Pregnen-3,20-dione.
Medroxyprogesterone	MPA	6α-methyl-17α-acetoxy-pregn-4-ene-3,20-dione.
Chlormadinone acetate	CMA	6-chloro-17α-acetoxy-pregna-4,6-diene-3,20-dione.
Norethisterone (Norethindrone)	NET	17α-ethynyl-17-hydroxy-estr-4-en-3-one.
Norgestrel	NOG	13β-ethyl-17α-ethynyl-17β-hydroxygon-4-en-3-one.
Norgestrienone	R-2010	17α-ethynyl-17-hydroxy-estra-4,9,11-trien-3-one.
Mestrinone	R-2323	13β-ethyl-17α-ethynyl-17β-hydroxygona-4,9,11-trien-3-one.
Desogestrel		13β-ethyl-17α-ethynyl-17β-hydroxy-11-methylene-gon-4-ene.
3-Keto-desogestrel		13β-ethyl-17α-ethynyl-17β-hydroxy-11-methylene-gon-4-ene--3-one.

Table 7-1: Trivial and systematic names of progestogens studied with vaginal rings

Dziuk and Cook[7] observed that the rate of passage of the more polar steroids (estradiol, cortisol, MGA and MPA) was less than the rate of the less polar steroids (progesterone, androstenedione and testosterone). The rate of passage appeared to them also to be affected by the presence of a side chain on the steroid nucleus.

Kincl et al[56] published a table of diffusion rates of different steroids from silicone tubes under controlled conditions, concluding that no correlation could be found between the diffusion of various steroids and their structure.

Initial studies of vaginal rings involved the release of medroxyprogesterone acetate (Provera)(MPA)(1).[11-13] Depo-medroxy progesterone acetate (DMPA), the intramuscular injectable form, has been extensively studied as a long acting

injectable contraceptive. Following discontinuation of the trimonthly DMPA injection, resumption of spontaneous ovulation occurred at prolonged and unpredictable intervals, which proved a serious drawback in this route of administration. No such problem was observed with vaginal ring users, where evidence of ovulation followed shortly after discontinuation of IVR use. It appeared that as little as 550 µg of MPA per day administered vaginally at constant rate is sufficient to inhibit ovulation. The systemic side effects were minor, such as breakthrough bleeding and spotting, which occurred in less than 10% of the cycles. Withdrawal bleeding occurred in most treated subjects within 3 days of withdrawal.[12]

In 1976 Vermeulen, Thiery et al[19,21] published the results of a small clinical trial with 100 mg and 200 mg MPA-silastic rings inserted vaginally and worn for 3 weeks; ovulation was suppressed in all women investigated, the method was acceptable and the device well tolerated. It was found that the inhibition of ovulation was achieved by suppressing the midcycle luteinizing hormone (LH) surge; except for the abolition of the midcycle gonadotrophin surge, the levels of LH and FSH were not affected appreciably.[11-13,19,21]

Victor and Johansson[22] published a comparative study of administration of MPA orally and vaginally using an homogeneously impregnated silastic ring. Peak plasma level declined from 1-5 ng/ml to 0.1-0.3 ng/ml within 12 hours after oral administration. During IVR treatment rather stable plasma levels between 0.4 and 0.6 ng/ml were reached: vaginal absorption of MPA was found to be very rapid with plasma levels already between 0.3 and 0.5 ng/ml 3 hours after insertion of the device.[22]

Chlormadinone acetate (CMA)(2) has been used as a low dose progestin oral contraceptive; it can achieve antifertility effects without inhibiting ovulation.[50]

The relative success of continuous oral administration of low dosage progestins, such as in the minipill, which does not consistently inhibit ovulation, led Mishell et al to adapt this concept to the vaginal route.[47] The rings were placed for 3 months undisturbed in the vagina. The hormonal effects of the absorbed progestin chlormadinone acetate were similar to those found during daily oral administration of low doses of CMA. Evidence of ovulation occurred during treatment and the development of a secretory endometrium was suppressed to some extent. A change in the cervical mucus was observed, which could be the reason for preventing conception, by interfering with sperm penetration.[12]

Subsequently, it was decided to utilize 19-nortestosterone derivatives in the rings because, in contrast to the C-21 acetoxy-gestagens such as MPA and CMA, administration of the former compounds was not associated with an increased incidence of mammary cancer in beagle dogs.[74]

Johannson[15] and Viinikka[75] reported studies of vaginal rings containing the progestin R-2323 (3). No ovulation was observed in the treated subjects and a strong suppression of ovarian function was produced by high doses of R-2323 progestin. R-2323 gave unsatisfactory and unpredictable bleeding patterns and hence it was not considered to be a suitable IVR steroid.[15,18]

Toivonen investigated the use of core-design vaginal rings containing 50 mg of R2010 (4). This is a synthetic progestin with structural similarities to norethisterone. The treatment was given in three week cycles, leaving one treatment-free week

between the cycles. The mean plasma concentration of R2010 produced by the rings was 0.9 ng/ml. Ovulation was observed in 25% of the cycles and the patterns of bleeding were unsatisfactory.[20]

The efficacies of orally active norethisterone (NET)(5) and l- or d,l-norgestrel (NOG)(6) led to various clinical trials administering the steroids vaginally.[14,16,17,49,65,72] Intravaginal release of norethisterone (5) was associated with a high incidence of bleeding while the device was in place and ovulation occurred in about one fourth of treatment cycles, proving unreliable for IVR use.[14] Another clinical study demonstrated that vaginal rings releasing low doses of NET at a rate of 50 µg/24h did not alter ovarian function, whereas those releasing 200 µg/24h exhibit a strong ovulation-inhibiting effect, but produced unacceptable bleeding profiles.[52,53] Norethisterone proved not to be a good gestagen to utilize in intravaginal rings; when bleeding did occur with the IVR in the vagina, most of the rings developed a very offensive odour.[24]

Vaginal rings containing dl-norgestrel(6) were associated with lower incidence of breakthrough bleeding and spotting (63 % of cycles) while signs of ovulation only occurred in 15% of the cycles. Rings were placed on the 1st or 5th day, and removed after 3 weeks for 1 week to allow withdrawal bleeding.[14,16]

D-Norgestrel or levonorgestrel is the active form of the synthetically manufactured racemic dl-norgestrel. The plasma levels of l-norgestrel reached (3-8 ng/ml) with vaginal rings impregnated with 50 mg of dl-norgestrel are higher than needed for contraceptive purpose.[23] Despite this, neither rings containing 50 mg or 100 mg of norgestrel were as effective as rings containing 100 mg of MPA in maintaining the

endometrium.[24] Serum l-NOG levels were highest 24 to 48 hours after the first 50 mg and 100 mg homogeneously impregnated IVR insertion and decreased gradually to some 30-40% of the initial peak level within the following 20 to 30 days, decreasing only slightly more during the following 2 months.[75] Plasma l-NOG was measured by radioimmunoassay.[75] A direct relationship was found between plasma l-NOG level and the degree of suppression in the ovarian function in women fitted with vaginal rings releasing 20 µg/24 hours at a nearly zero order rate.[69,70] A linear relationship was also found between the logarithm of l-NOG concentration in plasma and the duration of use, indicating an exponential character of decrease in l-NOG levels during the study year.[71]

In a clinical trial using IVRs containing l-norgestrel for 6 treatment cycles, ovulation occurred in only 3% of treatment cycles. Breakthrough bleeding and spotting occurred in 33 % of the cycles but on only 7% of the treatment days, whilst lack of withdrawal bleeding was observed in 5% of the cycles.[17] Odour was reported in 11.5% of the cycles. In most cases odour was associated with bleeding with the IVR in place. The odour may come from blood constituents being absorbed into the vaginal ring.[17,24]

To improve the bleeding patterns, Victor and Johannson carried out a new clinical trial with l-norgestrel containing vaginal rings, instructing the users to remove the IVR device from the vagina only after bleeding had started, thus transforming breakthrough bleeding into withdrawal bleeding. In this way the vaginal ring gave good protection against pregnancy, whilst causing acceptable bleeding patterns and requiring simple instruction to use.[65]

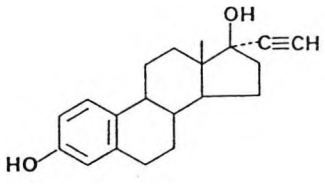
A clinical trial commissioned by WHO in 1978 found good correlation between daily release rates "in vitro" in an isotonic saline solution at 37°C under sink conditions and the estimated "in vivo" absorption of l-norgestrel, norethisterone or progesterone (7). These rings were designed to resemble the minipill, where enough l-norgestrel (21 μg/day), norethisterone (50 μg/day and 200 μg/day) or progesterone (1400 μg/day) was absorbed daily in the plasma to act as a contraceptive, but insufficient to inhibit ovulation (apart from the high dose releasing IVR norethisterone).[49,51] Sperm penetration of the cervix was inhibited by all four formulations, most consistently by the norgestrel-releasing devices. The rings were placed undisturbed in the vagina for 90 days. In vivo release rates were estimated after performing a post-use analysis of the residual progestin.[48]

A pharmacokinetic and pharmacodynamic study of WHO vaginal rings releasing 20 μg/day of l-NOG indicated that their mechanism of contraceptive effect could not depend solely on the suppression of ovulation (as there were only 50% anovulatory cycles during the treatment period) and as normal secretory endometria associated with normal ovulatory-like hormones profile was observed, it implied that the contraceptive effect may rely on the changes in cervical mucus. As menstrual disturbances mostly occurred in anovulatory cycles, this would suggest that the ideal device would be the one that changes the properties of cervical mucus, avoiding sperm penetration, without interfering with ovulation function.[54] In 1985 a similar clinical trial of IVR's releasing 25 μg/day of l-NOG concluded that the device causes too many bleeding irregularities at this dose level. However, the release profile of these rings were apparently not well characterised.[55]

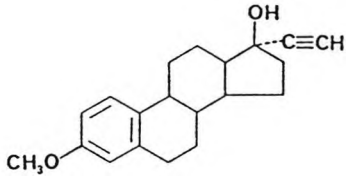
A progestogen only vaginal ring was associated with unacceptable bleeding profiles, therefore a combination of progestogen and estrogen, just like in the Pill, were investigated.

The use of oral combination steroid contraceptives is associated with increased risk of developing serious adverse side effects such as thromboembolism, stroke and myocardial infarction. Studies have shown that increased risk of these disorders is most significant in women ingesting a formulation of 50 µg or more of estrogen, therefore lower doses are considered to be more appropriate.

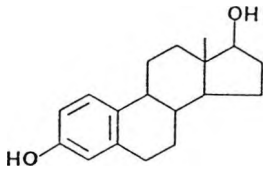
ESTROGENS



Ethynylestradiol(8)



Mestranol(9)



Estradiol(10)

All formulations with less than 50 ug of estrogen contain ethynylestradiol (8) which has been shown to be up to twice as potent as mestranol (9), the estrogenic component in most oral contraceptives containing 50 µg of estrogen.

New vaginal rings were formulated by adding estradiol (10) to l-norgestrel rings in the expectation of more efficient control of bleeding while suppressing ovulation. Breakthrough bleeding and spotting was significantly improved, occurring in only 7% of the cycles. There was no failure in withdrawal bleeding and no ovulation occurred in any of the cycles

studied. These rings released an average of 289 µg of l-norgestrel and 212 µg of estradiol daily, producing a constant serum l-norgestrel level of 1 - 3 ng/ml and an initial peak level of estradiol of about 100 pg/ml which rapidly declined. Presumably this initial surge of estradiol stimulates the endometrium sufficiently to provide improved bleeding control.[33]

A shell type vaginal ring with low dose d-NOG/estradiol (100-140 µg/day and 46-66 µg/day respectively) in continuous use for 6 months provided pituitary suppression without bleeding disturbances.[43] A smaller 50 mm ring produced plasma levonorgestrel concentrations too low to inhibit ovulation in some individuals.[44] Treatment with even lower dose NOG/estradiol vaginal rings gave very acceptable bleeding patterns while no ovulatory progesterone values were found.[42]

It is noteworthy that estradiol is not an orally active estrogen, owing to hepatic metabolism, but it is efficiently delivered into the circulation by the vaginal route.

A comparative clinical trial supported by WHO, the Population Council and the Ford Foundation started in October 1976 to determine the extent of serious adverse effects in contraceptive formulations containing estrogenic compounds administered via the oral and the vaginal routes. No statistical difference was found in the metabolic parameters due to the decreased estrogenic component of the oral contraceptive. However, administration of a gestogen-estrogen formulation by the vaginal rings eliminated the undesirable effect of hypertension and cardiovascular effects.[64]

In 1980 the Population Council commissioned an international multicentre clinical study to evaluate 2 different doses of l-norgestrel-estradiol vaginal rings on 1103

users. 533 women using oral contraceptives containing 150 µg d-norgestrel and 30 µg ethynyl estradiol were used as a control.[34,35,37-41] The vaginal rings were placed for 3 weeks and removed for 1 week to allow withdrawal bleeding. The rings were changed after 7 cycles (before depletion of the steroid could be noticed) for a new ring for a total period of 1 year. Amongst the vaginal ring users the pregnancy rate was less than 3%, approximately the same as the pregnancy rates observed amongst the control group taking oral contraceptives. Vaginal and menstrual problems were significantly more frequent amongst ring users. Headache, dizziness and nausea were reported significantly more frequently by the control group using oral contraceptives. The use of vaginal rings was not associated with a greater growth of pathogens in the vagina.[35]

De Leede et al[45] developed in 1986 a multicompartment vaginal ring system releasing at nearly zero rate 3-keto-desogestrel (12) and ethynylestradiol (8). 3-Keto-desogestrel is the active metabolite of desogestrel (11). Desogestrel has a unique and selective hormonal profile with strong progestogenic activity, low intrinsic androgenicity and no estrogenicity and it is successfully used orally in combination with ethynylestradiol. Due to its high solubility in silastic it can not be used in vaginal rings. Clinical trials are awaiting publication.

A relationship was found between bioavailability of levonorgestrel from intravaginal rings and undernutrition in women of low income groups: undernutrition decreases the half live of l-NOG which is compensated by the fact that undernourished women have little adipose tissue where l-NOG

deposits and hence a higher concentration of drug will be in circulation. As both effects compensate each other, inhibition of ovulation is observed in all subjects.[66]

The natural hormone progesterone (7) is not an oral progestin due to hepatic metabolism but it is efficiently delivered into plasma via vaginal rings. The administration of progesterone during lactation has been proposed as a convenient contraceptive for the postpartum period. Deleterious effects between lactation and infant growth and the administration of synthetic progestogens in lactating women has been reported; no such effect is connected with the use of the natural progestogen, progesterone.[70]

In 1978 Victor et al[68] studied progesterone-loaded vaginal rings with a 3.2 mm core diameter and 9 mm ring cross-sectional diameter. In an "in vitro" experiment (incubating in 1:750 benzalkonium chloride solution at 37 °C), they found that the rings released initially 3 mg/day which declined to 1.5 mg after 90 days incubation. Progesterone/estradiol combination rings of similar characteristics gave the same release for progesterone, and estradiol was released initially 180 µg/day declining to about 40 µg/day after 90 days incubation. There was a close correspondence between the in vivo release of progesterone and the" in vitro" measured release values, although "in vivo" release of estradiol was higher than "in vitro". Unfortunately, ovulation occurred in 60% of the treated cycles, indicating that higher release of progesterone was needed.

In 1978 WHO carried out a comparative clinical trial, coupled with an in "vitro" study of the release of three different loadings of progesterone vaginal rings;[49] 4000 µg/day, 1490 µg/day, and 1440 µg/day. These vaginal rings were

expected to release only enough steroid to function as a contraceptive device but not enough to interfere with ovulation. The rings were expected to be placed undisturbed in the vagina for 90 days. "In vivo" release rates were in general agreement with the release rates determined "in vitro". The measurement of steroids in vitro was done by GLC and UV.[48]

In 1985, Diaz et al[70] reported a contraceptive efficiency study of progesterone vaginal rings inserted at day 60 postpartum and replaced every 3 months with a new one during lactation. Two sizes of core type ring, supposed to deliver an average of 5 mg and 10 mg progesterone per day respectively, were tested. Initially, progesterone plasma levels were around 10 nmol/l and 15 nmol/l respectively, for rings of the two sizes, and declined slightly after 30 days; these levels were within the range shown to inhibit fertility in lactating women. No deleterious effects were detected in lactation and infant growth or maternal and infant health. "In vitro" measurements of the rings supposed to deliver 10 mg progesterone per day, gave initial values of 15 mg/day, which declined to 7 mg/day after 3 months; the rings supposed to release 5 mg progesterone per day had initial values of 7 mg/day, which declined to 4.5 mg/day after 3 months.

In 1988 Belsey[32] published a correlation between discontinuation of contraceptive use and vaginal bleeding pattern, emphasizing the urgency of developing vaginal rings which are safe and do not interfere with the menstrual period.

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CHAPTER 8

PROGESTERONE VAGINAL RINGS: RESULTS AND DISCUSSION

8.1 Introduction

This project, which started at the end of 1986, was supported by WHO to develop a vaginal ring required to release progesterone at a nearly zero order rate of 5 mg/day for a continuous period of 90 days. Maintaining an outside diameter of 55 mm and a cross-sectional thickness of 9.5 mm, rings were manufactured with different core diameters leading to different membrane thickness; it is well documented that the release rate is controlled by a membrane limiting process.[1,2] These core type rings were to be clinically compared with a ring produced for the Population Council, which was to release 10 mg/day. The rings were intended for use in lactating women who could not use synthetic progestins because of the problem of steroid transfer into breast milk.

The WHO rings were constructed by Dow Corning in Valbonne, France, from medical grade 382 silicone polymer. Each ring has a core designed to contain a homogeneous distribution of 25% w/w progesterone in the silicone polymer. The outside shell was constructed of the same type of silicone polymer but without the progesterone.

Chemistry of production of "Silastic 382"

"Silastic 382" is a medical grade elastomer supplied by Dow Corning. The correct proportion of end-capped dimethylpolysiloxane and silica are mixed together and after 16 hours at 175°C the base portion of the "Silastic 382" is formed. At the end of this period, the base is then cooled to room temperature and the correct proportions of

endcapped dimethylpolysiloxane and propyl orthosilicate are added, the latter serving as a crosslinking agent. This whole material forms a stable pre-polymer mixture. The addition of a catalyst, stannous octoate, initiates the crosslinking reaction and the silicone rubber, 382 Silastic, is formed within a few minutes at 80°C.

Outline of production of core type vaginal rings

The vaginal rings are toroidal-shaped, with an outside diameter of 55 mm and a cross-section of 9.5 mm. The core type vaginal ring is fabricated by moulding a drug-free diffusion layer of "Silastic 382" around a progesterone-loaded core of "Silastic 382". The devices were moulded using multiple steps, the moulding sequence being as follows.

1. Micronized progesterone was thoroughly mixed with the pre-polymer mixture to form the core mix. The catalyst was then added to the core mix and this mixture injected into a split cavity core mould, thermostatically controlled at 80°C. The core was allowed to cure for two minutes.
2. One-half of the core mould was removed and replaced by one having a cavity representing the outside dimensions of the device. Pre-polymer mixture (without progesterone) containing stannous octoate catalyst was injected into the thermostatically controlled cavity at 80°C and cured.
3. The remaining half of the smaller diameter mould was removed and replaced by the other half of the outside mould. Pre-polymer mixture (without progesterone) containing the catalyst was injected as explained above and cured to complete the device.
4. The devices were then sterilized with ethylene oxide and outgassed for 72 hours in a vacuum system before packing.

Objectives

Our objectives were:

1. To investigate the relationship between core diameter and release rate for a series of rings.
2. To determine the optimum core diameter necessary to produce a 5 mg/day release rate.
3. To carry out quality control of the first batches of rings to be used in clinical trials and thereby also help to define parameters and limits for a subsequent pharmacopoeia specification.

8.2 Solubility of Progesterone in Saline

As in previous WHO "in vitro" studies of steroid release rates from vaginal rings,[3] the new study used isotonic saline solution at 37°C as the bath fluid. The solubility of progesterone (USP) in a 0.9% w/v NaCl solution at 37°C was found to be 14.4 mg/l (UV comparison with a standard solution prepared by diluting a concentrated ethanolic solution with saline: final EtOH concentration 0.4% v/v).

In order to operate under "sink" conditions, which would avoid the release rate being affected by the external concentration of progesterone in the saline solution, it was decided to aim for a maximum solution concentration of about 10% of the saturation level. For a ring releasing 5 mg/day of progesterone there is a requirement for a volume of 4 litres per 24 hours. In principle it would be possible to carry out the experiment by immersing the rings in 4 plus litre vessels with a change of the immersion fluid every 24 hours. In practice however, this proved to present several problems, such as the difficulty of maintaining the high

number of 4 plus litre vessels at the correct temperature and of ensuring that the fluid used was adequately agitated, whether by shaking or by stirring. Consequently a flow system was designed, in which each ring was suspended in a small vessel containing the saline solution. The entire vessel was then immersed in a thermostatically controlled water bath, and a constant flow of pre-heated saline solution was passed through each vessel to give the required volume of fluid per 24 h. Using this approach it proved possible to correctly maintain up to 50 vessels in a single water bath, with the individual vessels contents being agitated by means of magnetic stirrers.

8.3 Design and operation of the flow feed saline baths

Distilled water was continuously prepared using an 8 KW water still with a capacity of 8 litres/hour. The distilled water was collected in a series of interconnected 54 litre glass tanks with a total capacity of about 350 litres. Distilled water was syphoned from this main reservoir into a 50 litre mixing tank. A predissolved concentrated Analar NaCl solution was then added to the mixing tank, the mixing tank brought up to exactly 50 litres, and the final mixture thoroughly mechanically stirred. The resultant isotonic saline solution was then pumped into a second set of interconnected holding tanks having a total capacity of 500 litres. These tanks were positioned at a level above that of the thermostatically heated water bath in order to provide a more easily controlled, positive gravity flow to the peristatic pump unit.

The vaginal rings were each suspended by 3 cotton threads in 250 ml glass Schott bottles. The bottles were fitted with screw caps with seals. Two 316 grade stainless steel tubes passed through each of the caps, with care taken to ensure that the caps were sealed around the tubes. The tubes served as inlet and outlet ports for the saline solution. The inlet pipe was positioned so that its end was below the bottom of the suspended ring while the outlet pipe was positioned to end above the ring, ensuring a large separation between the pipes ends. A 2.5 cm magnetic stirrer bar, teflon coated, was positioned in the bottom of each bottle and care taken to ensure that it was not fouled by any of the piping or the ring itself (Figure 8-1).

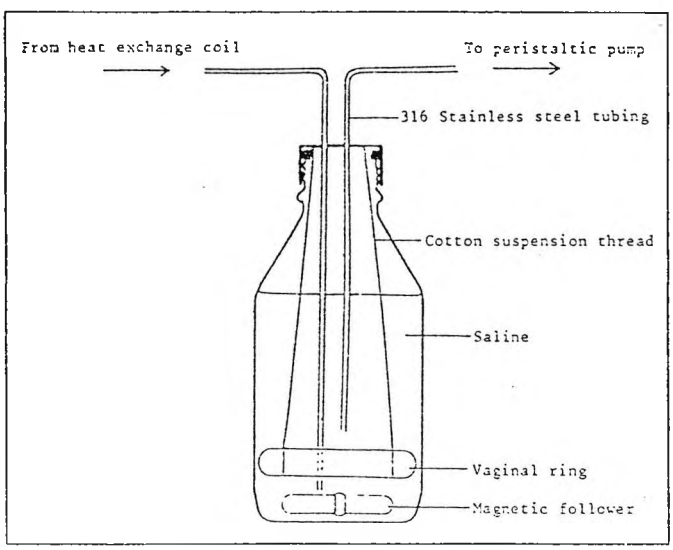


Fig. 8-1: Flow chamber for continuous elution of vaginal ring

The Schott bottles were immersed in a large thermostatic bath which was maintained at 37°C±0.1°C. The inlet tubes were connected to stainless steel heat exchange coils which were also immersed in the thermostatic bath. The heat exchange coils had a pre-coiled length of 1.75 m, and were made of 2 mm i.d. 316 stainless steel tubing. The heat exchange coils were connected to the saline reservoir via

long teflon tubes which were themselves connected through a Watson Marlow 50 channel peristatic pump model 502S. A single multichannel pump was used to ensure that all the heat exchange coils received the same feed rate. The outflow tubes from the Schott bottles were passed into 5 l glass receiver vessels. All the tubing involved, apart from the short 3 mm bore Tygon tubing used in the peristatic pump, had a bore of 2 mm and was either stainless steel or teflon (Figure 8-2).

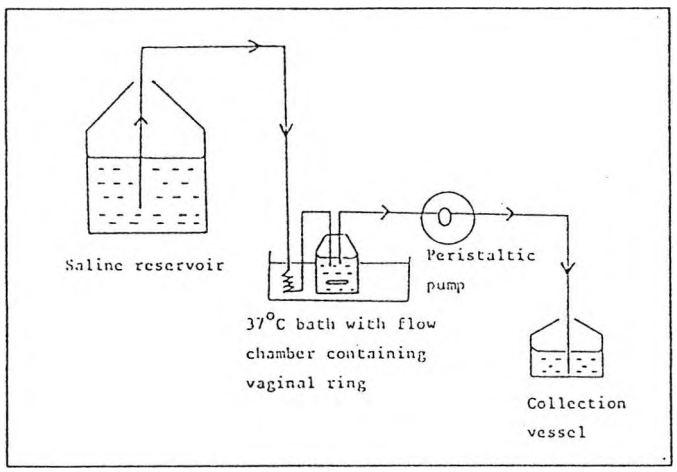


Figure 8-2: Schematic flow design

The peristatic pump was set to deliver approx. 4 litres/24 hour period. The volume of solution in each of the receiving vessels was measured daily and the quantity of progesterone was assayed as described later.

The individual Schott bottles were centred over magnetic stirrers. Initially a Variomag 15 position (3x5) electronic immersible variable speed magnetic stirrer was purchased for this purpose. The stirring positions that this provided were 7 cm apart, which meant that if 15 (3x5) 250 ml Schott bottles with 6.5 cm diameter bases were to be used, the bottles had to be very carefully positioned very close together. A plastic frame had to be produced to ensure

that the bottles were correctly positioned on the stirrer and that even stirring was obtained. An inexpensive alternative magnetic stirrer was built to meet the requirement for a wider separation between the bottles. The stirrer produced was powered by a central motor driving rubber belts which linked each of the stirring positions. Each unit provided two banks of six stirrers with a possibility of an additional stirrer position directly above the central motor if required. These units were capable of maintaining quite high stirring speeds during continuous operation over a period of several weeks with minimal maintenance. To avoid loss of stirring during maintenance, a spare unit was substituted for any requiring work. The replaced unit become the spare after servicing.

8.4 Analysis of progesterone concentration in saline

8.4.1 UV method

In order to process large numbers of samples on a daily basis, a fast and accurate analytical procedure had to be developed for determining the concentration of progesterone in the saline solutions produced. A Cecil 2112 variable wavelength spectrophotometer was fitted with a 75 µl 1 cm pathlength flowcell and the outlet tube from this was attached to a single channel Watson Marlow peristatic pump. The flowcell inlet was fitted with teflon tubing which was immersed directly in the solution to be determined. About 20-50 ml of each solution was pumped through the flowcell and the pump then switched off before a reading was taken.

The UV spectrophotometer was set to zero on saline at 250 nm (maximum absorbance of an aqueous progesterone sample). Calibration was then carried out in duplicate with

stock solutions freshly prepared daily by dilution of two independently prepared ethanolic progesterone standards in saline (final total EtOH content <0.5% v/v), and then the eluents of the vaginal rings were measured. Standards were re-run after every 5 or 6 determinations. The calibration graph was linear in the range under study, (0-6 mg/l) Figure 8-3.

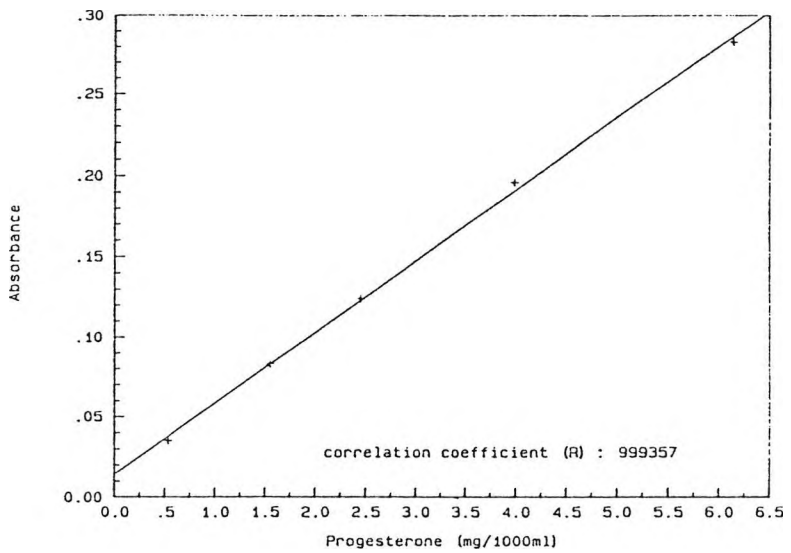


Fig. 8-3 Standard linearity calibration graph of the absorbance readings versus the progesterone concentration measured by an UV method at 250 nm.

8.4.2 HPLC method

To assess the reliability of the release data measured by the described UV method, a quantitative HPLC method was developed. It was thought possible that plasticizer components in the silicone rubber could be released along with the progesterone, and if these extra components have a UV absorbance at 240-250 nm they would lead to artificially high values for the apparent release rates. Similarly, any steroid impurities would contribute to the observed absorbance.

Direct 20 µl injections of the daily eluates of progesterone released from the vaginal ring on a 25 cm analytical HPLC ODS-Hypersil 5µm column, were too weak to be detected. Therefore an extraction procedure was devised to obtain a progesterone solution adequate for quantitative HPLC analysis. 200 ml aliquots of eluate were taken and exhaustively (4 times) extracted with CH_2Cl_2. The extracts were evaporated to dryness and redissolved in 10.0 ml of EtOH for HPLC analysis. Progesterone eluted at 4 minutes on the HPLC with 50:50 MeCN/H_2O eluent, monitored at 241 nm (λ_{max} of Progesterone dissolved in EtOH). 200 ml aliquots of standard solution containing known amounts of progesterone were similarly treated and analysed (Figure 8-4). Recovery of progesterone from the standards was found to be 101.3%.

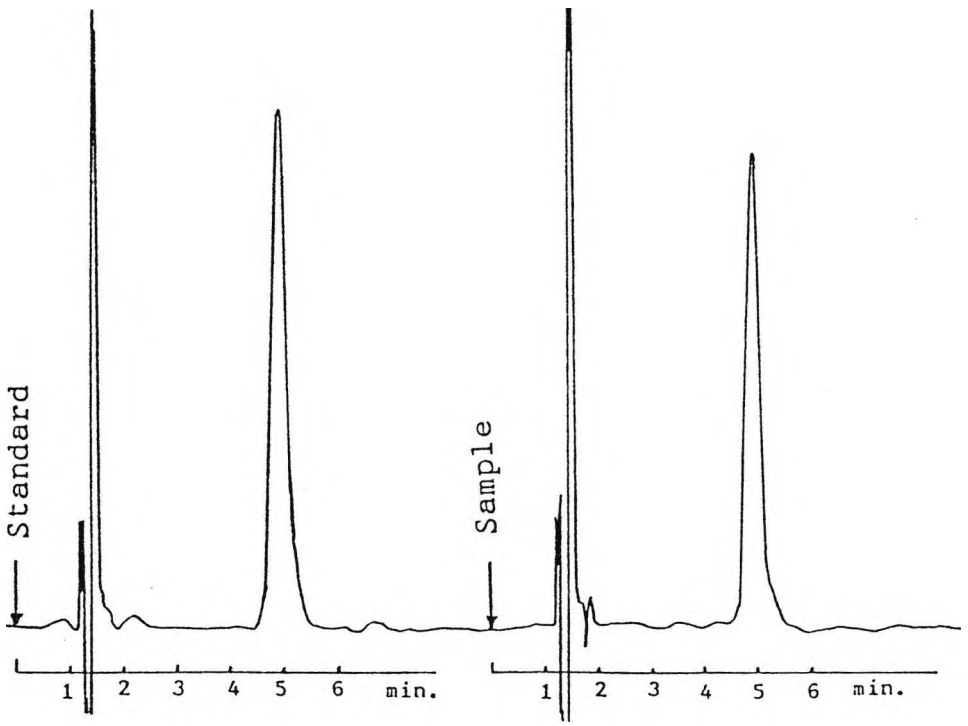


Fig. 8-4 HPLC chromatogram of standard and sample progesterone solution on a 25 x 0.45 cm Hypersil-ODS 5 µm column, eluted with 50:50 MeCN/H_2O at 2 ml/min, monitored at 241 nm. 200 ml saline aliquots of sample and standard progesterone solutions, were extracted into chloroform, and the residue following evaporation were dissolved in MeCN prior to injection.

8.4.3. Fast HPLC method

In order to speed up the evaluation, it was decided to examine the potential of a direct injection HPLC method which would avoid the need for time-consuming extractions. Since the concentration of progesterone in saline was rather low for HPLC detection, modifications to the earlier HPLC method were made. A short 5 cm column packed with 3 µm ODS-Hypersil was prepared that would ensure very fast analysis at high resolution whilst keeping the progesterone peak tall and narrow for a good signal/noise ratio. A shorter column also avoided the over dilution of the injected sample in an unsuitably larger solvent volume of a longer column. Additionally, the sample loop volume was increased first to 50 µl and later to as much as 200 µl to give a further 2.5 - 10 fold enhancement in peak size for quantitation. The analysis time of less than 2 minutes allowed sufficient repeat runs to obtain reliable averages (Figure 8-5). The samples were still detected at 241 nm and eluted with 50:50 v/v MeCN/H_2O at 2 ml/min.

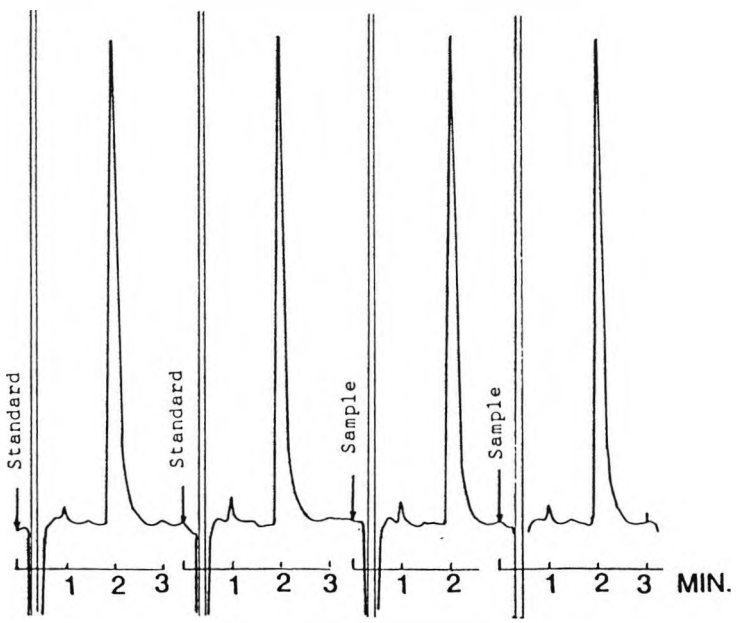


Fig. 8-5 Fast HPLC chromatogram of direct 200 µl injection sample and standard progesterone solutions on a 5 x 0.45 cm Hypersil-ODS 3 µm column, eluted with 50:50 MeCN/H_2O at 2 ml/min, monitored at 241 nm.

8.5 Release rate studies

8.5.1 Study 1.: Evaluation of Flowing Bath System

The flowing bath system was initially evaluated using preliminary batches of vaginal rings obtained by WHO from Mexico, with two different core sizes: 7.25 mm and 7.9 mm. These rings measured externally the same (55 mm O.D. and 9.5 mm thick) as the rings commissioned by the WHO for clinical trials.

In a preliminary study, 3 of each ring size were investigated. The individual release rates for these rings during the first few weeks are shown in Fig. 8-6 and the average value for each ring size over a longer period is plotted in Fig. 8-7. The 7.9 mm core rings released slightly more than the 7.5 mm core rings, however the differences in the average release rates are rather small, the rates being around 6 mg/day.

At day 35, the bath thermostat malfunctioned and the bath temperature rose to over 70°C for more than 24 h. A very large increase in the release rates occurred and after re-establishing normal bath temperature there was a sharp decline in release rates to below the previous values, and very erratic results. At day 50, the flow of saline to all the rings was suspended and the 7.9 mm rings were removed from the bath. However, the 7.25 mm rings were allowed to remain in the static saline solutions and after 3 days flow was resumed. The initial eluent reading was then very high for the first measurement, but on day 54 had declined and quickly re-established itself at preheated values, which then remained steady for a further month (Fig. 8-7).

These observations are of interest with regard to handling of the rings, in that they suggest that excessive

heat treatment of the rings (whether by storage or washing in hot water to sterilise) may cause prolonged erratic performance: on the other hand, steady performance eventually recovers after prolonged immersion in a static fluid at normal temperatures.

It was also observed during this pilot study, that the rate of stirring of the solution which surrounded the suspended vaginal ring was critical. A minimum of 100 rpm constant stirring speed was required to achieve a uniform rate of progesterone release into the flowing solution travelling via the outlet tubing into the collecting vessels. If this requisite was not met, or if the stirring was stopped for any significant period of time, a significant drop in progesterone release would be measured for that particular ring on the troublesome day. Despite restoring stirring conditions, the previous drop in progesterone release would be followed the next day by a proportional extra high steroid release which would finally be restored to the normal releasing level on the following day. This observation suggests that when, due to external circumstances, a ring releases less than that observed under sink conditions, a build up of steroid occurs in the outer diffusion polymer layer. Once the external circumstances have been returned to normal, this accumulated steroid in the outer layer is released into the medium within the next few hours.

To investigate the reliability of the results by the UV method and to assess the possible release of plasticizer components from the silicone rubber, HPLC analysis on the 6 rings for the first 3 days collections were carried out.

Analysis of the saline eluents from the 6 rings gave progesterone contents averaging 85% of the values which had been estimated by the direct UV method. It is assumed that this discrepancy is accounted for by the presence of UV-absorbing plasticizers and polymer components in the eluents: additional peaks were observed in the HPLC traces of the extracts from the ring solutions, compared with the extracts from the standards (Fig. 8-4), accounting for 5-10% of the discrepancy. The remainder of the discrepancy is attributed to components which elute under the solvent peak or remain on the column under the conditions employed for the HPLC analysis.

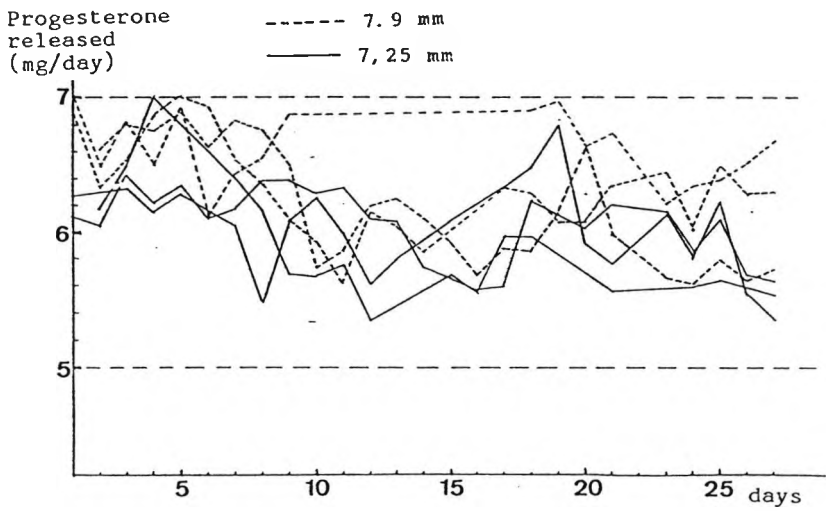


Fig. 8-6 Individual release rate of progesterone from 7,25 mm and 7.9 mm core Mexican vaginal rings.

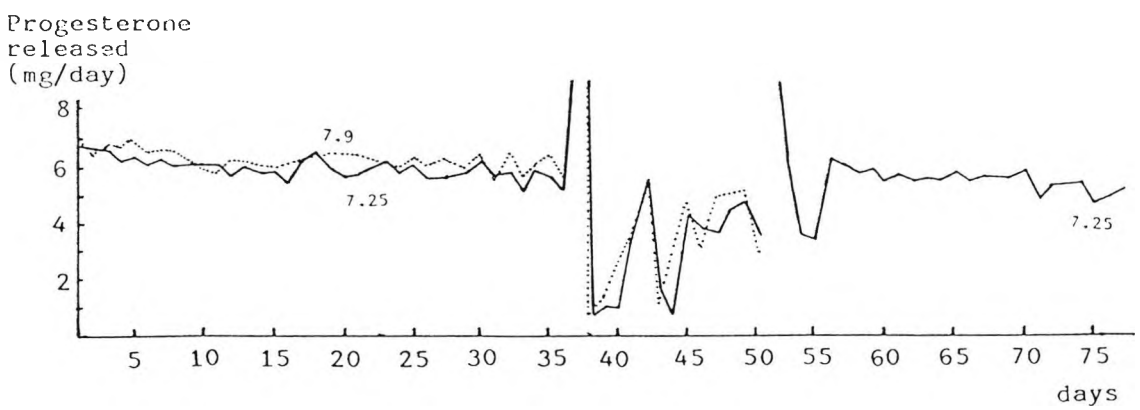


Fig. 8-7 Release rate averages of progesterone from 7,25 mm and 7.9 mm core Mexican vaginal rings.

8.5.2 STUDY 2: PROGESTERONE RELEASE RATE VERSUS VAGINAL RING CORE SIZE

Dow Corning rings with 4. 5 and 6 mm cores

Rings with 4, 5 and 6 mm cores from Valbonne were received and 5 of each size were studied. The results for all rings are shown in Figures 8-8, 8-9, and 8-10 (averages are shown in Fig. 8-13). The release rates were found to be relatively uniform for each size, compared with the Mexican rings: however, for the 4 and 5 mm sizes, there was one ring in each batch which released significantly less than the other four.

Again, it was considered desirable to assess the possible release of plasticizer components from the silicone rubber by a direct injection HPLC method using a short length 3μm ODS-hypersil column.

HPLC analysis of the saline eluents from the 4 mm and 5 mm Valbonne rings on day 70 gave progesterone contents which showed no statistically significant difference to the values which had been estimated by the direct UV method.

Fig. 8-8 Daily release rate of progesterone from 4 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

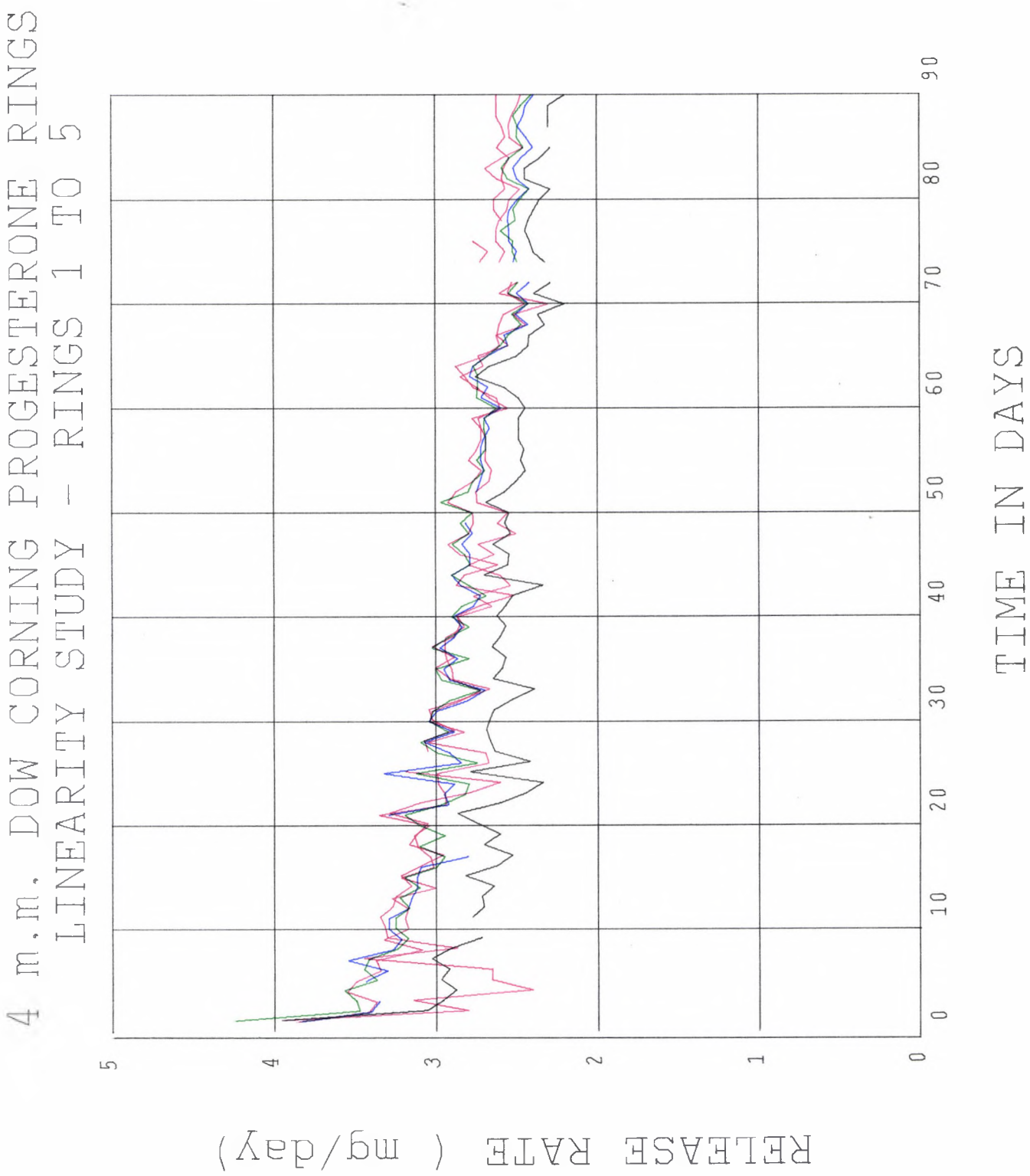


Fig. 8-9 Daily release rate of progesterone from 5 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

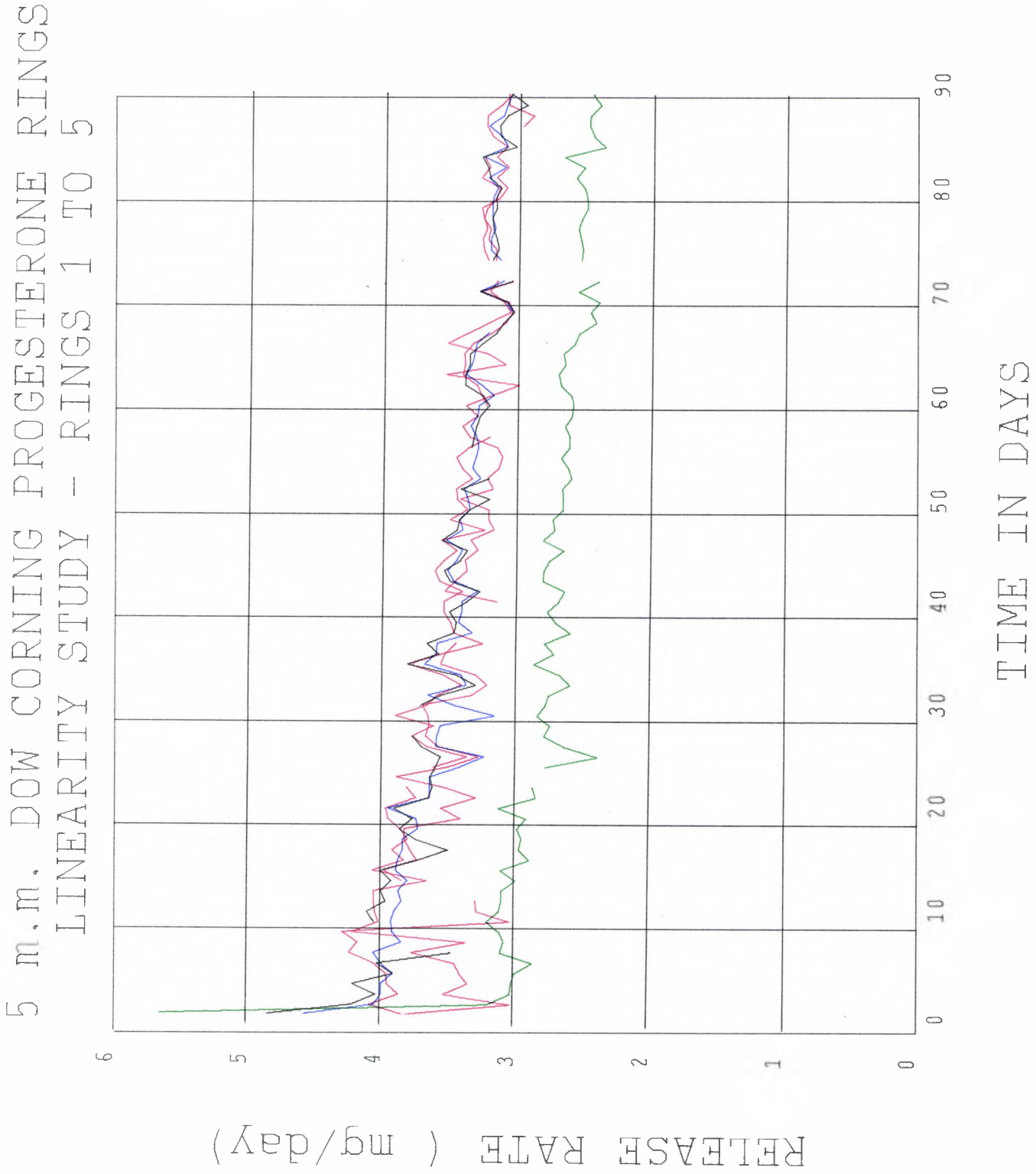
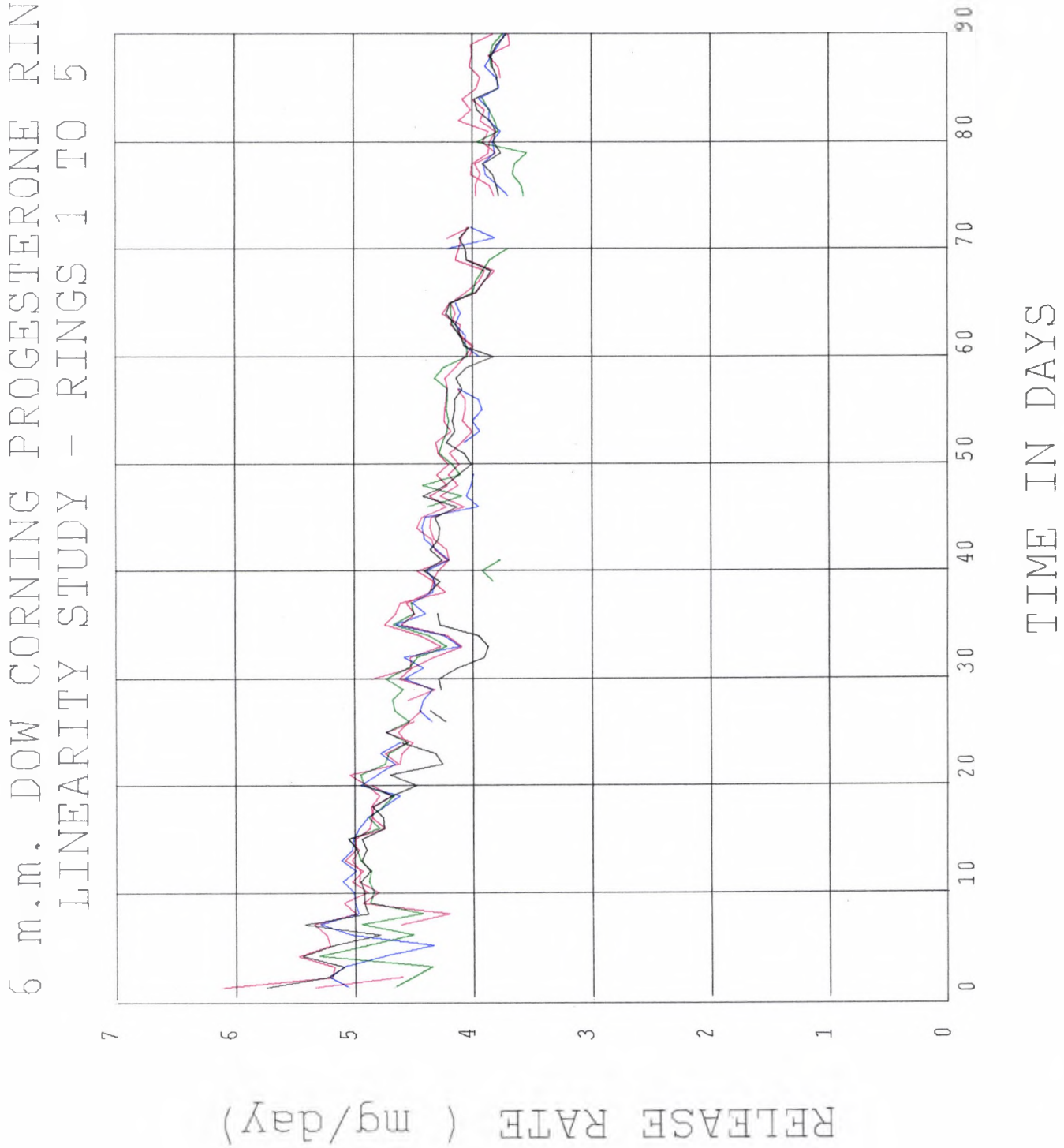


Fig. 8-10 Daily release rate of progesterone from 6 mm core diameter Dow Corning vaginal rings prepared for a linearity study.



Dow Corning rings with 6.7 and 7.25 mm cores

The first study of Valbonne rings suggested that the required size of ring core for a 5 mg/day release rate at the end of the 3rd. month would be about 7 mm. Further trial batches were therefore fabricated with 6.7 and 7.25 mm cores and release rates for 4 rings of each size were measured. Data for each individual ring is presented in Figures 8-11 and 8-12, the average release values are plotted in Fig. 8-13. The release rates are a little more erratic than those of the earlier Valbonne rings; moreover, one of the 6.7 mm is releasing at a high rate compared with the other 3, whereas one of the 7.25 mm size is releasing at a low value (the rings were packaged in pairs and we took care during setting up to avoid mixing rings: possibly there was an exchange of rings prior to packaging in the factory, however).

The absolute release rates of progesterone were again checked by the direct injection HPLC method. HPLC analysis of the saline eluents from all the 6.7 mm and 7.25 mm Valbonne rings on day 23 gave progesterone contents which were 97.6% $\pm$ 2.6% of the values which had been estimated by the direct UV method.

Overall, the Valbonne rings (unlike the Mexican rings) appear to release little or no UV-absorbing material (at 240-250 nm) other than progesterone itself. The close agreement between the UV data and the independent HPLC data gives the results obtained with these rings a high degree of reliability.

Fig. 8-11 Daily release rate of progesterone from 6.7 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

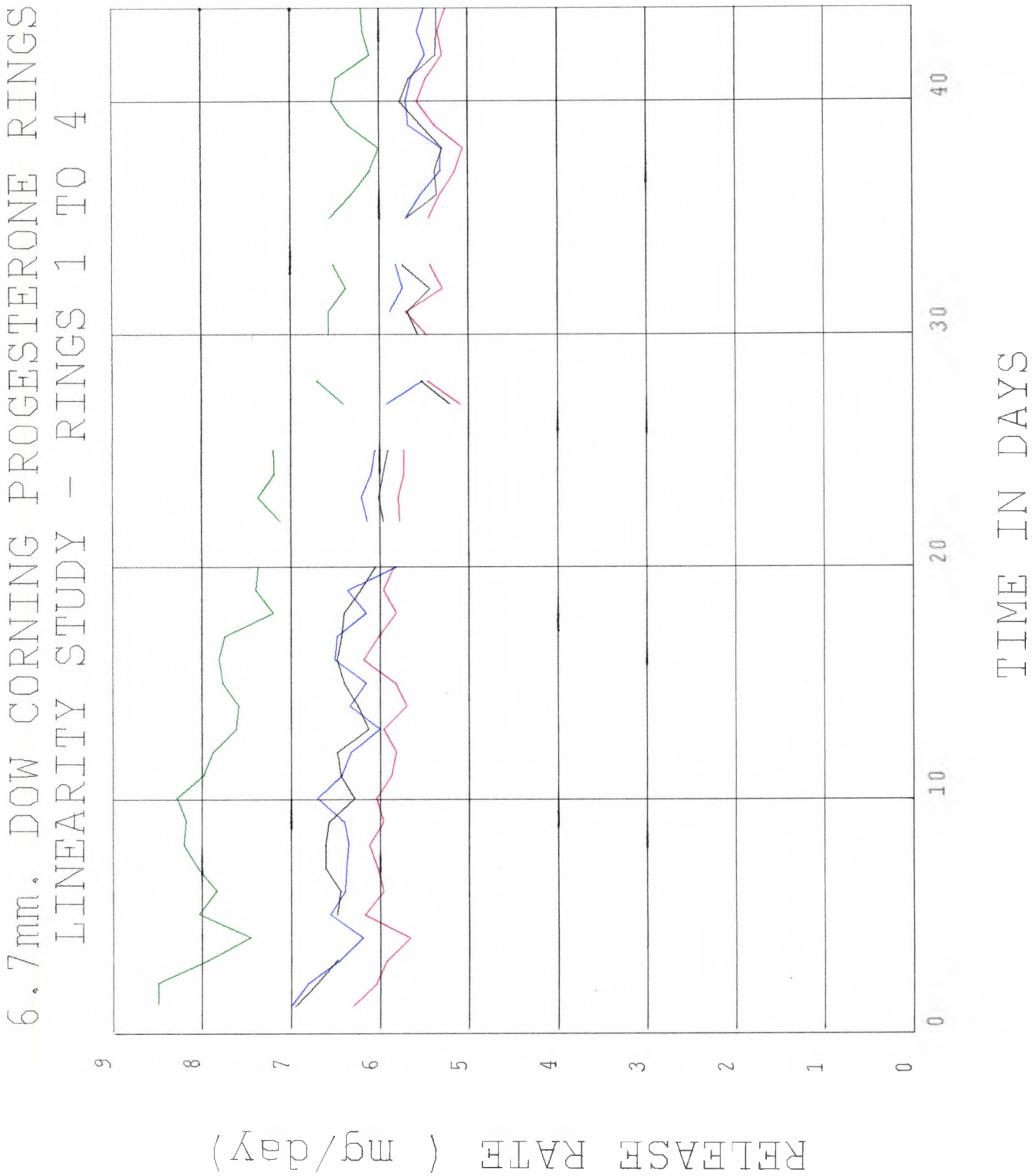


Fig. 8-12 Daily release rate of progesterone from 7.25 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

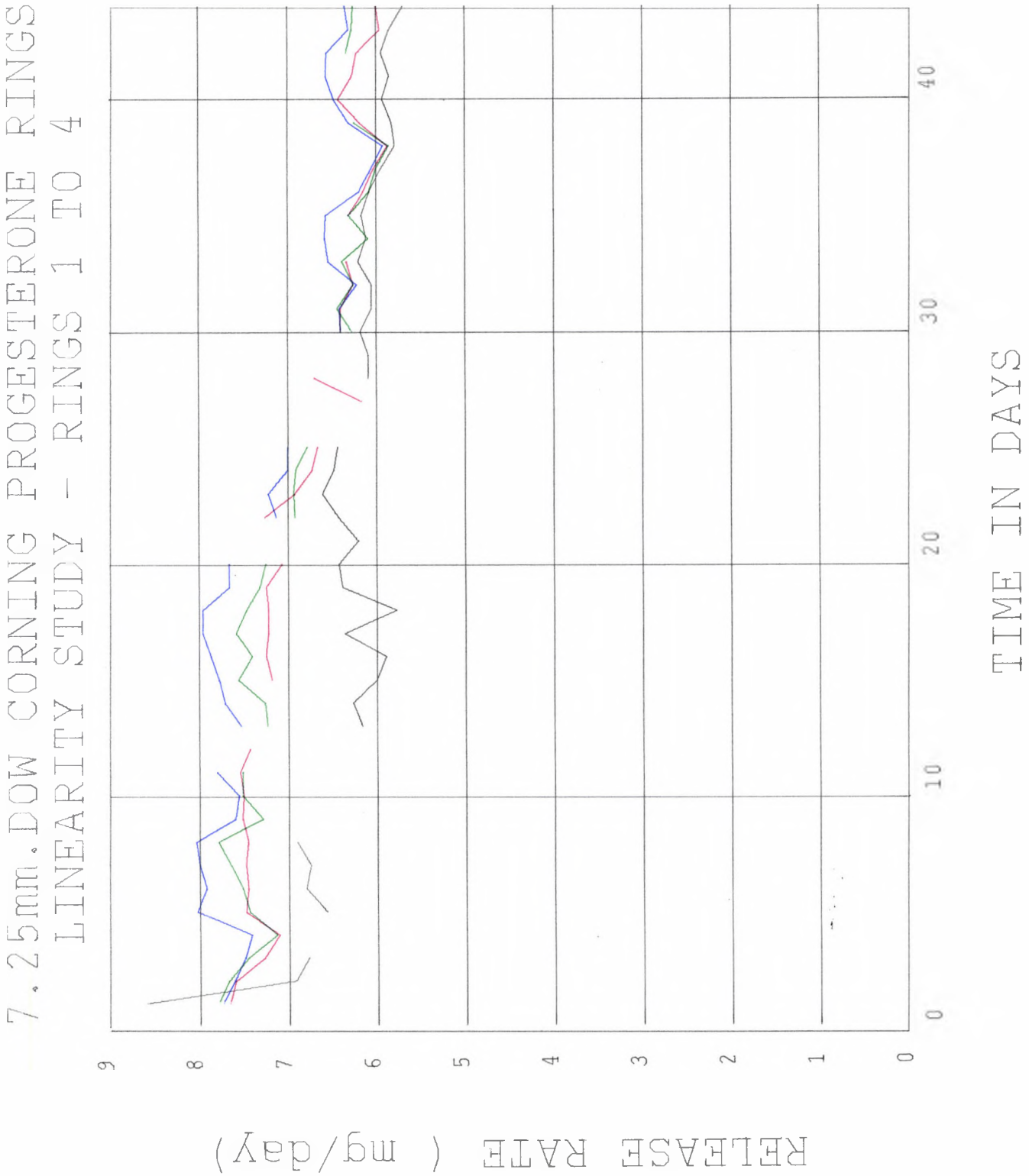
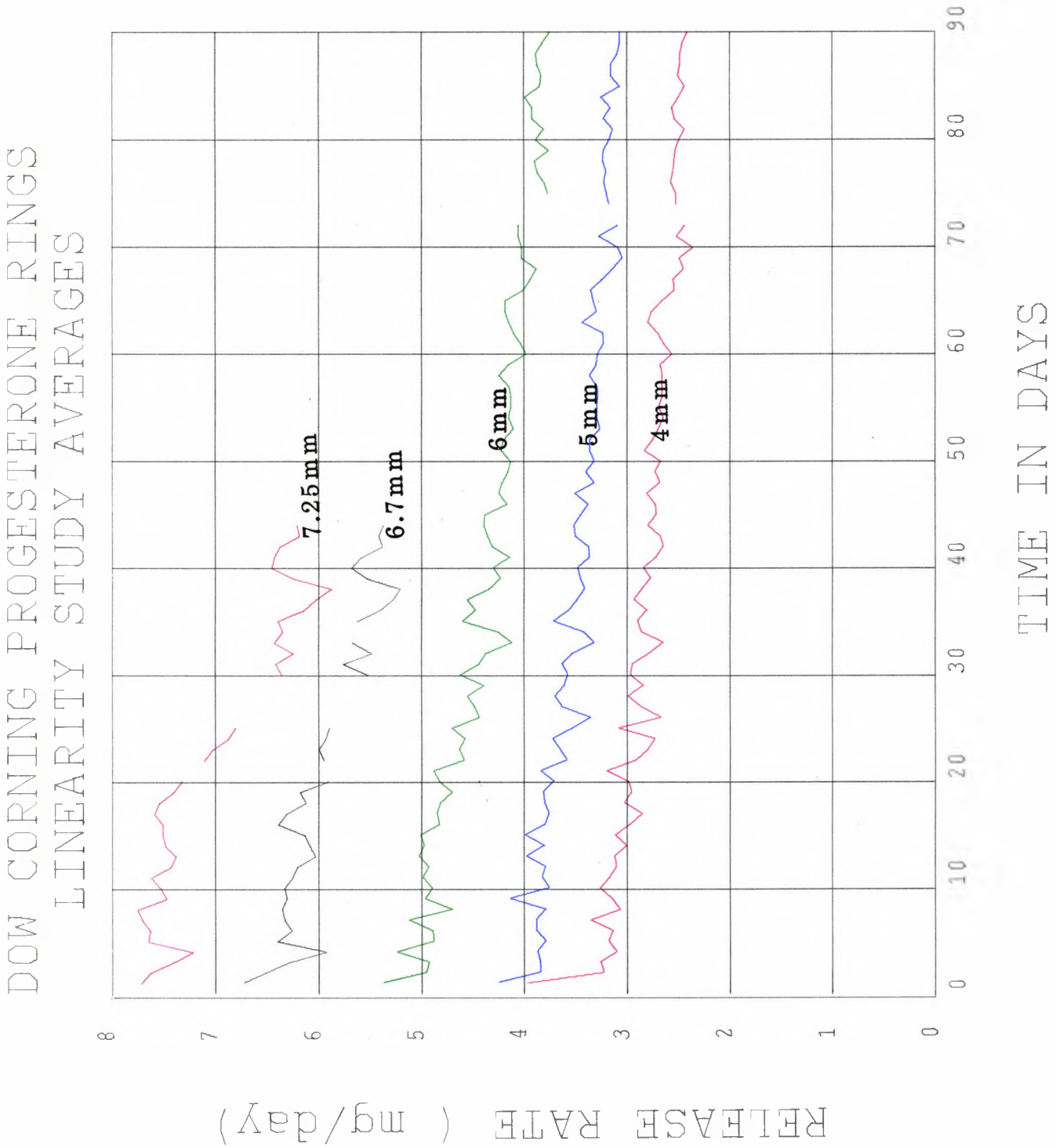


Fig. 8-13 Comparison of the daily release rate of progesterone of 4 mm, 5 mm, 6 mm, 6.7 mm and 7.25 mm core diameter Dow Corning vaginal rings prepared for a linearity study.



Zero order rate release for core design rings

As Chien[59,60] predicted and demonstrated in his mathematical model for core design vaginal rings, the accumulated release rate is nearly linear with time, under a matrix controlled process, which is achieved under true sink conditions. This relationship also applies to Dow Corning vaginal rings; Figure 8-14 to 8-18 demonstrate the zero order release rates for 4 to 7.25 mm core respectively, during the first month.

The increasing deviation of the accumulated release value with time from the initial linearity observed in the Dow Corning rings under study is due to a depletion effect of the steroid from the core; as the steroid content from the core releases into the diffusion matrix, the outermost layers of the core suffer a depletion and consequently while the effective core diameter decreases, the effective pathlength involved in the steroid diffusion increases. The continuous increase of the diffusion pathlength causes a continuous decrease in release rate, as can be seen in Figure 8-14 to 8-18. This effect has been extensively reported.[68,71] A correlation between depletion of the core versus time released has been plotted using rings with the diffusion membrane made out of clear polymer; photographs were taken at different stages of the release and the core size measured. A non linear correlation was found.[63]

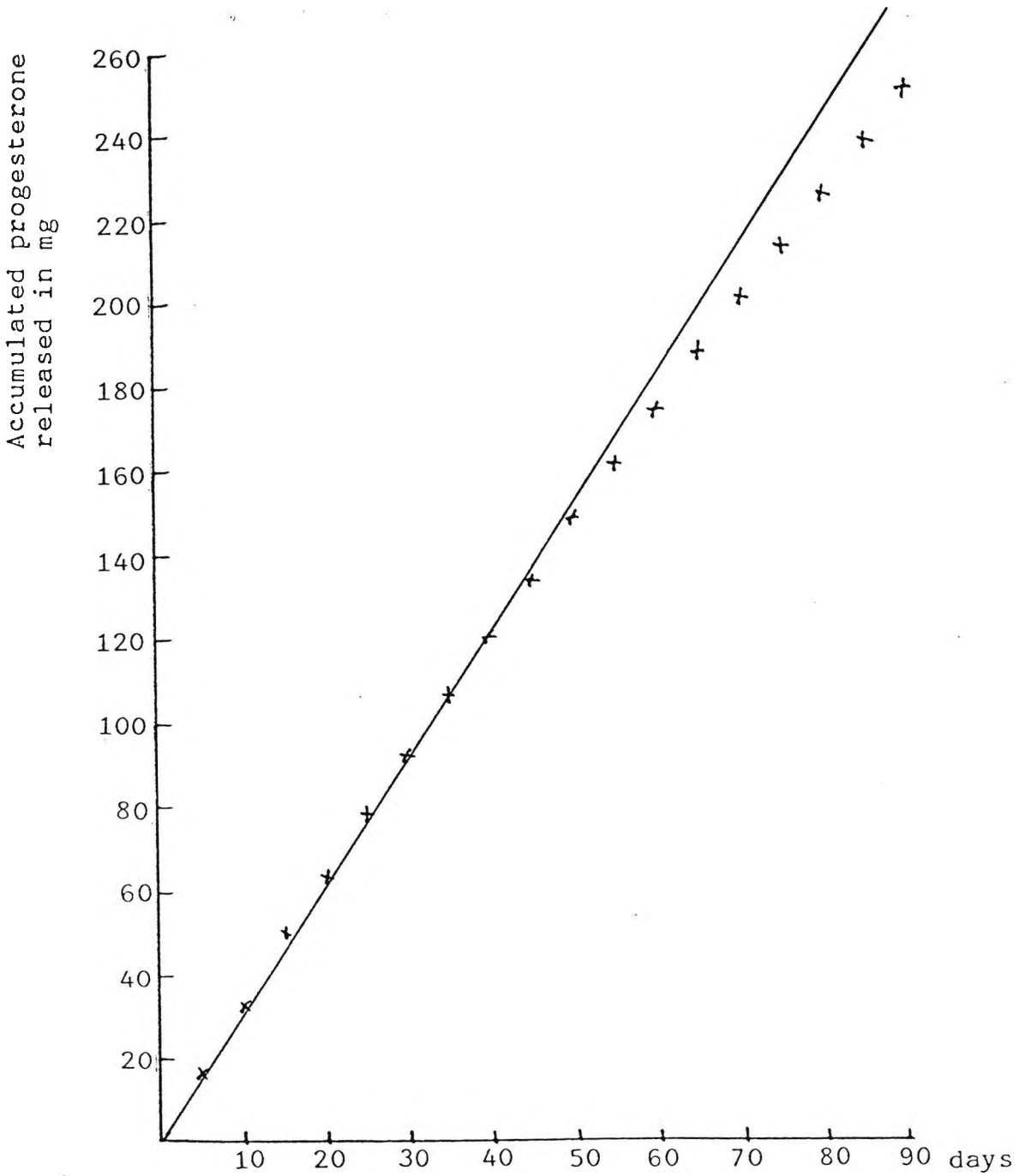


Fig. 8-14 Graphical representation of accumulated progesterone released from 4 mm core diameter Dow Corning vaginal rings versus days release.

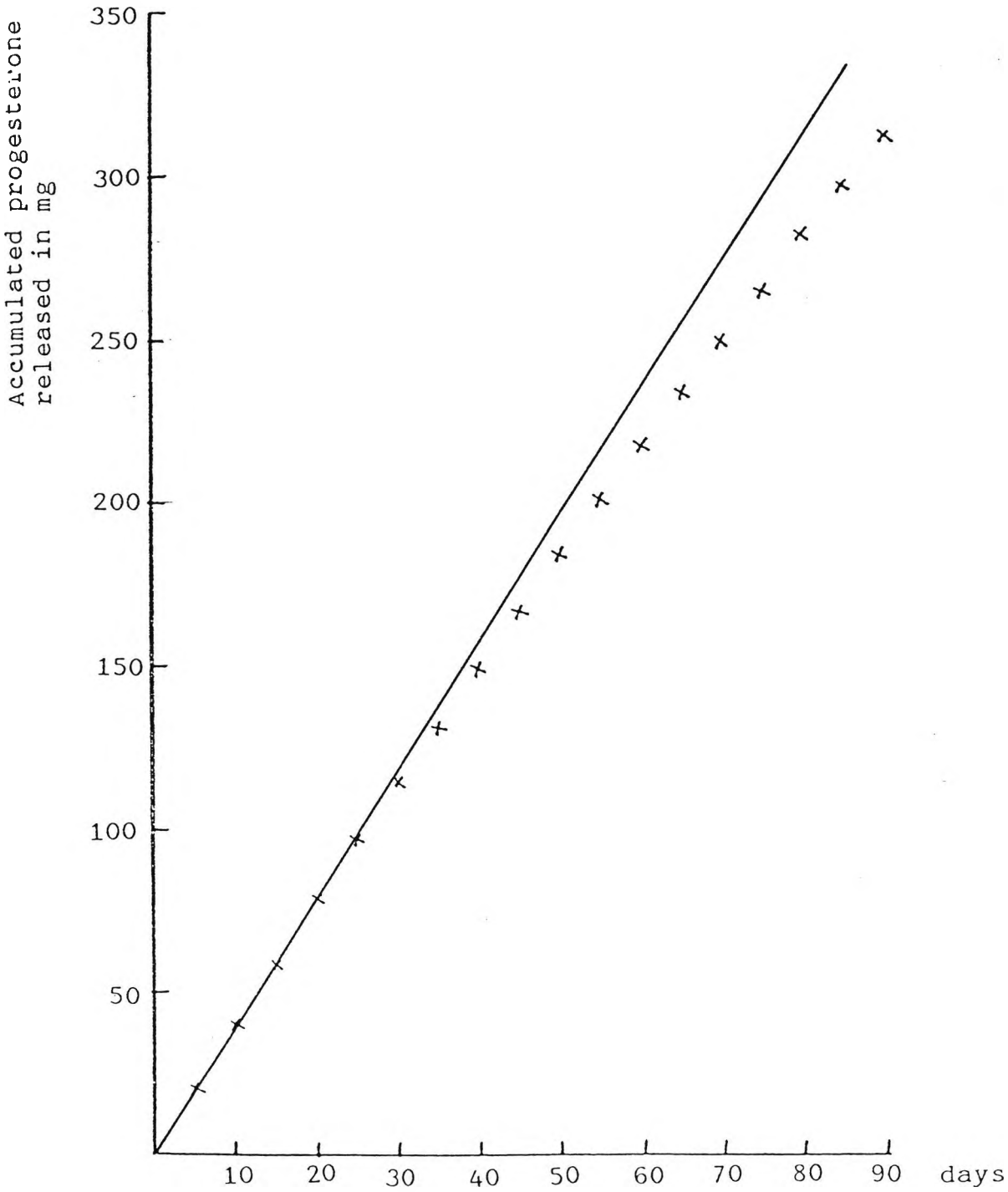


Fig. 8-15 Graphical representation of accumulated progesterone released from 5 mm core diameter Dow Corning vaginal rings versus days release.

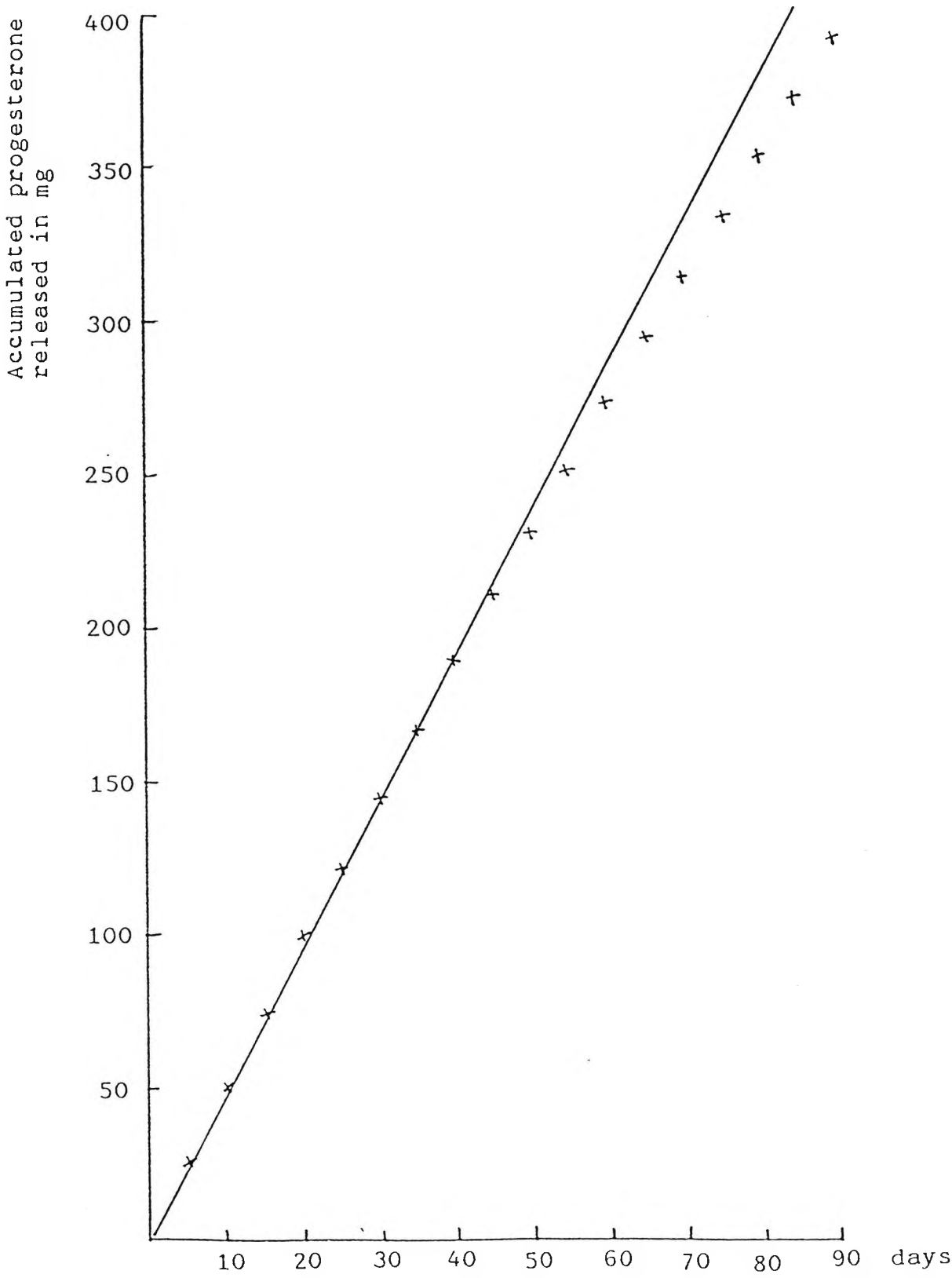


Fig. 8-16 Graphical representation of accumulated progesterone released from 6 mm core diameter Dow Corning vaginal rings versus days release.

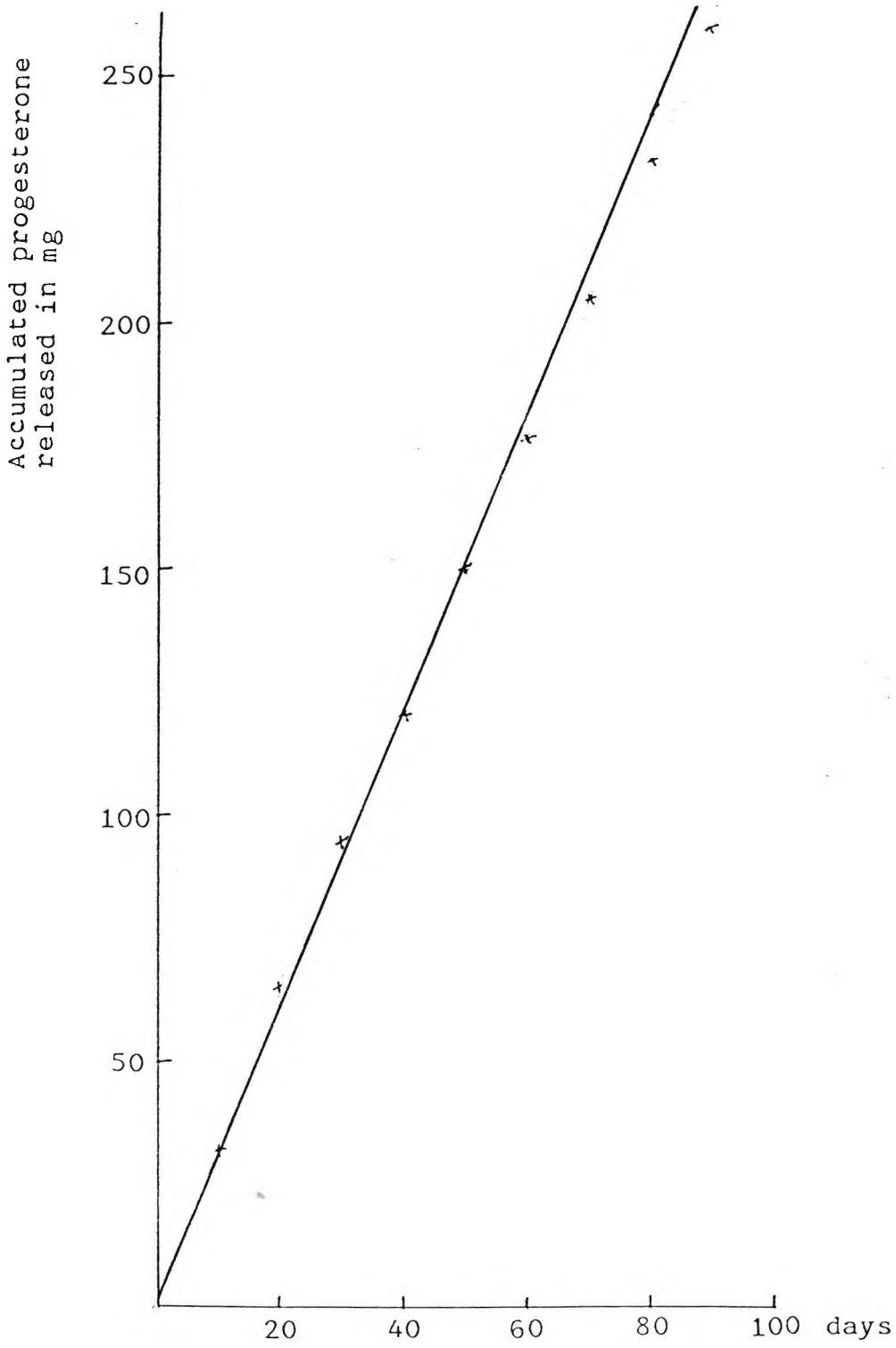


Fig. 8-17 Graphical representation of accumulated progesterone released from 6.7 mm core diameter Dow Corning vaginal rings versus days release.

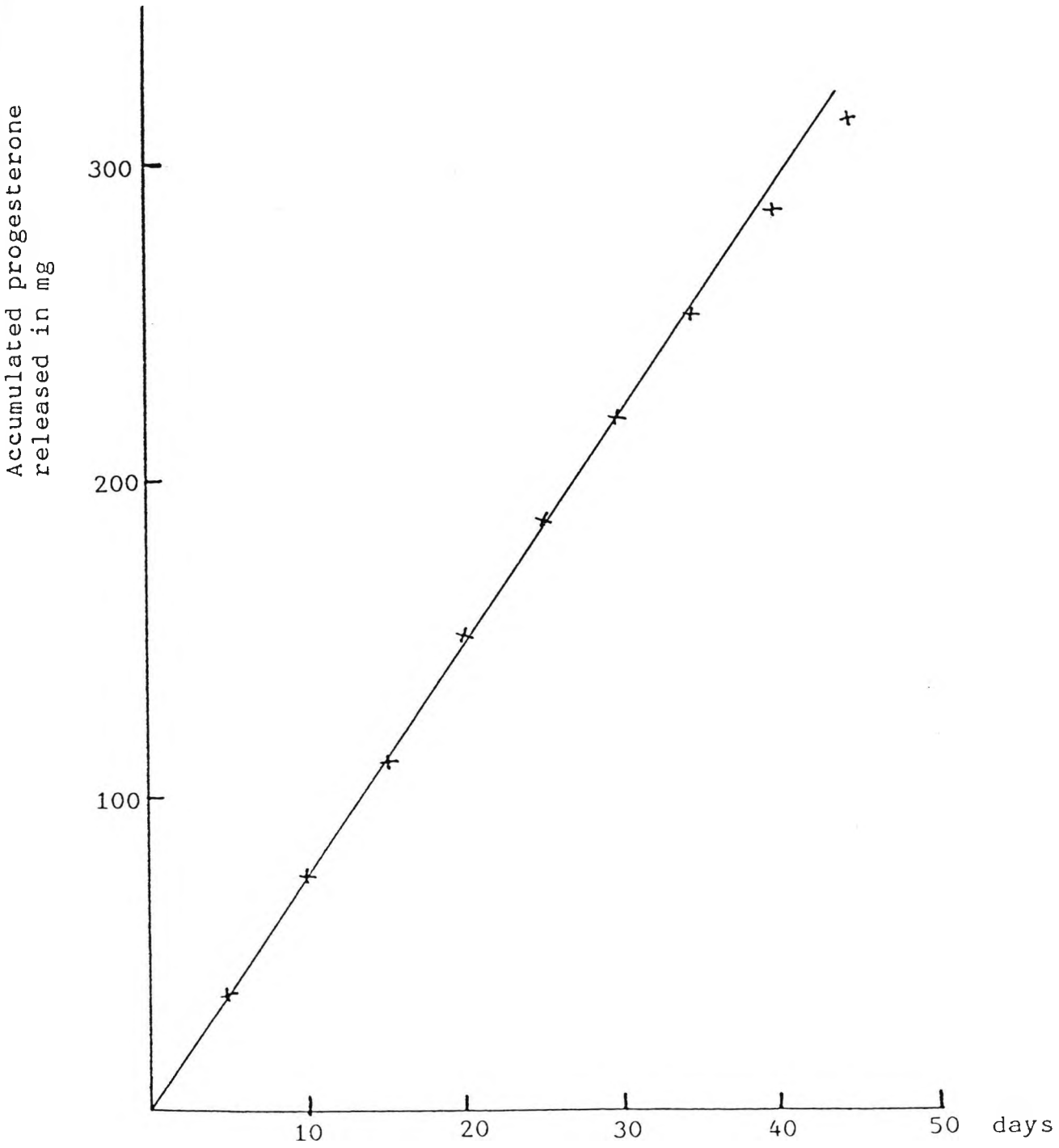
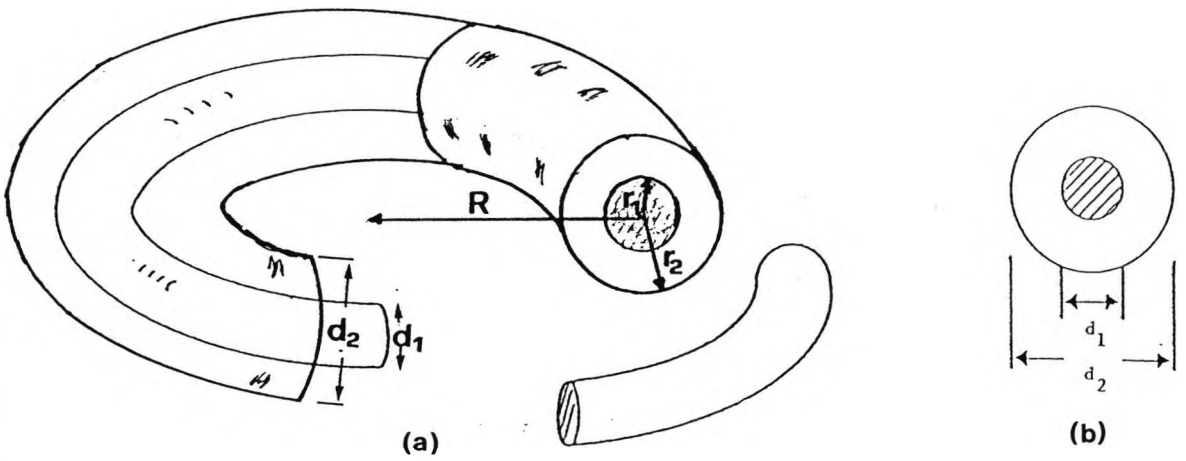


Fig. 8-18 Graphical representation of accumulated progesterone released from 7.25 mm core diameter Dow Corning vaginal rings versus days release.

Determination of Core Diameter Requirements

The above study of steroid release rates from the trial rings fabricated by Dow Corning with different core sizes in the range 4 mm to 7.25 mm was aimed at determining experimentally the most suitable core size for a release rate of 5 mg/day of progesterone. In general, daily release rates increased from ca. 3 mg to ca. 7.5 mg as core diameter increased from 4 mm to 7.25 mm (Figure 8-13).

A vaginal ring resembles a toroid, and the area and volume of the core are calculated to be:



Area toroid (core) $4\pi^2 R r_1$

Volume toroid (core) $2\pi^2 R r_1^2$

Fig. 8-19 Geometric parameters of core type vaginal rings

where R is the major radius to the centre of the ring and r_1 is the core radius (Fig. 8-19). Therefore while the area of the core is related to the core radius and hence to its thickness, the volume of the core is related to the square of the core thickness.

The nature of the relationship between core diameter and steroid release rate was studied by graphical correlation. For rings with a homogeneous steroid content,

previous studies have suggested that release rate is proportional to the ring surface area, and hence to its thickness. For the core type of ring under study, the release rate from the surface would be proportional to the outer surface area of the core, and hence the core's thickness d_1 (Fig. 8-19b), if diffusion of the steroid through the outer layer was sufficiently fast so as not to be rate-limiting.

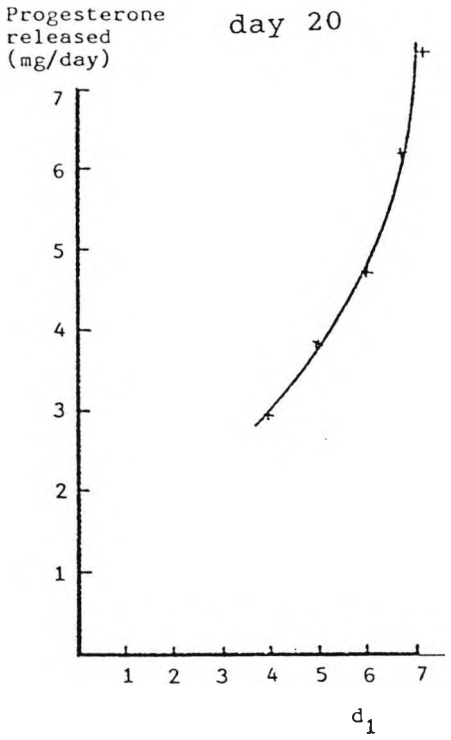
A plot of release rates versus the core diameters for the investigated series of rings on 3 typical release days, such as the 20th., 32nd. and 45th. day, is shown in Fig. 8-20, respectively, and is evidently non-linear.

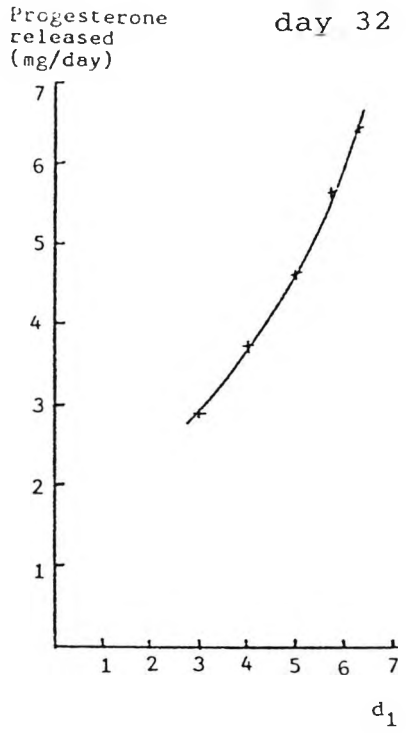
It is also possible, for a given steroid, that the diffusion rate through the outer layer could determine the overall release rate. A plot of the steroid release rate versus the reciprocal outer layer thickness ($1/(d_2 - d_1)$) i.e. the inverse of the diffusion distance from the core to the ring surface, shows a linear relationship (Fig. 8-21).

The correlation coefficient values for the linear correlation at different release periods are tabulated in Table 8-1.

Release day	correlation coefficient of release rates versus $1/(d_2-d_1)$
2	0.994
9	0.998
12	1.000
20	0.998
25	0.993
36	0.992
45	0.995

Table 8-1: Correlation coefficient calculated for different release days.





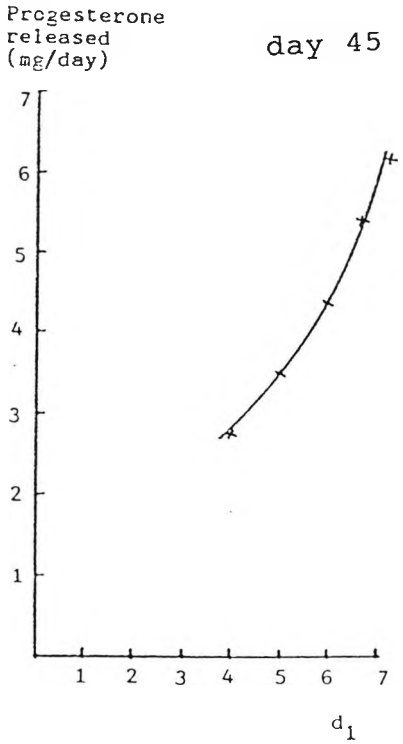
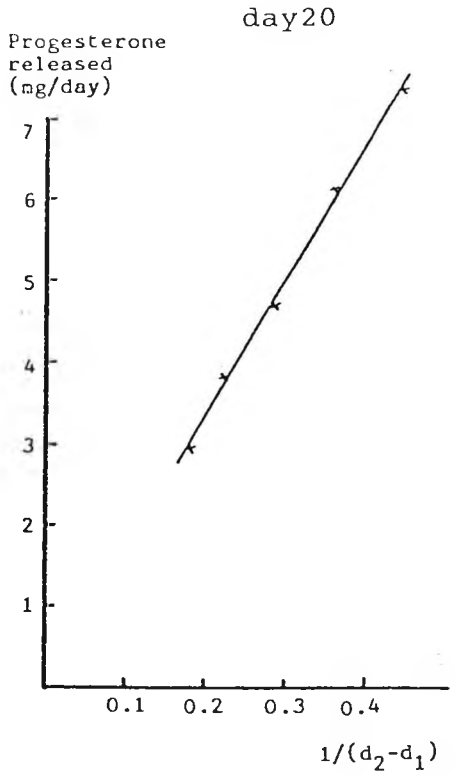
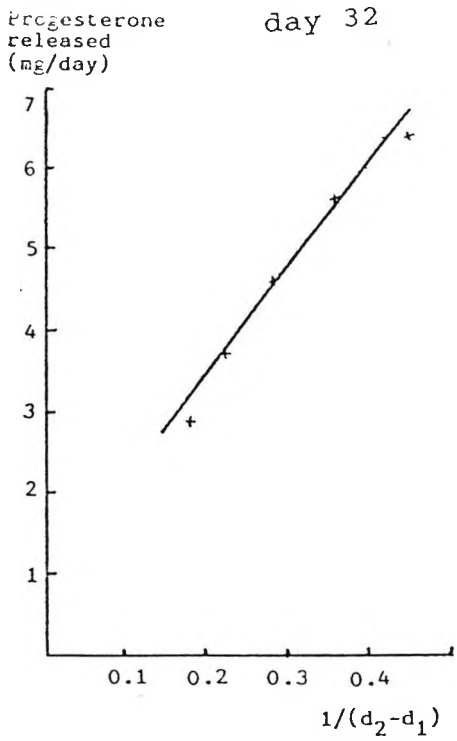


Fig. 8-20 Graphical correlation between release rate of progesterone at day 20, 32 and 45, and the core diameter of the vaginal rings (d_1).





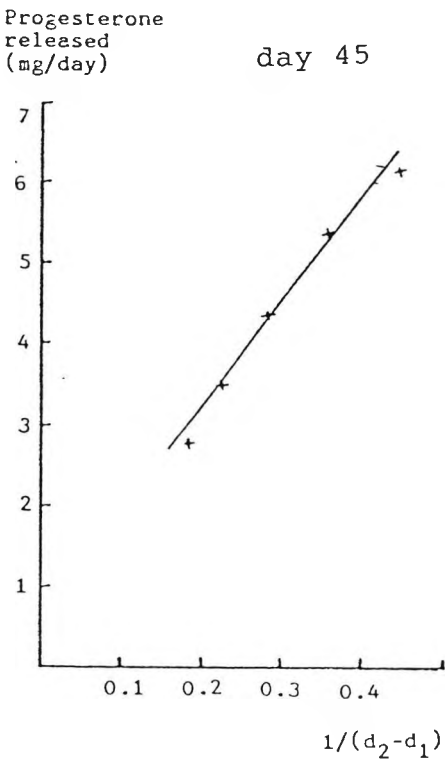


Fig. 8-21 Graphical correlation between release rate of progesterone at day 20, 32 and 45, and the inverse of the diffusion distance of the vaginal rings ($1/(d_2-d_1)$).

8.5.3 Study 3.: Population Council Rings

A few rings prepared for the Population Council were available with a designated release rate of 10 mg/day of progesterone and 4 of these were studied alongside the Valbonne rings (i.e. simultaneously in the same bath and with saline from the same reservoir and using the same standards for calibration).

The data obtained (Fig. 8-22) shows the following features: -

1. Rather erratic profiles in the first 3 weeks.
2. After this time, three of the rings became fairly uniform, but the release rate of the fourth declined steeply and never recovered.
3. Average in vitro release rates for the Population Council rings in saline solution declined from ca. 8 mg/day in the first month to 6-7 mg/day by the third month. Release rates of 10 mg/day were not observed at any time.

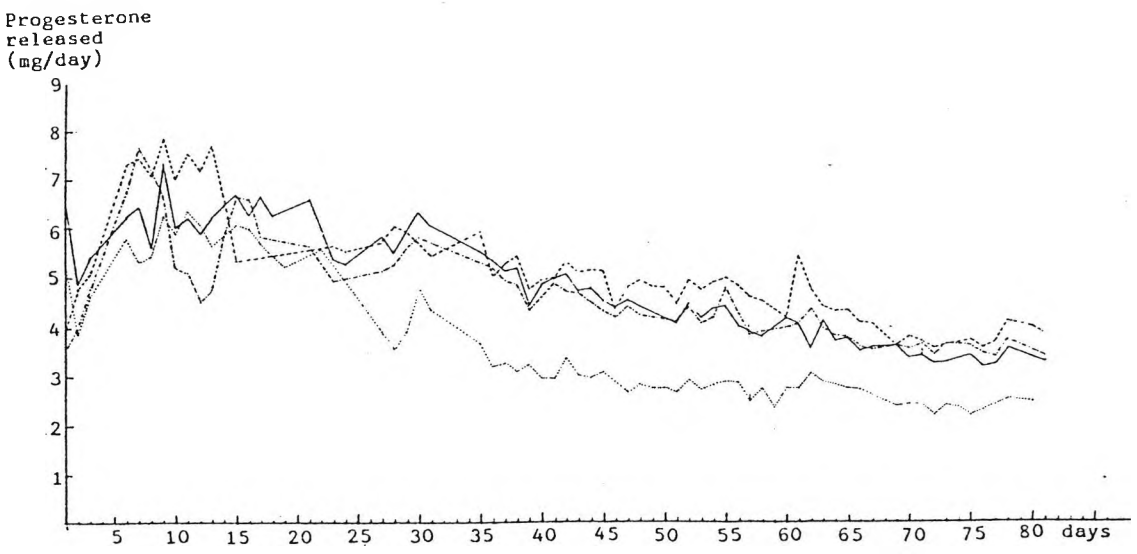


Fig. 8-22 Daily release rate of progesterone from "Population Council" core design vaginal rings.

8.5.4 Study 4.: Comparison of Saline and Benzalkonium Chloride Solution as Eluents

Information from the Population Council indicated that their own in vitro release rate studies utilised 1/750 w/v benzalkonium chloride solution as eluent and gave higher release rates for their rings than we observed using saline. Experiments to examine this discrepancy were therefore carried out.

Solubility of Progesterone in Benzalkonium Chloride Solution

Solubility was estimated by the same procedure as described earlier for the determination of progesterone solubility in saline: its solubility in 1/750 w/v benzalkonium chloride solution was found to be 2.6 times higher than in saline.

Comparison of Release Rates

A new experimental design was used for this comparison. A pair of 6.7 mm core Valbonne rings from the same production batch was selected and each was suspended in a large, closed glass vessel filled with 4.0 l of solution. Each jar was placed in a constant temperature bath at 37°C, stirred continuously by a magnetic follower and the eluting solution was changed once every 24 hr. One ring was immersed in 0.9% saline, the other in 1/750 w/v benzalkonium chloride solution for 10 days, then the eluents were switched so that the ring which had been in saline for 10 days was immersed in benzalkonium chloride solution and vice versa. Separate standardization of the progesterone levels in the benzalkonium chloride was performed, since correction must be made for background UV absorption of the organic salt.

The results of this study are presented in Fig. 8-23. The data confirms that a substantially higher release rate is observed when the ring is immersed in benzalkonium chloride solution. The readings for the two rings reverse nicely after the eluents are switched on day 10, demonstrating that this is a genuine effect and not a chance difference between the two rings.

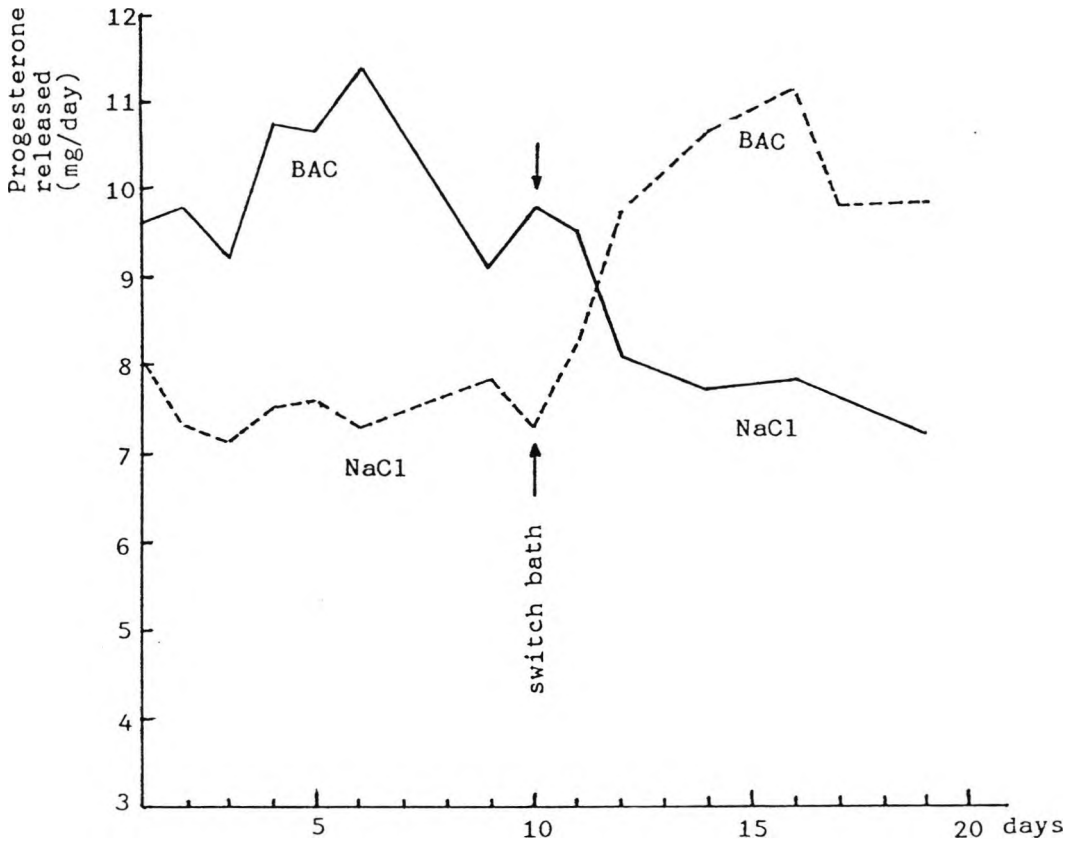


Fig. 8-23 Comparison of release rates of two 6.7 mm Dow Corning rings in NaCl and benzalkonium chloride (BAC) bath media, with switch of baths after day 10.

8.6. Vaginal Rings For Clinical Trial

8.6.1. Selection of Ring Size

For clinical trials to be conducted by the World Health Organisation, vaginal rings were required which would give initial release rates of ca. 5 mg/day of progesterone and remain effective for 3 months. The data obtained indicated that rings with a core diameter of 6 mm would be appropriate, giving an initial release rate of 5 mg/day of progesterone, declining to less than 4 mg/day by the third month (close to 0.5 mg/month loss in rate of progesterone release).

8.6.2. Effect of Quarantine Period Following Ethylene Oxide Sterilization

Two batches of 6 mm core rings were manufactured as trial batches for the designed clinical study. A sample of 20 rings from each batch was sent to us for quality control immediately after sterilization of the finished rings with ethylene oxide. A release study was started 3 days after sterilization on 10 of the 20 rings from each batch (Fig. 8-24 and 8-26). The remaining 10 rings from each batch were kept in quarantine for a further 15 days, open to the air and then submitted to a release study lasting over 30 days (Fig. 8-25 and 8-27). It can be seen therefore that the quarantine period following ethylene oxide sterilization of the rings has no significant effect on release rates.

Fig. 8-24 Effect of ethylene oxide sterilization on the release rate of 6 mm core diameter Dow Corning progesterone vaginal rings. Batch 1: study started 3 days after sterilization.

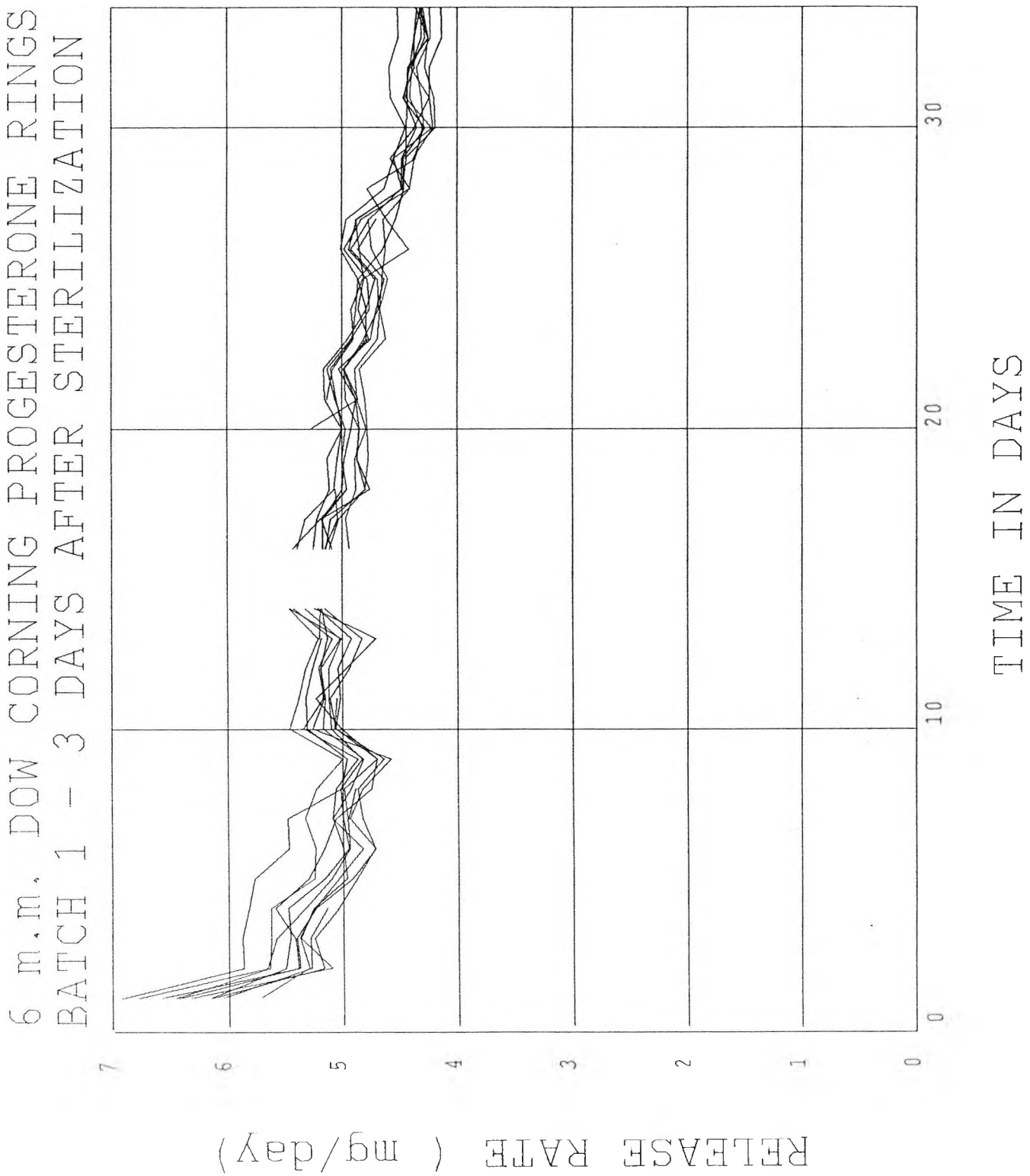


Fig. 8-25 Effect of ethylene oxide sterilization on the release rate of 6 mm core diameter Dow Corning progesterone vaginal rings. Batch 1: study started 18 days after sterilization.

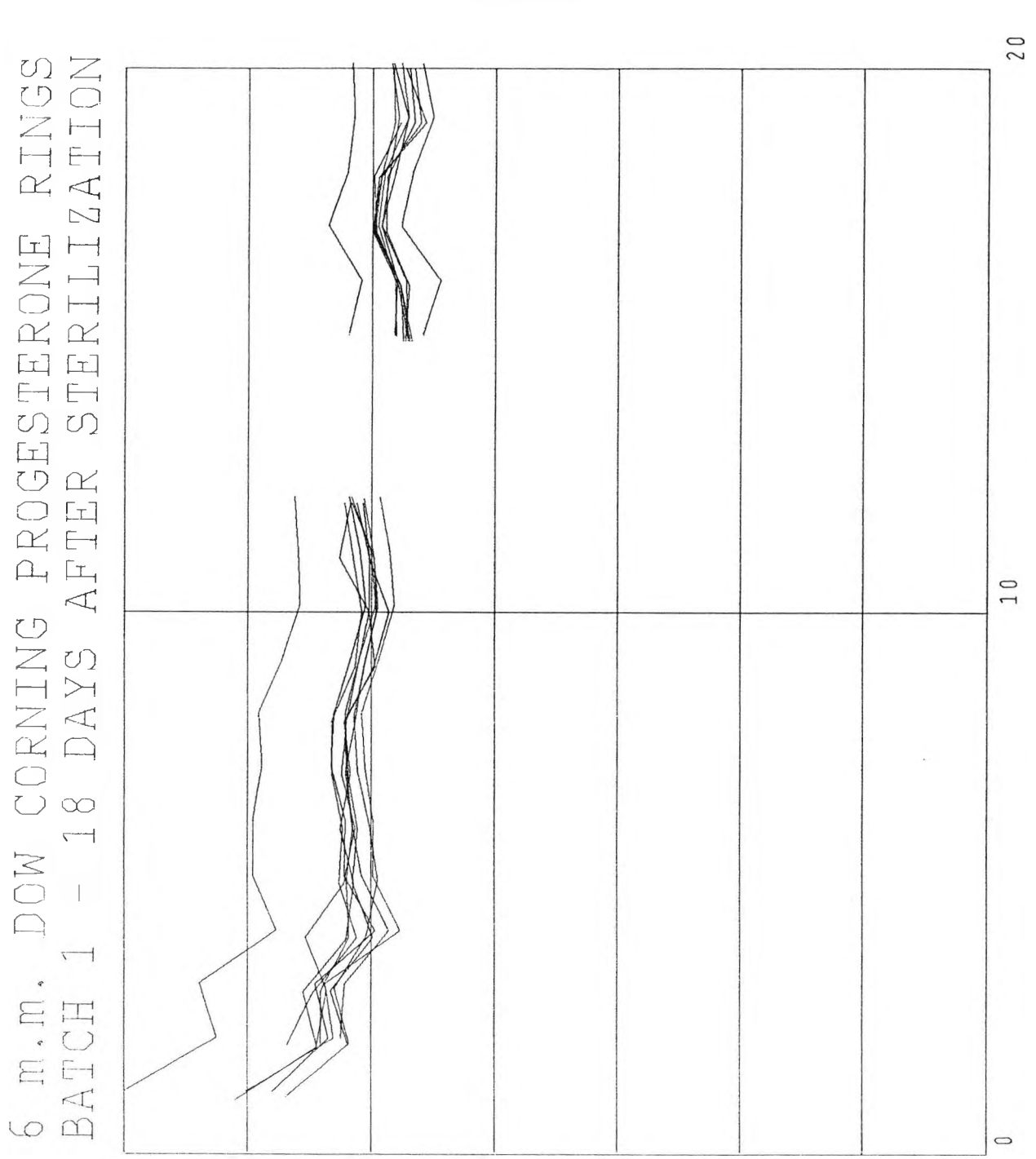


Fig. 8-26 Effect of ethylene oxide sterilization on the release rate of 6 mm core diameter Dow Corning progesterone vaginal rings. Batch 2: study started 3 days after sterilization.

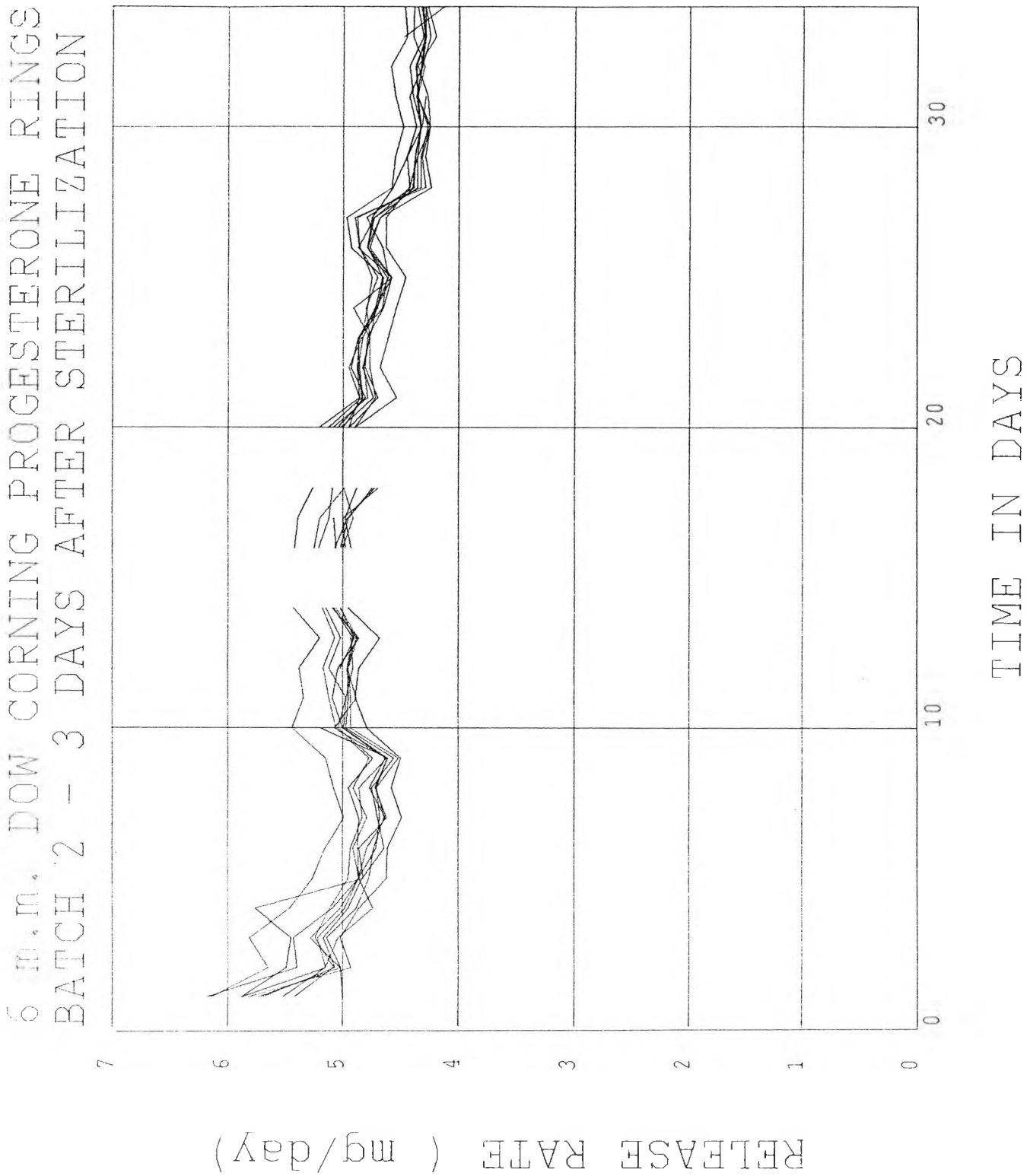
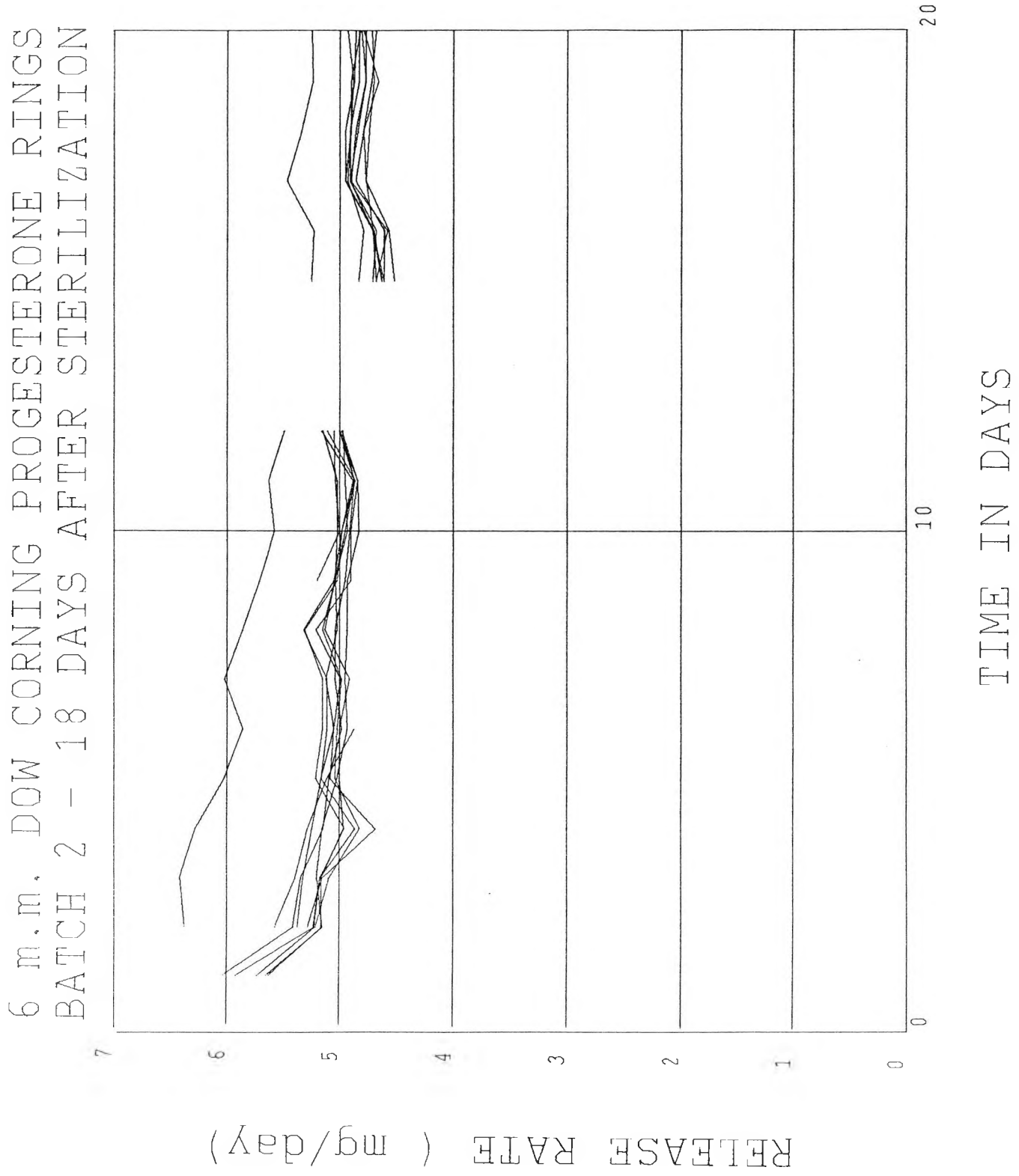


Fig. 8-27 Effect of ethylene oxide sterilization on the release rate of 6 mm core diameter Dow Corning progesterone vaginal rings. Batch 2: study started 18 days after sterilization.



8.6.3 Quality control of 6 mm core rings for clinical studies

Samples of rings from four batches prepared for clinical trials were received during the period September 1986-January 1987. 20 rings from each batch were initially placed in the flowing saline baths and release rates monitored daily. At the end of 28 days, 10 rings were removed and study of progesterone release from the remaining 10 rings was continued for a full 90 day period. The progesterone release rates of the 4 individual batches of vaginal rings are represented in Figs. 8-28 to 8-31, and the average release rates of the four clinical trial batches can be seen in Fig. 8-32.

Shortly after the start-up of analysis of the first clinical trial batch, a sharp drop in the progesterone levels in the eluents was noted, as measured by the UV method. HPLC analysis confirmed the decrease of the progesterone concentration while showing the appearance of early eluting peaks. It was suspected that this was due to biodegradation of the steroid. This was eventually traced to contamination of the saline: this problem, which had not been encountered in the previous year of studies, coincided with a move of the elution rig to a different part of the building. Studies of water samples by an outside microbiologist indicated that the new water supply was probably contaminated (even after distillation) and it was necessary to find a suitable additive to inhibit bacterial growth in the saline. Eventually, it was found that glutaraldehyde could be used as a bactericide without

interfering unduly with the analysis and without affecting release rates. Initially 25 ppm glutaraldehyde was used, but later a recurrence of apparently very resistant bacteria led to this being increased to 50 ppm and finally to 150 ppm.

In addition to some losses of data with batches A and B due to bacterial contamination, one day's data for individual rings, or the entire set has occasionally been lost due to operational failure of the peristaltic pump tubing, magnetic stirrer system, UV monitor, etc. On one occasion, thermostat malfunction resulted in a temperature increase to 40°C over a weekend and the consequent loss of an entire block of results of a few days. Another time, the thermostat heater coil burnt out and the temperature fell to ambient during a weekend.

Because of the problems mentioned, there are occasional gaps in the overall release rate profiles plotted in Figs. 8-28 to 8-32. The solid lines represent data which is considered to be entirely reliable and obtained under well controlled conditions, always with the aid of at least two independent analytical standards.

8.6.4 Prolonged release study

To determine whether rings used for longer than 90 days would show a sudden decline in release, 10 rings (batch C) were studied for a further month. The results (Fig 8-30) show that the release rate continues to decline at the same steady, slow pace, reaching 3 mg/day at the end of the fourth month. There was no evidence for sudden ring failure during this extra period of time.

Fig. 8-28 Graphical representation of 90 days daily release rate of progesterone from 6 mm core diameter Dow Corning vaginal rings: clinical batch A.

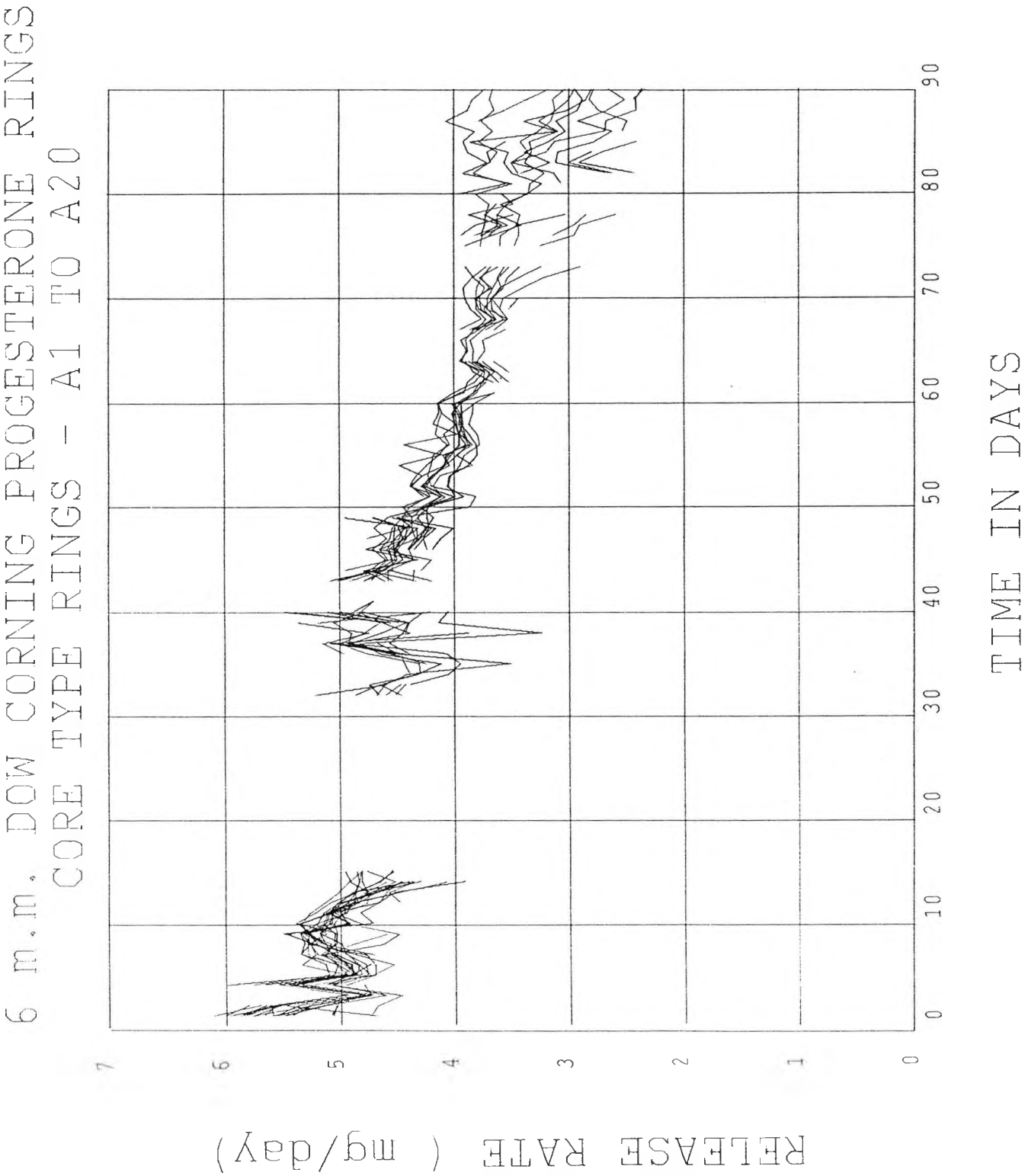


Fig. 8-29 Graphical representation of 100 days daily release rate of progesterone from 6 mm core diameter Dow Corning vaginal rings: clinical batch B.

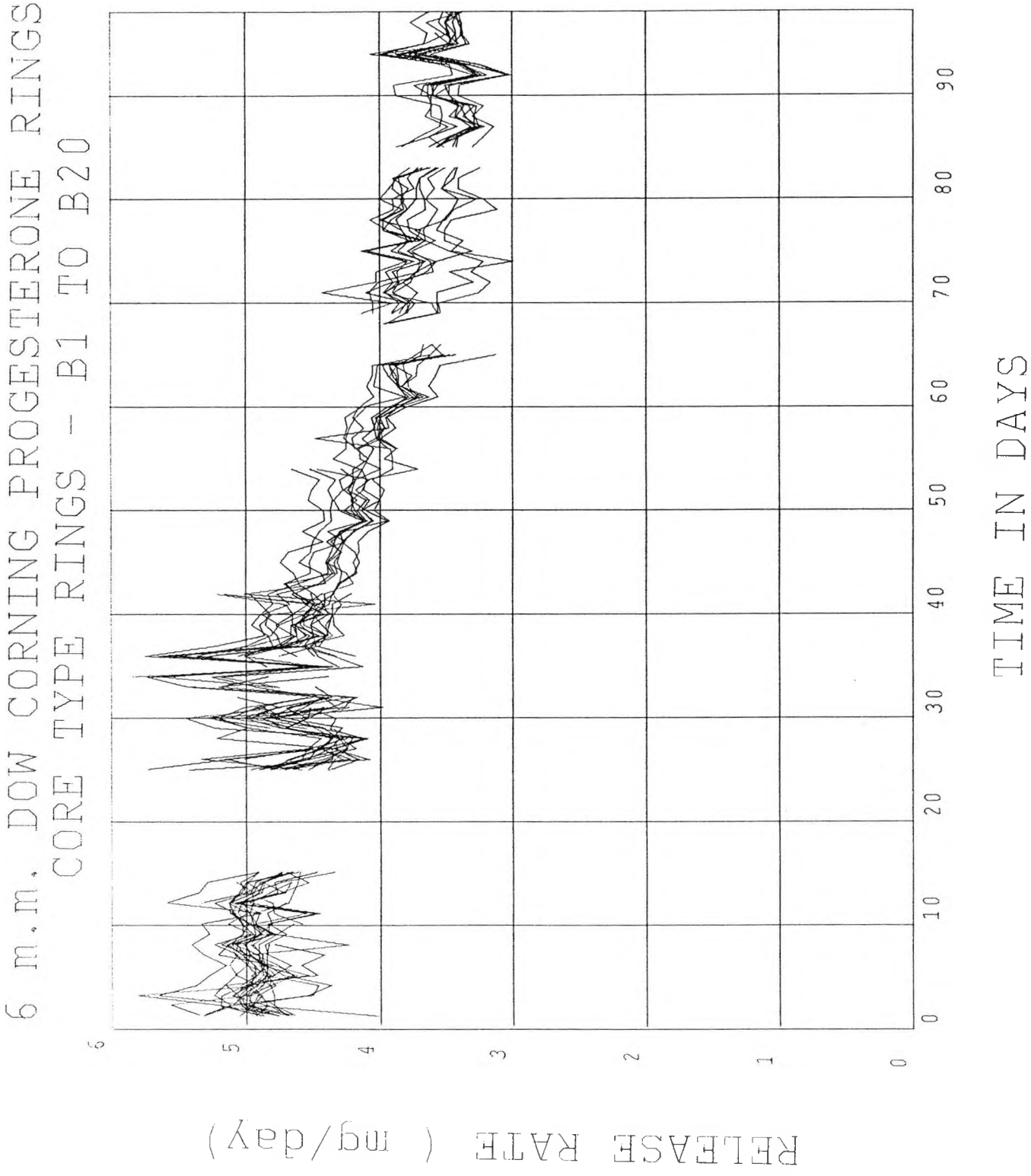


Fig. 8-30 Graphical representation of 130 days daily release rate of progesterone from 6 mm core diameter Dow Corning vaginal rings: clinical batch C.

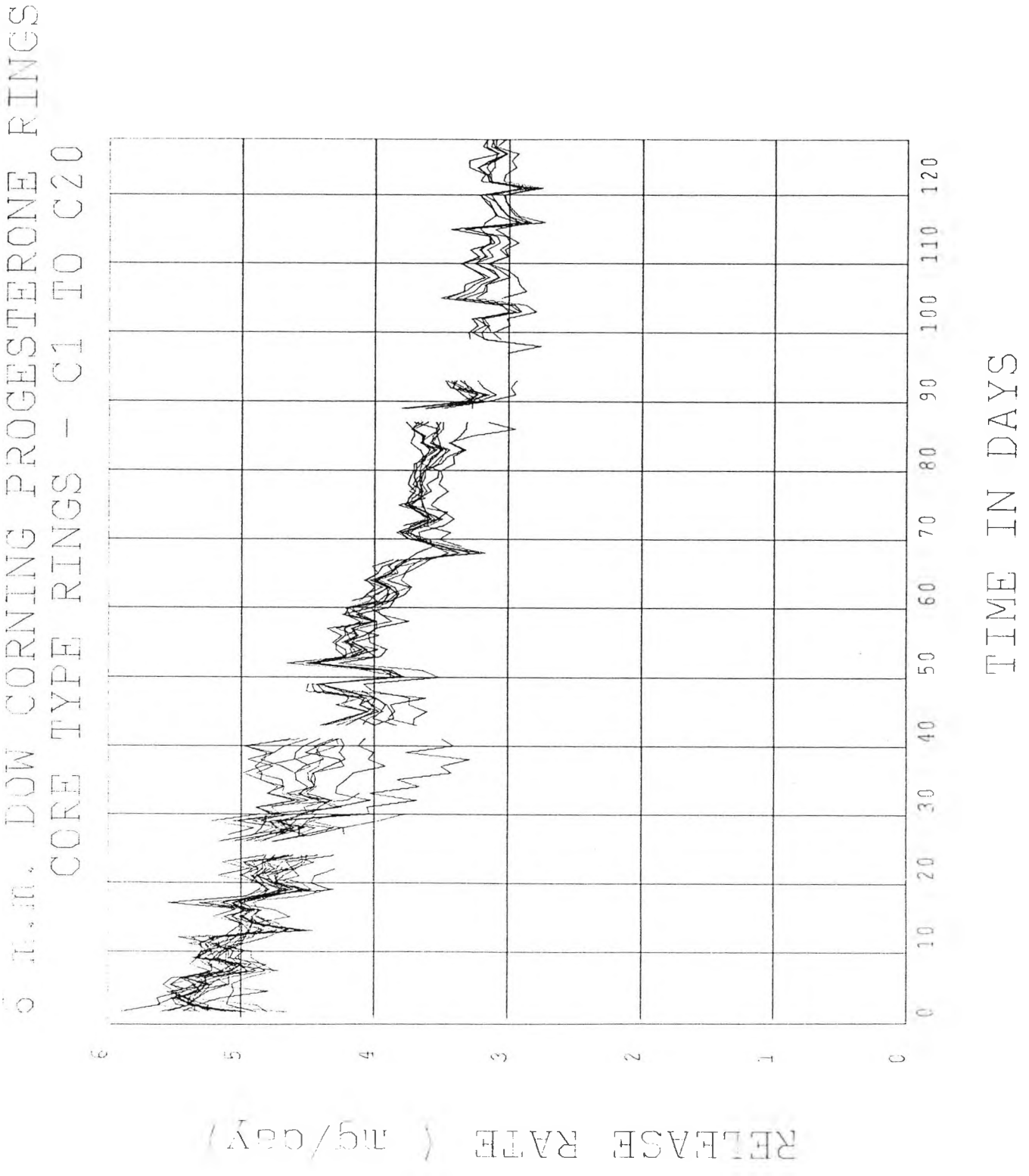


Fig. 8-31 Graphical representation of 90 days daily release rate of progesterone from 6 mm core diameter Dow Corning vaginal rings: clinical batch D.

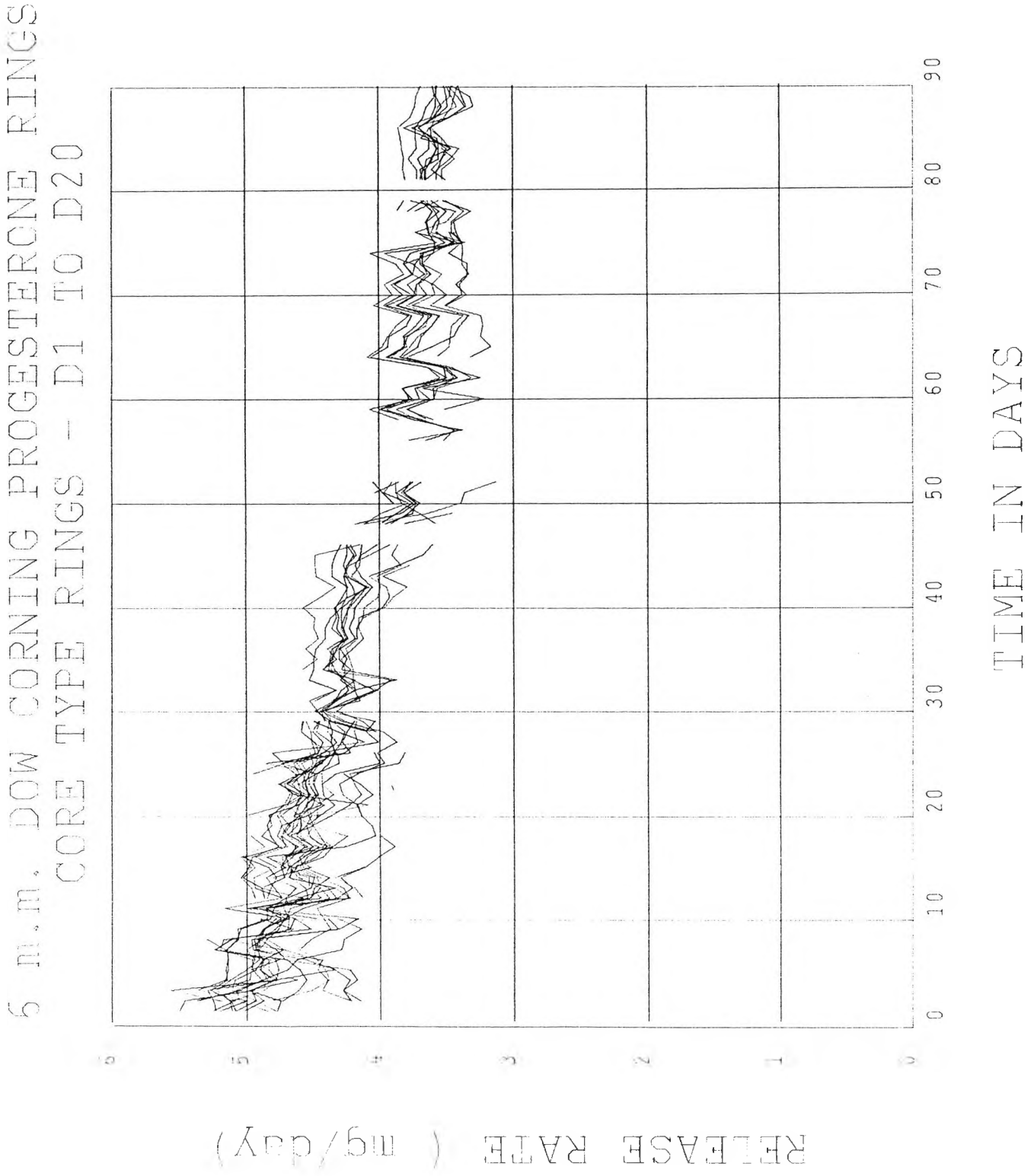
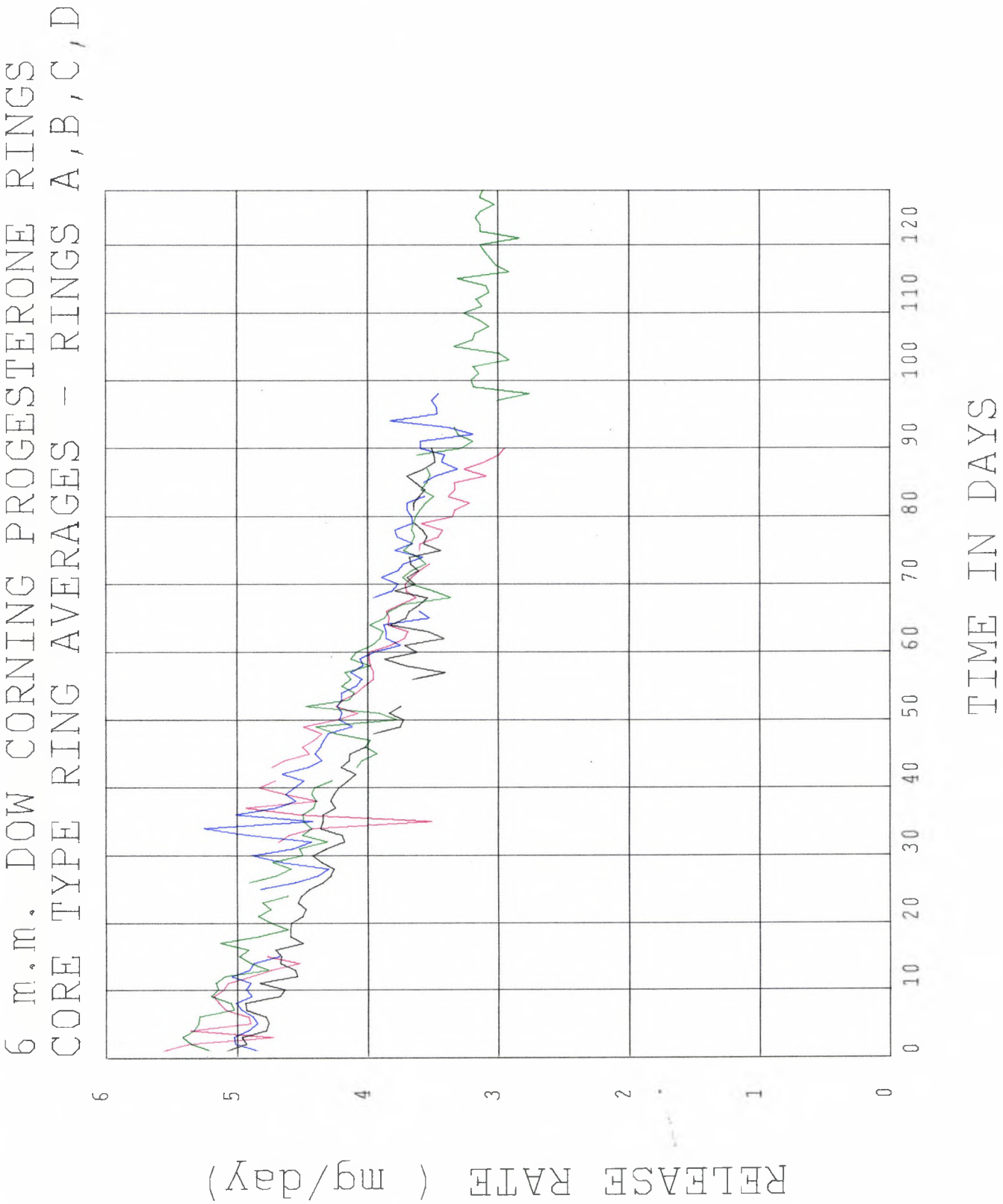


Fig. 8-32 Comparison of the daily release rate of progesterone of four clinical batches A, B, C and D from 6 mm core diameter Dow Corning vaginal rings.



8.6.5. Quality control of prepolymer/progesterone mixture used in manufacture of 6 mm core rings for clinical studies

Samples of the prepolymer mixtures, prepared to contain 25% w/w progesterone, used in fabricating the cores of the rings by Dow Corning, were analysed by the following method.

Initially, a weighed (100-150 mg) sample was dispersed in 50 ml n-hexane. Dilution of 1 ml of the clear supernatant to 100 ml with n-hexane was followed by UV determination at 250 nm. Each sample was analysed by independent duplicates against a "blank" of silastic prepolymer and two independent standards also containing the silastic prepolymer. The results show consistently 25 - 27.5%w/w progesterone content in all the samples analysed, as listed in table 8-2.

Lot. No. VL-	Progesterone %w/w	Lot. No. VL-	Progesterone %w/w
076267	26.7	106259	25.9
		106271	26.0
086202	26.6	106278	26.3
086273	26.6	106292	25.8
086280	27.4	106299	25.8
086285	27.0		
086290	26.8	116207	25.0
		116212	25.7
096213	25.1	116218	25.5
096218	26.8	116223	25.7
096234	26.6	116233	25.7
096242	26.1	116240	26.5
096251	26.8	116247	25.5
		126257	25.5
		126275	25.7
		126285	25.9

Table 8-2 Progesterone content in pre-polymer silastic mixture for 6 mm core rings

8.7. Population Council rings

Initially, four core type rings prepared for the Population Council with an intended release rate of 10 mg/day progesterone were studied simultaneously with those rings from Dow Corning for the linearity study. The data obtained showed rather erratic release rate profiles, declining from 8 mg/day in the first month to 6-7 mg/day by the third month (Figure 8-22).

A new set of 10 rings from the Population Council was studied alongside the clinical batches from Dow Corning. Apparently, these new rings were of the homogeneous rather than core design and therefore were expected to show an initial high burst of steroid release, followed by a sharp decline and an eventual flattening out of release rate. Results for the individual rings are shown in Fig. 8-33 and the average release and comparison with Dow Corning clinical batches are plotted in Fig. 8-34.

8.7.1 Correlation between accumulated release rate and time

As Chien had documented,[1,2] the accumulated release of steroid of a homogeneously impregnated ring under matrix controlled conditions (sink effect) was linearly correlated with the square root of time. This is also the case for this 10 mg Population Council ring as seen in Figure 8-35.

Fig. 8-33 Graphical representation of 90 days daily release rate of progesterone from an homogeneous type Population Council vaginal rings batch for clinical trial.

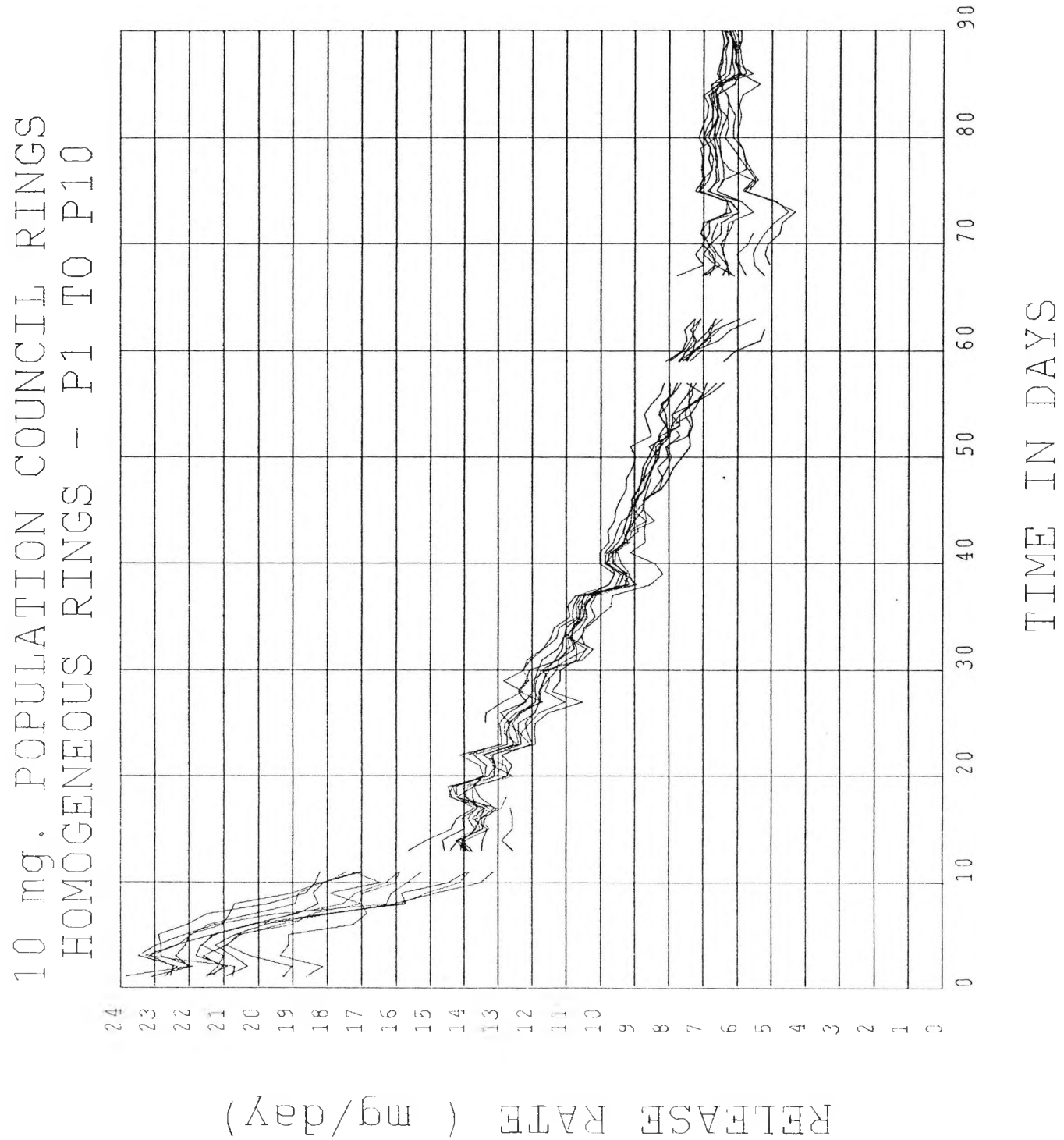
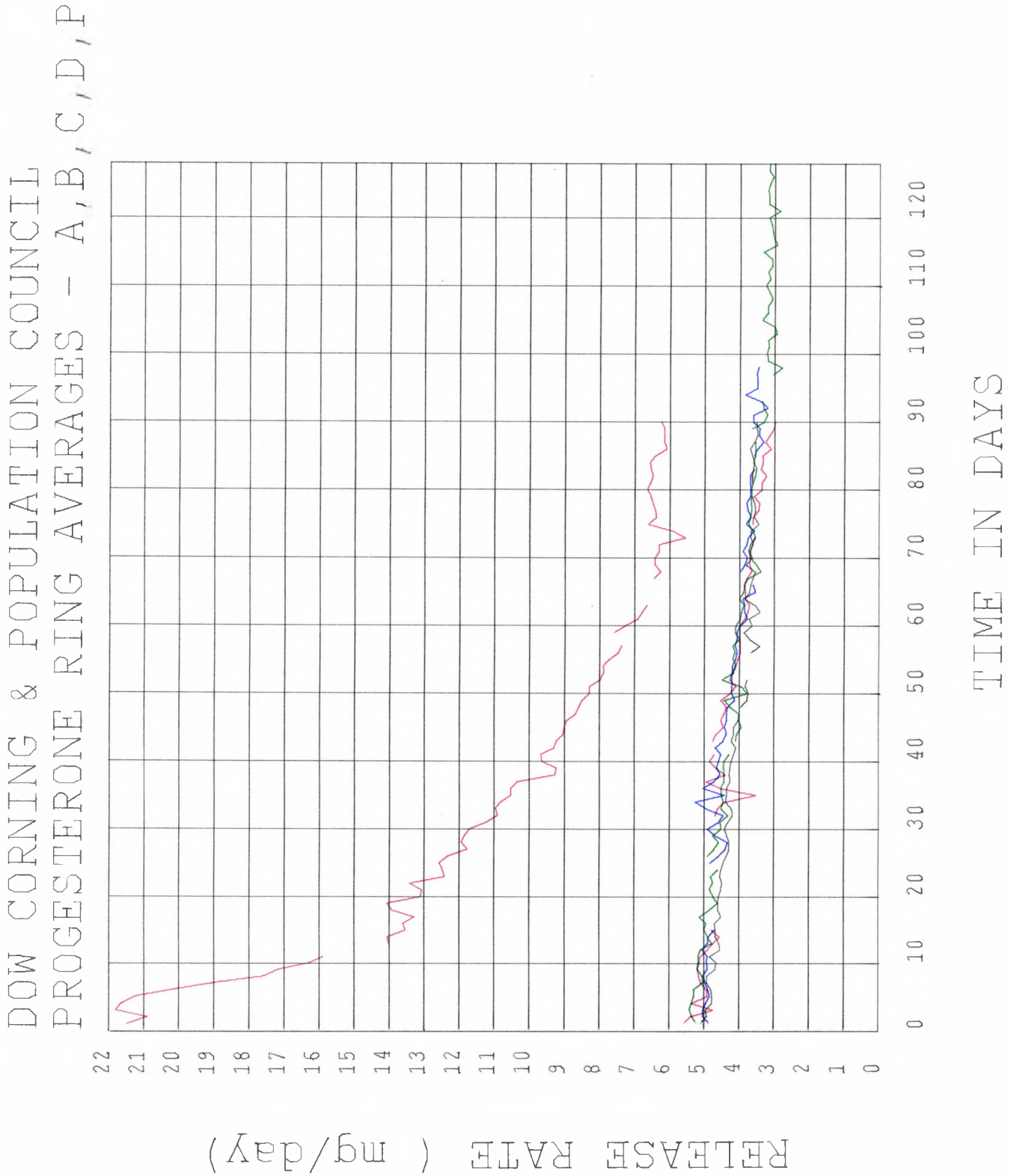


Fig. 8-34 Comparison of the daily release rate of progesterone of four clinical batches A, B, C and D from 6 mm core diameter Dow Corning vaginal rings with another clinical batch of an homogeneous type vaginal ring from the Population Council.



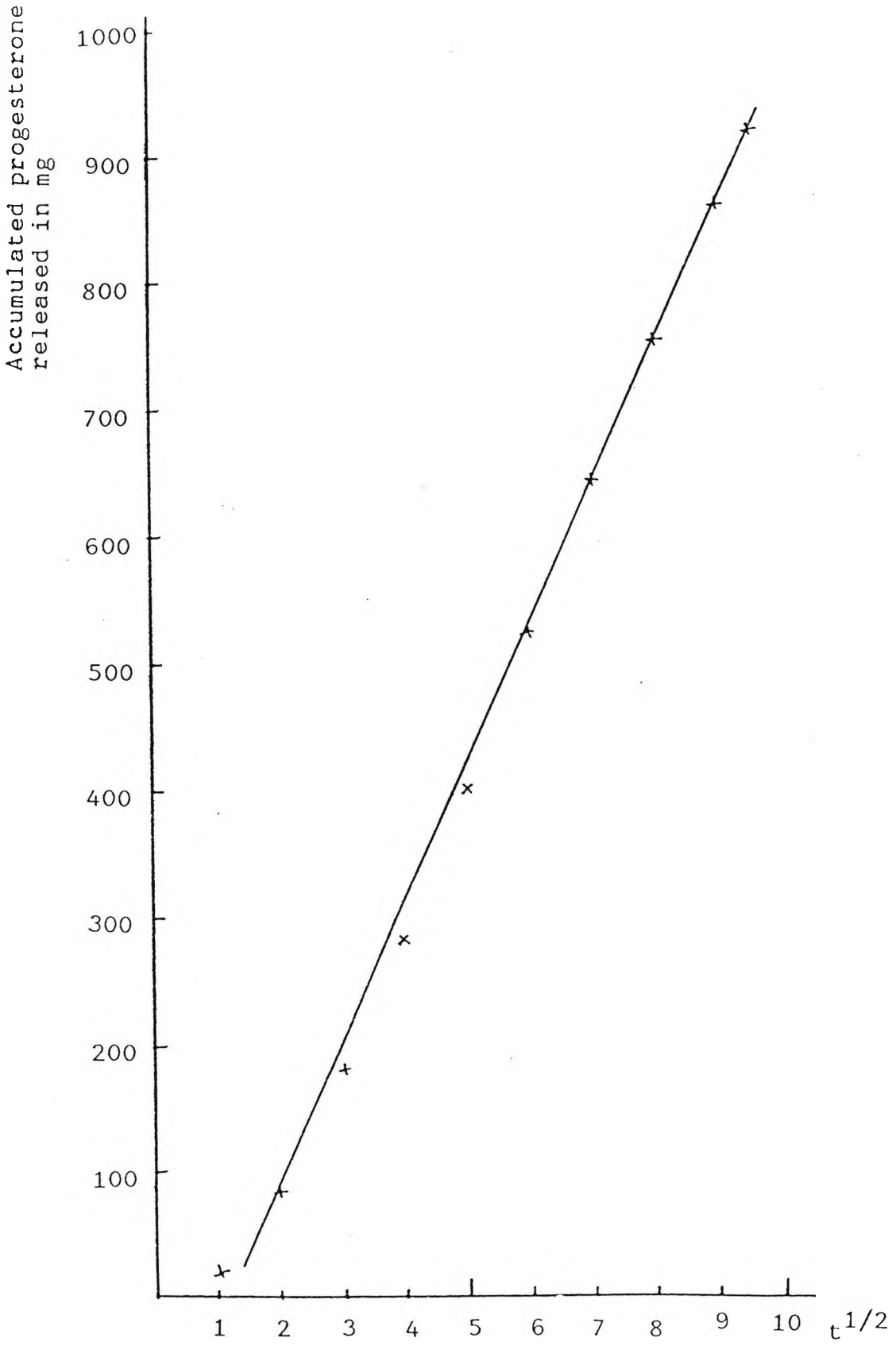


Fig. 8-35 Graphical representation of accumulated progesterone released from Population Council homogeneous type vaginal rings versus the square of the days release.

8.8. Conclusions

1. In this project methodologies were developed for in vitro elution of progesterone from vaginal rings by a continuous flow of saline. Eluate concentrations of progesterone could be measured either by UV analysis or by HPLC, either by extraction procedure and HPLC analysis on conventional columns or, more conveniently, by direct, large volume injection onto short columns containing a 3um packing. The ring elution and eluate analysis procedures were shown to give a reproducible and accurate measure of 24 hours release rates and good agreement was obtained between the two methods of progesterone analysis.
2. Application of the in vitro method to core-design vaginal rings fabricated by Dow Corning showed that rings of a given core diameter and fixed outside dimensions generally gave consistent release profiles. The release rate increased with core diameter. A good, linear correlation was found between release rate and reciprocal diffusion distance.
3. From the results obtained for sets of rings of different core diameters, it was determined that 6 mm core rings would give an initial release of 5 mg/day. Four batches of rings of this size were fabricated by Dow Corning for WHO clinical trials.
4. QC analysis of rings from each of the four clinical batches was carried out. It was confirmed that all the rings gave reproducible release rates which declined gradualy from 5 mg/day to 3.5 mg/day during 90 days.
5. One batch was followed for 120 days and continued to give 3-3.5 mg/day release during the fourth month.

8.9 References

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CHAPTER 9

LEVONORGESTREL VAGINAL RINGS: RESULTS AND DISCUSSION

9.1 Introduction

Following the success of clinical trials in the early 1980's,[1-4] WHO decided to make available for general clinical use a zero order vaginal ring releasing a daily dose of 20 µg/day levonorgestrel continuously for 90 days, suitable as a non-ovulation-inhibiting contraceptive in non-lactating women.

9.2 Results and Discussion

The subject of this project is a study commissioned in 1987 by the WHO to investigate the appropiate core size of a vaginal ring which should release a steady 20 µg per day of levonorgestrel for a 90 day period. Clinical batches would be subsequently prepared and tested with the optimum core size. A set of 7 different core sizes were prepared for WHO by Dow Corning, for the release of the synthetic progestin.

Our project involved examination of 4-5 rings of each experimental batch which all had the same outside dimensions as the previously described progesterone vaginal rings, in order to determine a suitable size for the release of 20 µg/day.

In this study, fixed daily volumes of saline at 37°C were used and 24 hour collections were analysed every few days by HPLC. Levonorgestrel concentrations were much too low for UV readings on a conventional scanning spectrophotometer.

An HPLC method for direct injection analysis was developed on a similar system to that used for the previous

study of progesterone: a short 5 x 0.45 cm Hypersil-ODS 3 µm column and mobile phase of 50/50 (v/v) MeCN/H_2O was found to elute levonorgestrel within two minutes and well resolved from any possible impurities (Chromatogram shown in Fig. 9-1). Detection was at 241 nm, 0.05 AUFs.

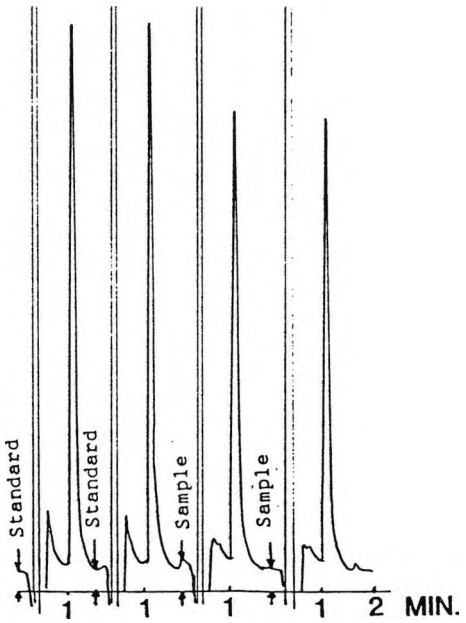

Fig. 9-1 Fast HPLC chromatogram of direct 200 µl injections sample and standard levonorgestrel solutions on a 5 x 0.45 cm Hypersil-ODS 3 µm column, eluted with 50:50 MeCN/H_2O at 2 ml/min, monitored at 241 nm.

Release rates for the individual ring for each core size are plotted in Fig. 9-2 to 9-8, averages and maximum and minimum values observed are plotted in Fig. 9-9. Initial collection volumes were set to provide an adequate dilution for a sink effect and some volumes were increase subsequently (Table 9-1) : for the 6 mm rings, this led to a significant increase in release rate.

Fig. 9-2 Daily release rate of levonorgestrel 1 mm [illegible] diameter Dow Corning vaginal rings prepared for a linearity study.

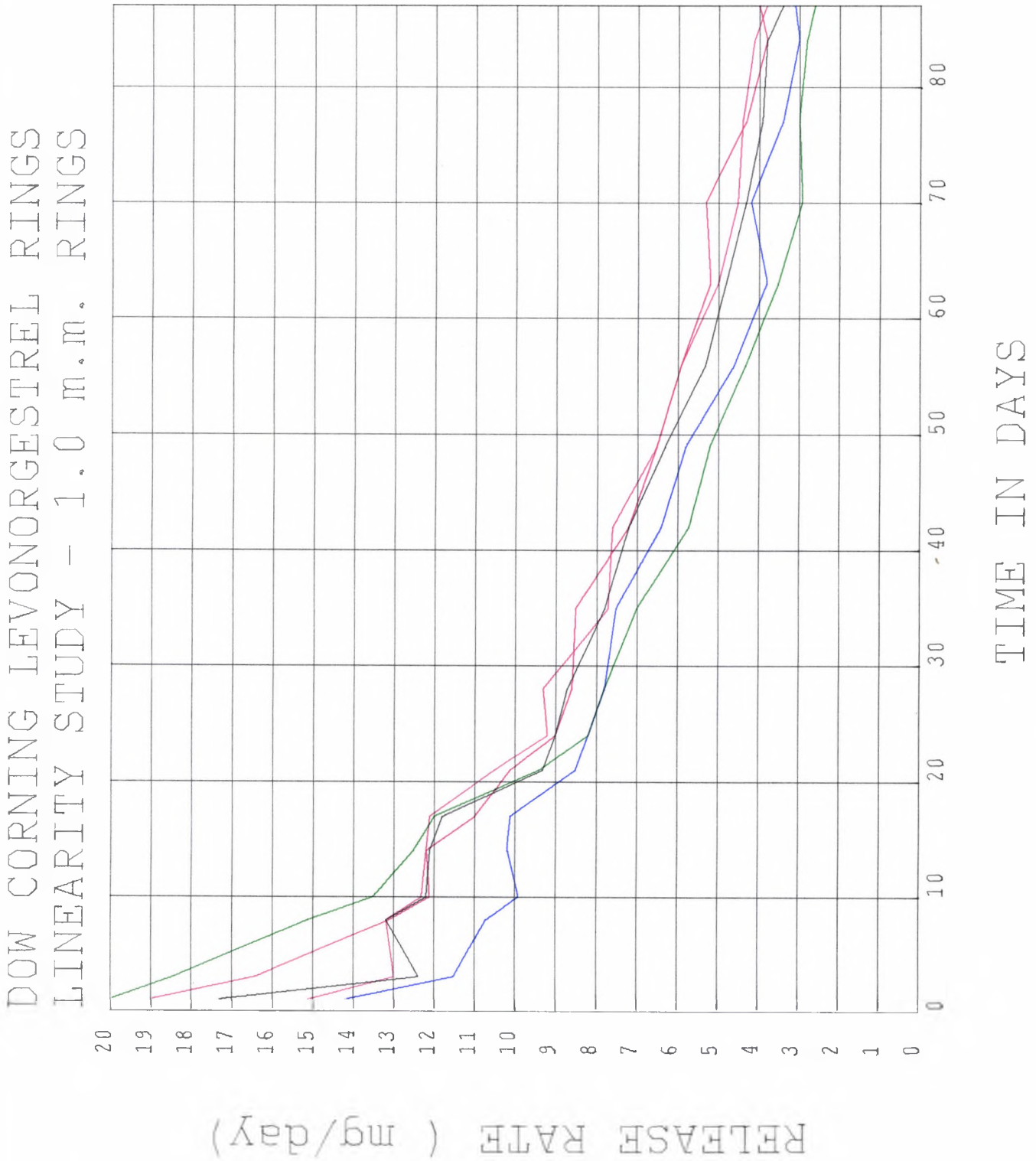

Fig. 9-3 Daily release rate of levonorgestrel 1.5 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

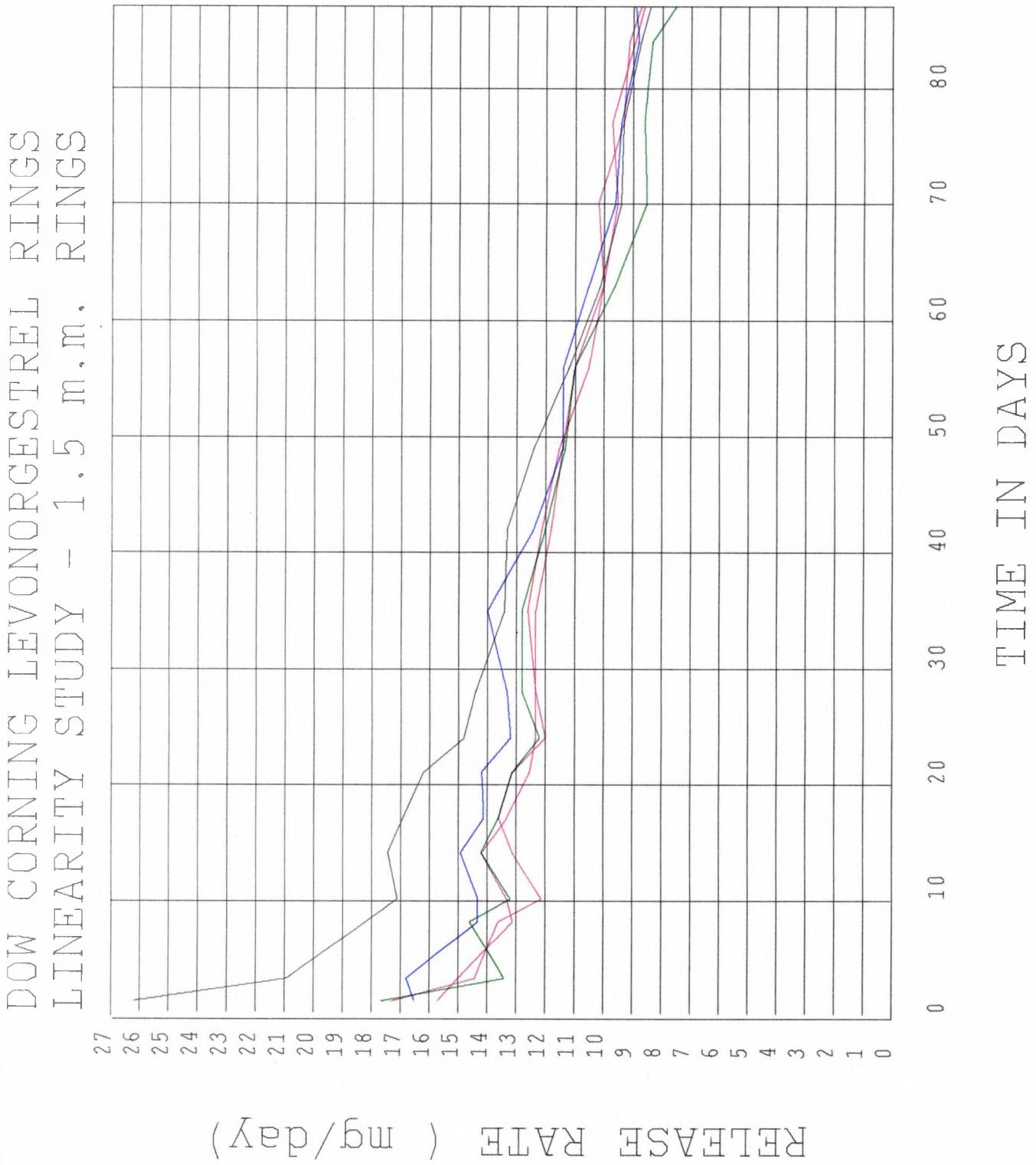

Fig. 9-4 Daily release rate of levonorgestrel 2 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

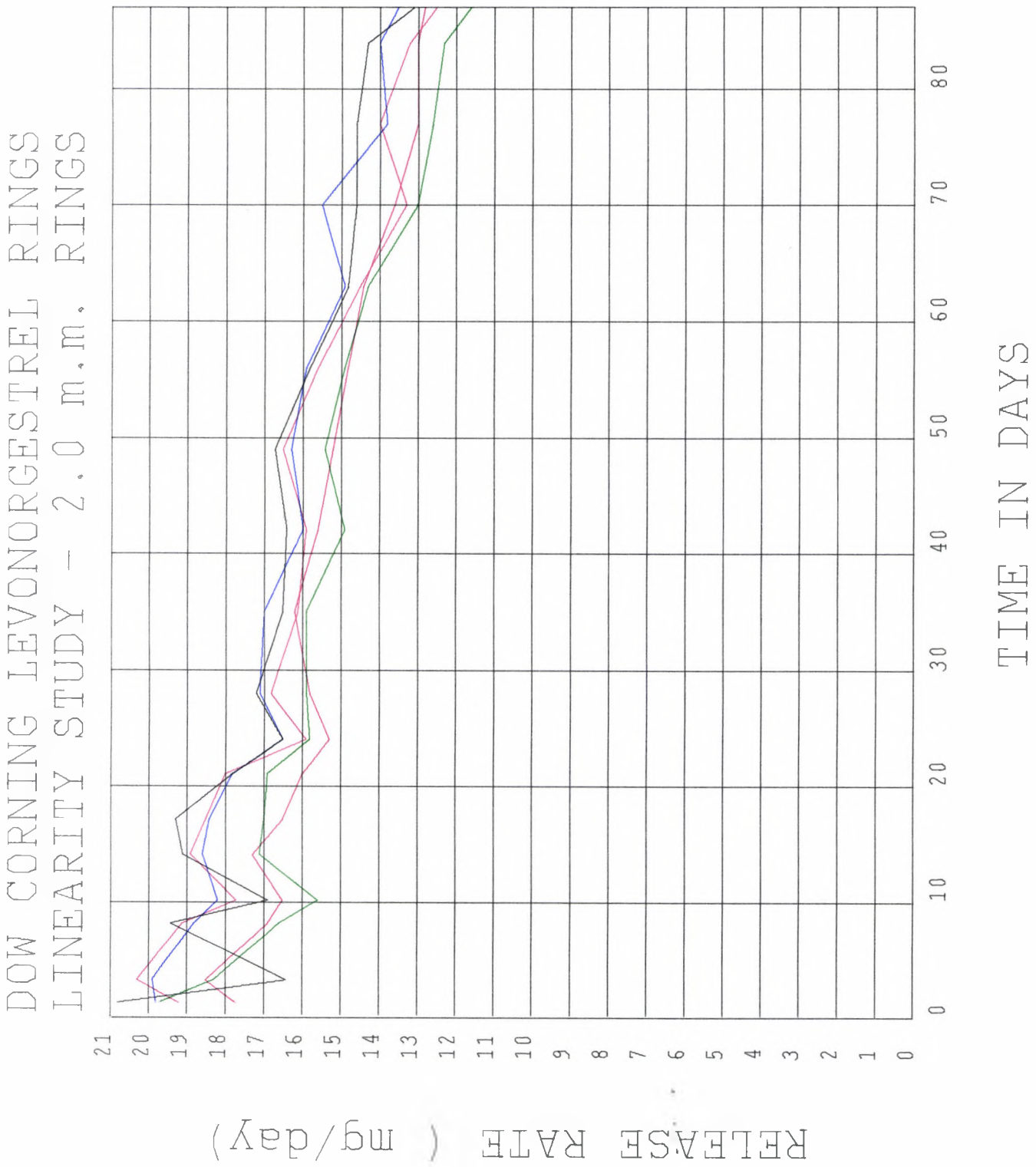

Fig. 9-5 Daily release rate of levonorgestrel 3 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

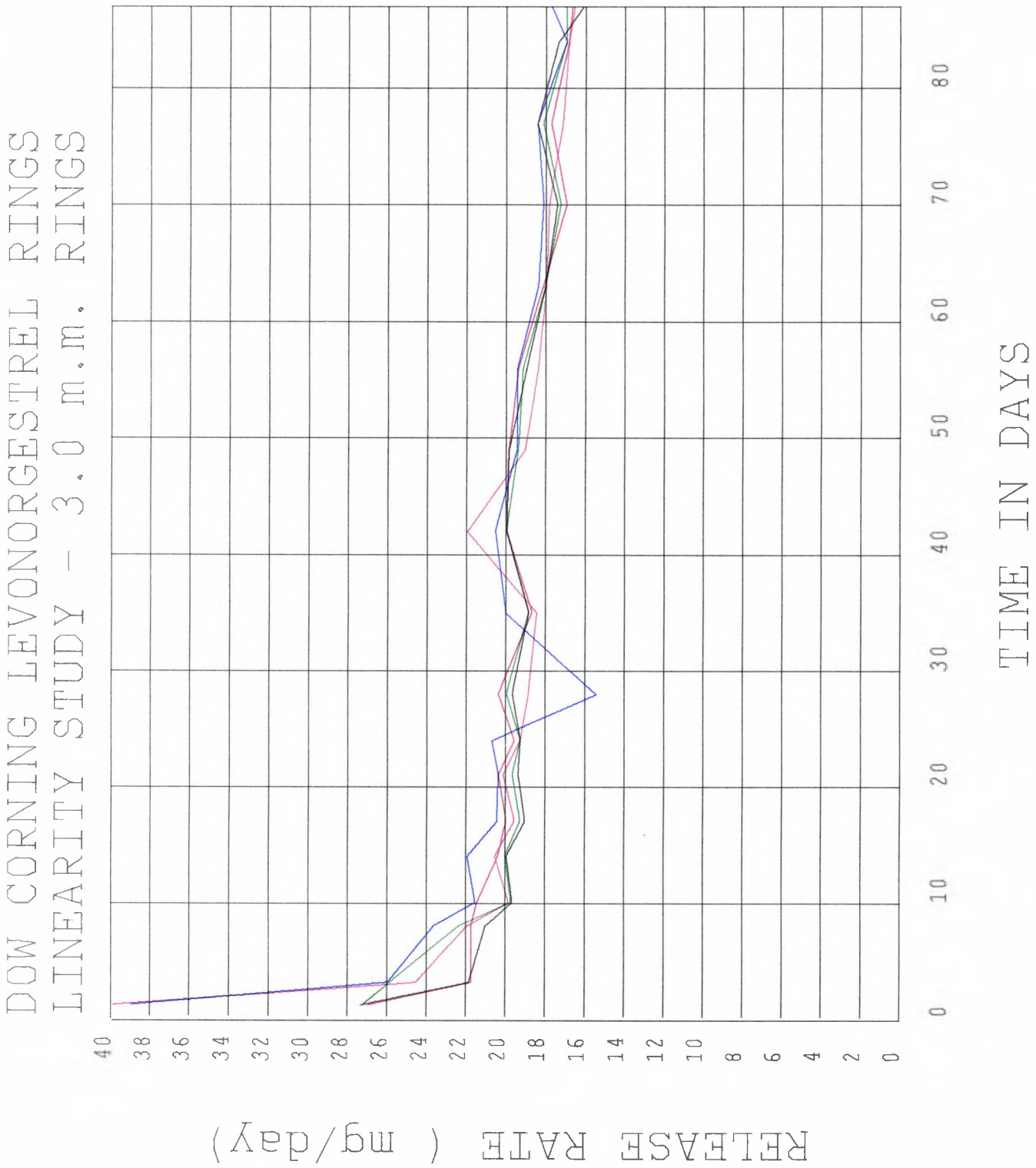

Fig. 9-6 Daily release rate of levonorgestrel 4 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

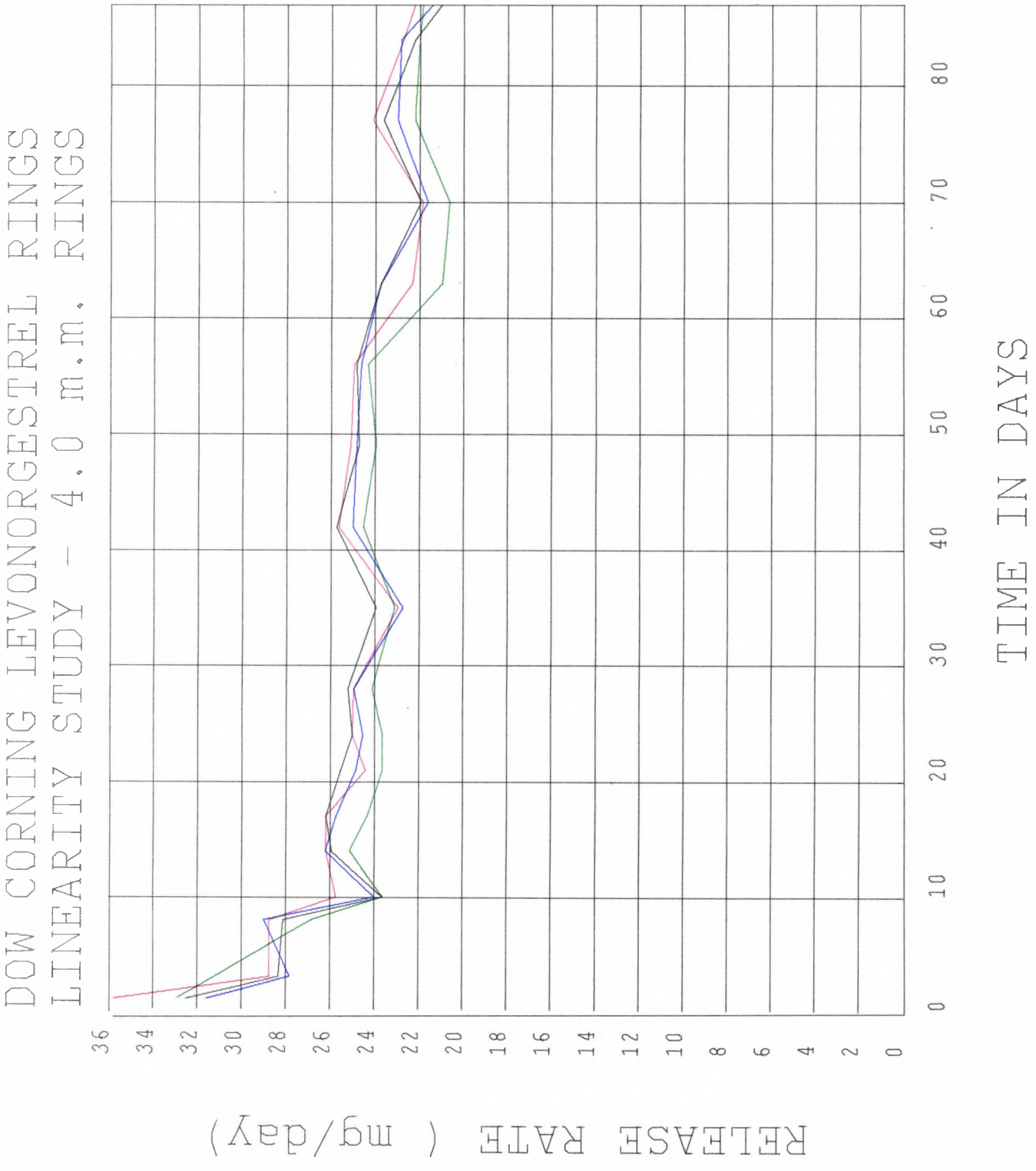

Fig. 9-7 Daily release rate of levonorgestrel 5 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

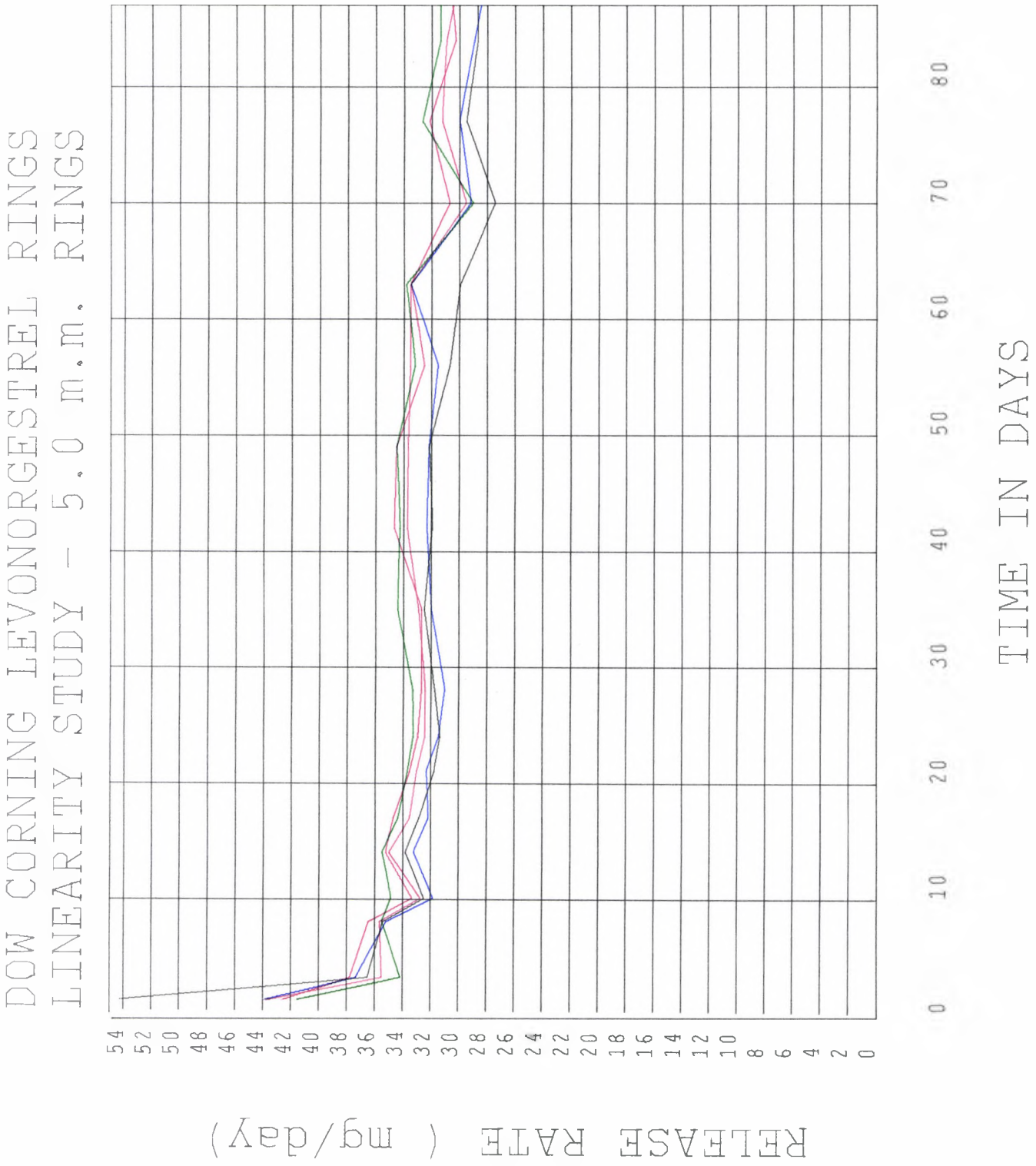

Fig. 9-8 Daily release rate of levonorgestrel 6 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

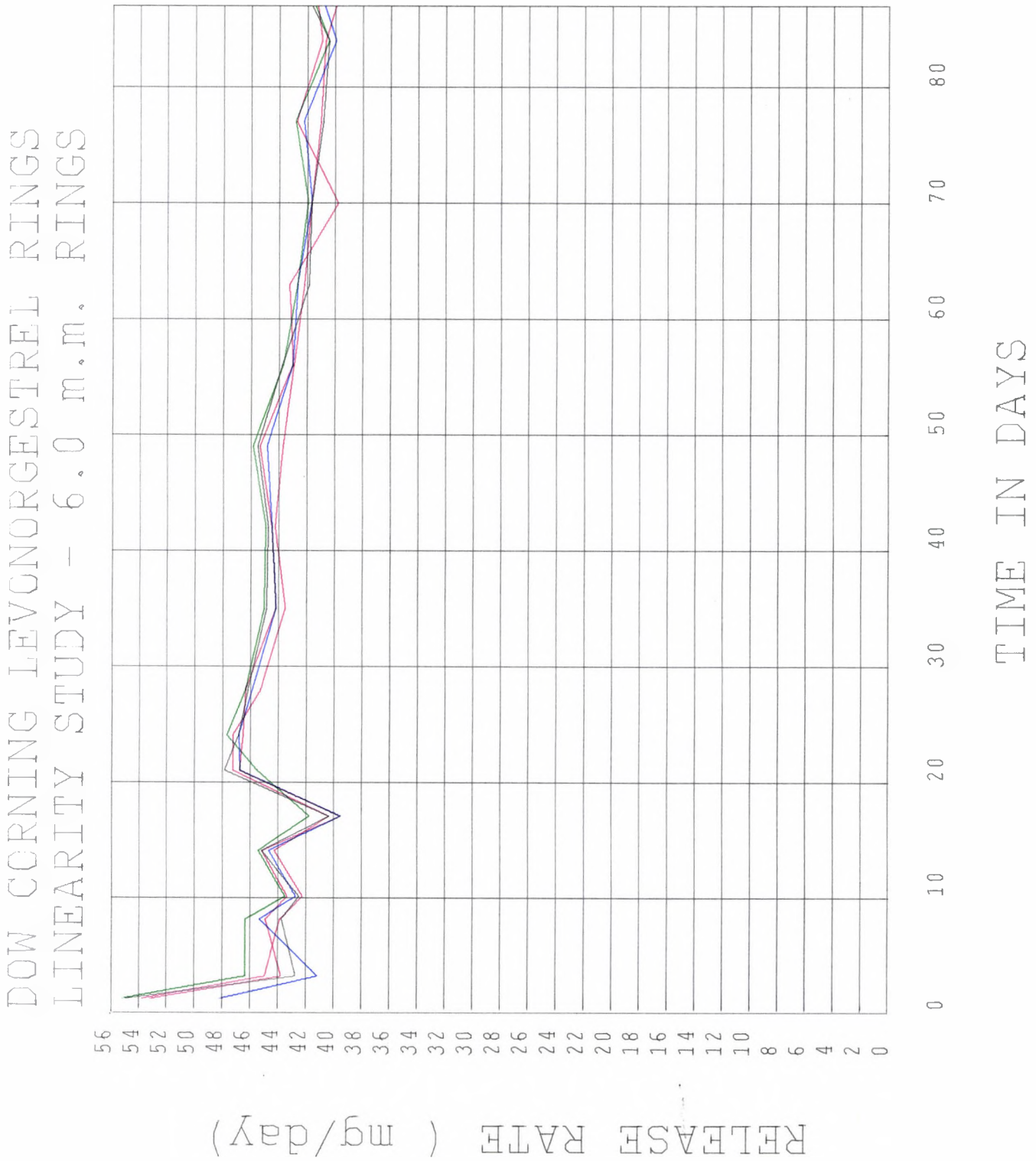

Fig. 9-9 Comparison of the daily release rate of levonorgestrel of 1 to 6 mm core diameter Dow Corning vaginal rings prepared for a linearity study.

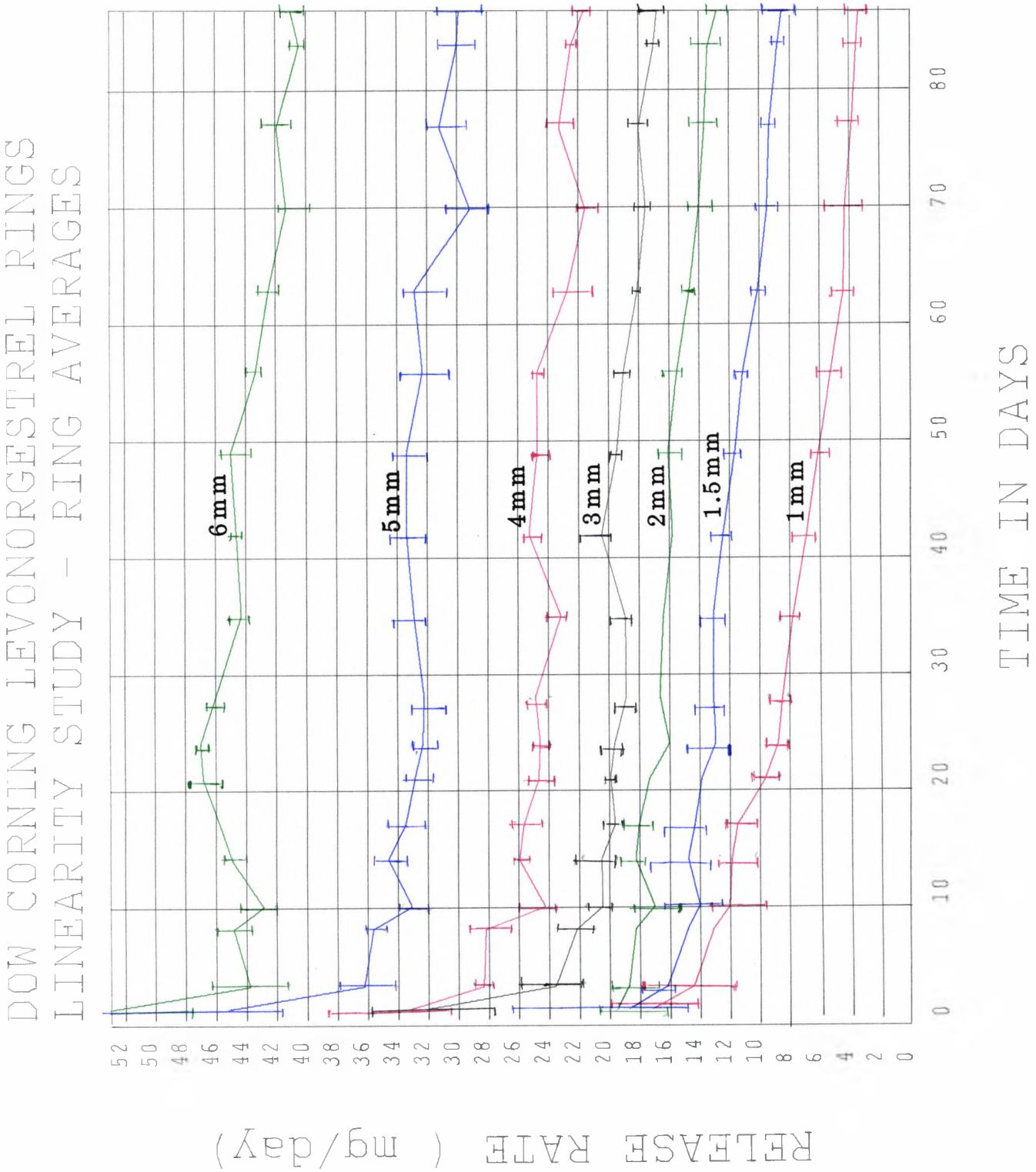

Table 9-1

Ring (mm)	Start (ml)	Change day	End (ml)
1	50	10	60
1.5	50	10	60
2	50	10	60
3	75	-	75
4	75	-	75
5	100	35	120
6	100	21	150

The plotted release rates show nearly constant behaviour for larger ring sizes , but steep decline for 1 mm rings from early on and decline in release from the 1.5 mm rings a couple of weeks later.

9.3. Determination of core diameter requirements.

The nature of the relationship between core diameter and levonorgestrel release was studied by graphical correlation. For the levonorgestrel-containing rings in the present study, a linear correlation was found between the reciprocal outer layer thickness and release rates for most core diameters (Fig. 9-10). Thus, the release of levonorgestrel from these silastic rings appears to be controlled exclusively by its rate of diffusion from the core to the outside surface. The correlation breaks down for the smallest ring sizes at the point where they have become depleted and are under-releasing as seen in day 24 and 49 (Fig. 9-10). Similar to the progesterone rings studied before, a non linear correlation was found between the release rate and the core diameter of the rings.

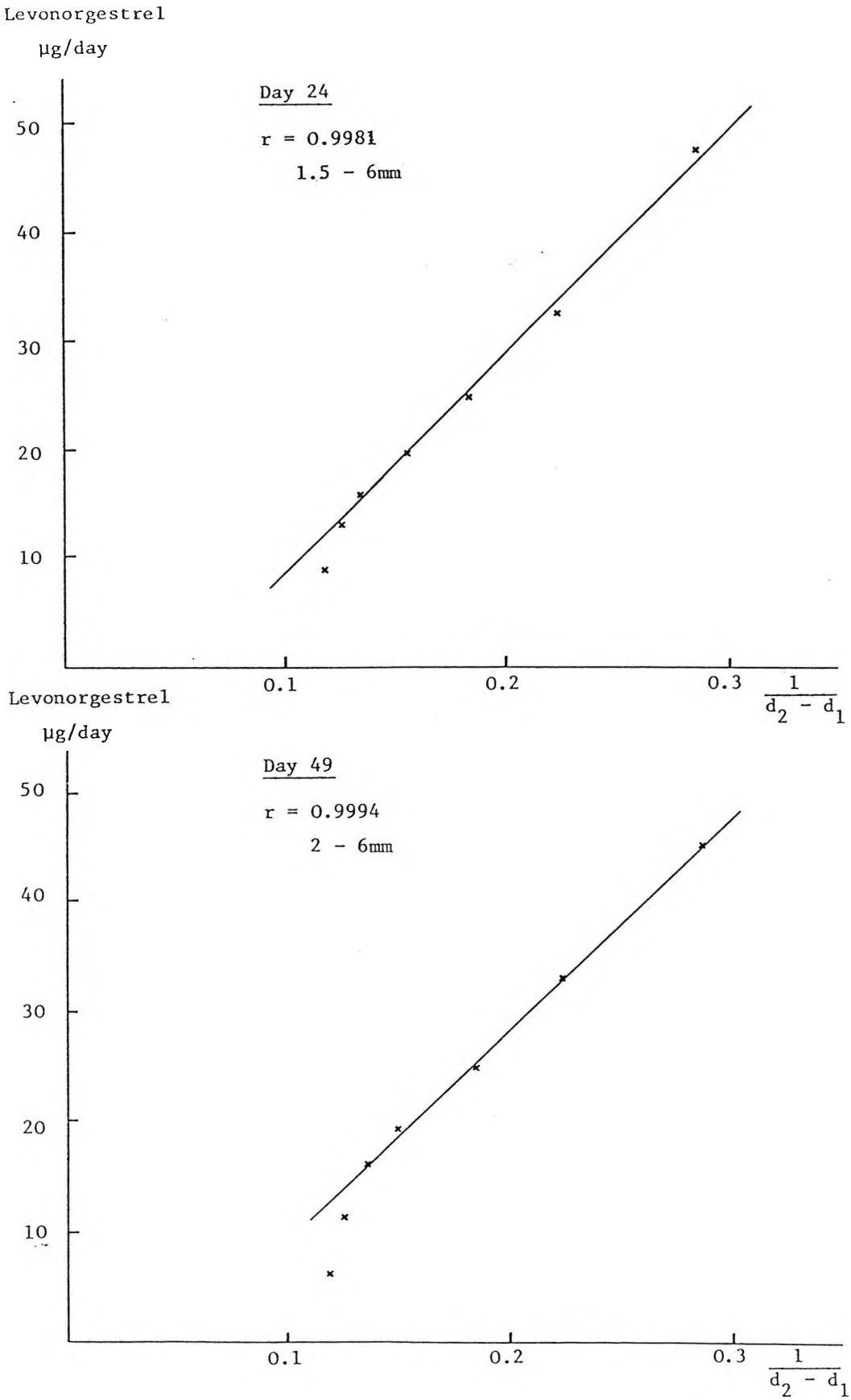

Fig. 9-10 Correlation between daily release rate and the reciprocal diffusion distance for levonorgestrel vaginal rings with fixed outside dimensions and a core diameter from 1 to 6 mm; A) at day 24, B) at day 49.

9.4. Conclusions

1. The in vitro release rates of these levonorgestrel rings were evaluated using a fixed daily volume of saline solution, complying with sink conditions. The volumes of saline solution used were adjusted to obtain similar levels of levonorgestrel concentration for the different core size rings. Eluate concentrations of levonorgestrel could not be measured by UV analysis as the levonorgestrel concentration was too small to obtain a good signal to noise level. It was neccesary to meassure the levonorgestrel concentration by HPLC analysis, with a direct, large volume injection onto a short 3 µm column, as was also used earlier for the progesterone eluates.
2. Application of the in vitro method to core-design vaginal rings fabricated by Dow Corning showed that rings of a given core diameter and fixed outside dimensions generally gave consistent release profiles. A good, linear correlation was found between release rate and reciprocal diffusion distance. For the very small core size rings, a depletion effect was observed after 20 days for the 1 mm core ring and after 40 days for the 1.5 mm core size rings.
3. From the levonorgestrel release plotted in Fig. 9-10 for the experimental core ring sizes, it is apparent that a vaginal ring with a core measuring between 3 and 4 mm would provide nearly zero-order daily release of 20 µg levonorgestrel. Subsequently, WHO has signed a contract with Roussel Laboratories (Swindon) for the manufacture of 20 µg releasing levonorgestrel rings, based on the data obtained in this study.

9.5 References

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