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NON-HUMAN PRIMATES IN CONTRACEPTIVE RESEARCH

By

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and Duane C. Kraemer*

ABSTRACT

An extensive review of the use of non-human primates in contraceptive research was compiled in December 1971. An effort has been made to update certain topics from that symposium, specifically (1) similarities and differences between humans and other primates with respect to gametogenesis and gamete transport, implantation and ovum development, sexual cycles and sexual behaviour; (2) metabolism of steroids in non-human primates; (3) certain metabolic effects of steroids in non-human primates, canines, and humans; and, (4) the relevance of certain aspects of toxicity, such as tumorigenesis, when studied in non-human primates and other species

*I. Comparative reproductive physiology*

*Introduction*

In December 1971 a symposium was convened by the World Health Organization in collaboration with the Ministry of Health of the USSR to consider the value of non-human primates as animal models for the study of problems in human reproduction. The proceedings have been published in book form (*The Use of Non-Human Primates in Research on Human Reproduction*; Karolinska Institutet, 1972) and as Supplement 166 of *Acta endocrinologica*. The

present communication is intended as a supplement to that volume, summarizing information which has become available since that time and presenting certain data not covered in that symposium. We have tried to avoid repetition as much as possible, and we acknowledge our great indebtedness to the wealth of information which was compiled. Other relevant surveys which have appeared since that time deal with comparative anatomy of the reproductive tract of non-human primates (*Hill 1972*), comparative biology of non-human primates (*Hafetz 1971*), the biology of human conception and contraception (*Hafetz & Evans 1973*), and the pharmacology of progestational compounds in man and non-primate laboratory animals (*Tausk 1972*).

#### *Gametogenesis*

Basically, the process of gametogenesis appears to be very similar in man and other primates, although there are some differences in detail, and there is much that is not documented in many species. There are two recent reviews of gametogenesis in non-human primates (*Butler 1971; Baker 1972*). It is unlikely that gametogenesis is the same in any non-human primate species as in man, if for no other reason than that the chromosome number (in the 110 of the 180 living primate species for which chromosome number is known) is never  $2n = 46$ , as in man (*Chiarelli 1971*). The  $2n$  chromosome number for the apes varies from 44 to 52; Old World monkeys vary from 42 to 72, New World monkeys from 44 to 62, and prosimians from 38 to 62 (*Buettner-Janusch 1963; Hill 1966; Chiarelli 1971*). In addition to differences in chromosome number, there are also differences in chromosome morphology and DNA content. Chimpanzee and human karyotypes have been compared in some detail. Ten of the chromosomes show remarkable similarity, seven display some analogy, and six appear quite different (*Chiarelli 1971*). The remarkable similarity between the features of a chimpanzee with trisomy of a small acrocentric chromosome and Down's syndrome in man (trisomy-21) demonstrates marked genetic homology of a particular chromosome in these species (*McClure et al. 1973*). *Weiss et al. (1973)* have also described a rhesus monkey with an XO anomaly, ovarian dysgenesis, and other stigmata resembling Turner's syndrome in man. The most striking differences between gametogenesis in non-human primates and man are (1) the timing of various events in the process, probably related to differences in gestation length and age at puberty and (2) the seasonality of gonadal activity in some of the non-human primate species.

#### *Gamete transport*

Transport of sperm in the female reproductive tract, including that in man and non-human primates, has been reviewed recently by *Hafetz (1973a)*. Anatomically, the uterine cervix is more complex in the smaller non-human primates than in the apes and man. However, the basic epithelial structure of the

cervical mucosa appears to be similar, as is the percentage of ciliated cells: 7-9% in the macaque and 5% in women (Fluhmann 1961; Hafez 1973b). The *Macaca senica* and *Macaca radiata* appear to be unique in that they produce large amounts of cervical mucus (Hafez 1973a; Ovadia et al. 1971). Jaszczak & Hafez (1973) have studied sperm migration through the uterine cervix of *Macaca fascicularis*; however, comparable studies have not been reported for man. The pattern of movement of the spermatozoa through the cervical canal was primarily random. However, in the mid-cervix and endocervix many spermatozoa were aggregated along strands of cervical mucus. Some of these spermatozoa were migrating directly into the uterus, while others were moving along strands which led into the cervical crypts. The pattern of sperm movement through the uterus has not been determined in the primate. Appearance of spermatozoa in the human oviduct occurs within 5 minutes of insemination in women (Settlage et al. 1973). Sperm distribution is uniform throughout the oviduct by 15 minutes after insemination and this state is maintained for at least 12 hours. Manabe & Mastoianni (1967) recovered occasional spermatozoa from rhesus monkey oviducts 90 min after artificial insemination. Hafez (personal communication) observed 200 or less spermatozoa in serially sectioned *Macaca fascicularis* oviducts 3 hours after mating. Sperm distribution and ovum transport in human and non-human primates are apparently unaffected by plastic intrauterine devices (Marston et al. 1969).

Anatomically, the non-human primate oviduct is generally more tortuous than that of man, with the exception of the marmoset and squirrel monkey (Hill 1972). Transport of the ovum through the oviduct requires approximately 8 days in both man and non-human primates, although the lack of a method for exact timing of ovulation has limited the precision of this determination (Croxatto et al. 1972; Hendrickx et al. 1971; Hartman 1944). Pharmacologically, the oviduct of the rhesus monkey and man tend to respond similarly to epinephrine and pituitrin (Woodruff & Pauerstein 1969). For a recent bibliography and review of the physiology and pharmacology of the primate oviduct, see Coutinho (1972).

#### *Implantation and ovum development*

Implantation has been covered in great detail by Blandau (1972) and in the commentary by Lindner (1972) in the Symposium, and no significant addenda can be made at this writing. This subject is of particular interest for problems of teratogenesis, which have received a great deal of attention following the thalidomide tragedy.

Interpretation of the findings in non-human primates depends on proper staging of embryogenesis, and considerable work has been done along these lines (Hendrickx 1972 and in press). All four macaque species studied to date develop on the same time scale, which is somewhat faster than that of man.

The galago and African green monkey embryos develop at a rate more like that of man. This is remarkable in that the gestation period for the galago is  $133 \pm 4$  days and for the green  $155 \pm 7$ , compared to 165 for macaques and 175 for baboons. The embryo development rate evidently must be accelerated in both galago and green.

Non-human primate teratological studies have largely been directed toward developing a model using thalidomide as the noxious agent, to show human relevance. *Poswillo et al.* (1972) have recently shown the marmoset to be sensitive to thalidomide, bringing the list of primates to nine (man, baboon, rhesus, four other macaques, African green and marmoset). Only the galago has been insensitive. The sensitive period for the marmoset is poorly defined; for all other species it falls in the interval between 24 and 30 days except in the green monkey, whose slower developmental rate moves the sensitive period up to 30-33 days. Other studies (*Poswillo* 1972) have shown that solanine, the toxin in spoiled potatoes, produces malformations in the marmoset which are similar to those in man. Corticosteroids have been associated with cleft palate in humans, and triamcinolone has now been shown to produce similar orofacial defects in the baboon and the bonnet monkey (*Hendrick*, in press), as well as in the usual laboratory rodents.

### *Sexual cycles*

Exhaustive studies of the integrated mechanism which controls reproductive cycles in rat (*Schwartz* 1969) and man (*Vande Wiele et al.* 1970) have been published. Investigation of the mechanism in non-human primates has been made possible by radioimmunoassay techniques for measurement of gonadal steroids in plasma and for assay of releasing factor and gonadotrophic activities. Since the results of experiments in this field are so critically dependent on the precise experimental situation, on dose-duration characteristics, on the time of the oestrous or menstrual cycle (or absence thereof), and on previous hormonal events, it is extremely difficult to compare experiments performed in the various species.

Much of our knowledge of neurophysiological control of the ovarian cycle rests upon studies in rodents, for example the functions of the preoptic nucleus as a "biological clock" regulating the tonic output of the ventromedial-arcuate region. Studies in the baboon by our colleague, *N. Hagino*, indicate substantial differences in the effect of electrolytic lesions and deafferentation: in contrast to the rat, injury to the area of the preoptic nucleus of the baboon produces transient or a delayed effect on menstrual cycle rhythm (unpublished). Another important difference between these two species is the inability of constant illumination to alter ovarian cycles in the baboon, whereas the phenomenon of anovulatory persistent oestrus is readily produced in the rat (*Hagino* 1971). On the other hand, a "critical period" during rat prooestrus (the time

when ovulation can be blocked by pentobarbital) has now been shown to exist in a non-human primate (Baboon) as well. A further interesting development has been the demonstration that synthetic (but not natural) corticosteroids can block induced ovulation in the immature rat; a similar observation has now been made in non-human primates (*Hagino 1972*). In the baboon, the blocking effect of triamcinolone acetonide can be overridden by administration of gonadotrophin releasing factor (unpublished).

Synthetic gonadotrophin releasing factor has been shown to be active in a wide variety of animal species including man. In the rhesus, for some reason, there seems to be less response than in other species tested (*Ehara et al. 1972*).

The pattern of plasma levels of FSH, LH, oestrogen and progesterin has been examined throughout the cycle in numerous species, and remarkable similarities may be observed in the data from rat, macaque, baboon, chimpanzee, and man. Quantitative differences exist, as may be expected. Qualitative differences in steroid response have been touched upon elsewhere. In rhesus, baboon, and man, independence of corpus luteum function from a uterine factor has been convincingly demonstrated. The similarity of corpus luteum function during pregnancy between human and rhesus has also been commented upon (*Knobil 1973*).

Rapid pulsatile discharge of FSH and LH in the normal and/or the castrate situation has been demonstrated in both human subjects and rhesus. The dynamics of positive and negative feedback effects of oestrogens and progesterone or synthetic progestins have been extensively investigated in the rat and in man, and recently by *Knobil* and his group in the rhesus. *Karsch et al. (1973)* have shown that in the rhesus, as in man, "the negative feedback control of LH secretion by oestrogen is a finely tuned system with a set-point which appears to change as a function of time after castration." Differences in the effect of various steroids from rat to rhesus to man (*Karsch et al. 1973*, *Leyendecker et al. 1972*; *Nillius & Wide 1971*) may, as indicated above, be the consequence of small but important differences in experimental conditions, and are difficult to interpret.

*Nixon et al. (1971)* examined the similarity of antigenic determinants in pituitary and chorionic gonadotrophins from primates. A marked similarity between chorionic and pituitary gonadotrophins exists both in the chimpanzee and in man. Pituitary gonadotrophins from rhesus, chimpanzee, baboon and man gave similar responses in radioimmunoassay (RIA) and these were similar to the response to HCG. The RIA slope of chimpanzee chorionic gonadotrophin was also similar to that of HCG, while the responses of baboon and rhesus chorionic gonadotrophin were different. These data suggest a large degree of similarity in antigenic sites, and thus by inference in primary structure, of pituitary gonadotrophins in several primate species. Substantial differences appear to exist, however, in the gonadotrophins of chorionic origin.

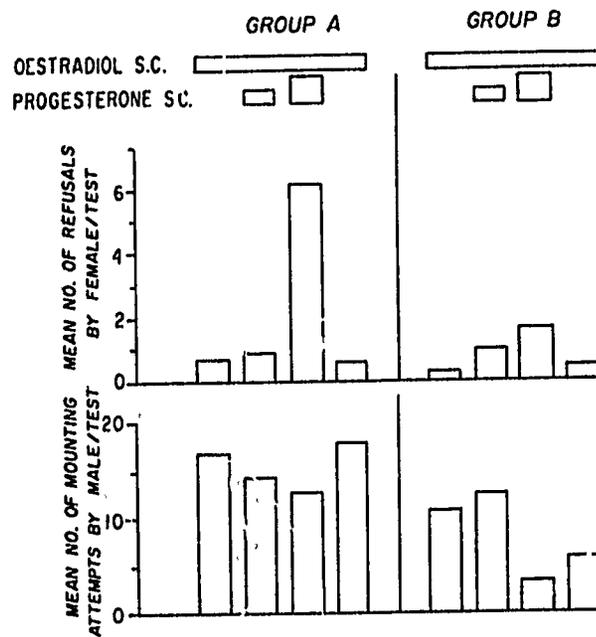
### *Sexual behaviour in human and non-human primates*

As one ascends the phylogenetic scale, it is generally believed that gonadal hormones assume less importance in the interaction between sexes. However, studies of the sexual behaviour of non-human primates (reviewed by *Michael et al.* 1972 and *Goy & Resko* 1972) demonstrate that endocrine factors play a significant role in their behaviour.

In lower primates the endocrine control of sexual behaviour is somewhat similar to that in non-primate mammals. Thus, in *Lemur catta*, which has an ovarian cycle length of 33–41 days, receptivity to the male is limited to about twelve hours (*Evans & Goy* 1968). In *Galago crassicaudatus*, in which oestrus persists for about 12 days, the onset of sexual behaviour occurs after 3–4 days of endogenous oestrogen stimulation. The intensity of behaviour reaches its peak promptly and remains elevated for 1–2 days, after which there is a rapid decline. In contrast, many monkeys and apes copulate throughout the menstrual cycle, although completed copulation is increased at mid-cycle and diminishes shortly after ovulation. In studies of *Michael et al.* (1972) on thirty-two pairs of rhesus monkeys followed over a five-year period, the maximum incidence of ejaculation occurred 17 days before onset of the next menstruation. Frequency then declined progressively throughout the luteal phase and the nadir was reached two days before menstruation. In other studies on rhesus monkeys (*Goy & Resko* 1972), the percentage of tests culminating in ejaculation increased markedly at the time when female oestrogen levels reached their peak. The establishment of the corpus luteum was promptly followed by a rapid decline in the incidence of full copulatory activity. However, the inhibitory effect of the corpus luteum on sexual behaviour was incomplete. Some pig-tailed macaques continue to copulate for prolonged periods even after bilateral ovariectomy. The effect of pregnancy on primate sexual behaviour is generally one of suppression of ordinary sexual activity with occasional passive cooperation with the male.

Various behavioural parameters have been used to study the effects of endogenous and exogenous hormones on sexual behaviour (*Michael et al.* 1972). Since the number and frequency of female invitational gestures depend not only on the attitude of the female to the male, but also upon the male's attitude towards the female, several behavioural parameters of the pair need to be observed in order to assess hormonal effects on female behaviour. Ovariectomy markedly reduces the interactions between males and females. Administration of oestradiol to females restores both male sexual activity and female receptivity and attractiveness; progesterone treatment counteracts the effects of oestrogen (Fig. 1).

Implantation of steroid hormones in specific areas of the female rhesus monkey brain has been used to study direct effects of hormones on neural mechanisms. The behaviour of the male partner is influenced by oestrogenizing



Redrawn from: R.P. Michael, 1971

Fig. 1.

Two types of response to the administration of progesterone in castrate, oestrogen-primed rhesus monkeys. In one group, female rejection was predominant, while in the other, male disinterest was the major effect of progesterone.

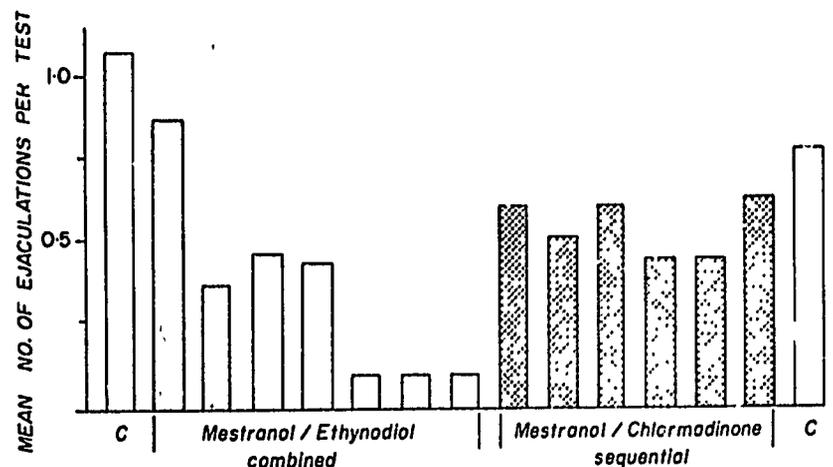
the deeper, phylogenetically older parts of the female brain. Testosterone propionate, administered intracerebrally (hypothalamus, amygdala and thalamus) elicited a well-marked increase in female invitations but only a moderate increase in male mounting behaviour. However, the intracerebral testosterone implants raised plasma testosterone levels so that interpretation of the results is equivocal (Plant & Michael 1971).

The effects of oestrogen on female sexual motivation have been studied in a free-cage operant conditioning situation where the female rhesus monkey was trained to press a lever and operate a partition which permitted free movement of the female between compartments but obstructed passage of the male. A marked increase in operant behaviour by the female was observed during a period of time around ovulation which correlated with increased excretion of urinary oestrone and increase in serum oestradiol. A similar dose-dependent behavioural response was produced in ovariectomized females by graded amounts of oestradiol.

The influence of sexual behaviour by olfactory communication *via* phero-

mones has been recognized in the breeding of farm animals. Prosimians are believed to possess specialized apocrine scent glands which are used for self marking, territorial marking and for marking each other. However, as one ascends the phylogenetic scale, the size of rhinencephalic structures is correspondingly reduced. Olfactory stimuli might therefore be expected to play a lesser role in communication between male and female non-human primates. However, under both natural and laboratory conditions males of several species of macaque display scenting activity related to oestrogen-induced stimuli aroused by vaginal secretions. Such substances have been termed "copulins" and five or six short-chain aliphatic acids have been identified. It has been possible to obtain behavioural effects in the rhesus monkey by using vaginal secretions from baboons (Kevenic & Michael 1971).

In humans, sexual behaviour is the result of complex interactions of endocrine, sociocultural and psychological factors. Women are reported to display a premenstrual peak in sexual desire when levels of endogenous ovarian hormone are low and falling rapidly (Davis 1929). Ovarietomy or spontaneous menopause do not significantly alter sexual response (Masters & Johnson 1967). On the other hand, adrenalectomy or hypophysectomy decrease libido and orgasm, suggesting that adrenal androgens may be endocrinologically important.

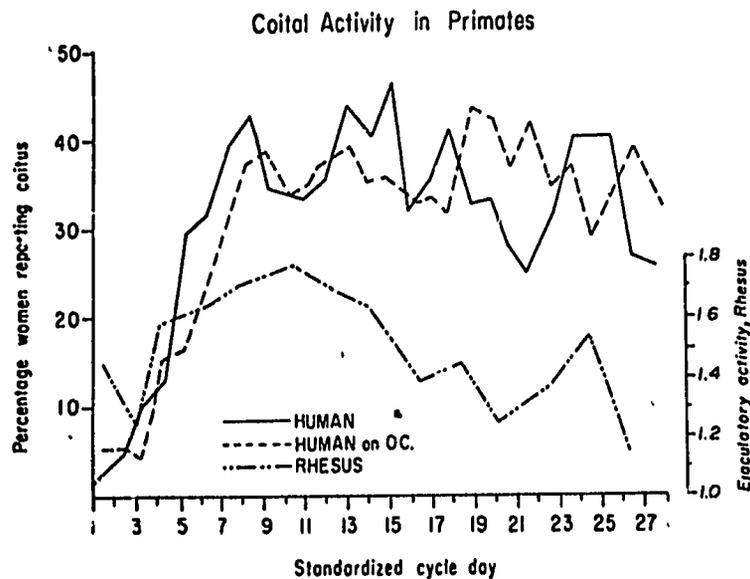


Redrawn from R.P. Michael, 1971

Fig. 2.

Effect of contraceptive steroid preparations on sexual behaviour in rhesus monkeys (after Michael). Administration of an agent which incorporated a synthetic progestin throughout the treatment cycle had a depressant effect on sexual activity, while a "sequential" preparation, in which progestin administration occurs only at the end of the regimen, did not.

The effect of progestational substances in decreasing sexual activity in laboratory primates has been amply demonstrated (Fig. 2). Widespread use of contraceptive preparations containing a variety of progestational substances has afforded an opportunity for related studies in humans. The vast clinical literature on the psychogenic effects of oral contraceptives is largely of an anecdotal character, and its interpretation is highly equivocal (Goldzieher *et al.* 1971). However, a few well designed studies are relevant. Nilsson & Solvell (1967) administered four oral contraceptives varying widely in oestrogen and progestin content in a randomized, double-blind crossover study; no changes in libido were demonstrated upon institution of the medication, nor were there any differences between the four preparations. Udry & Morris (1970) carried out an even more significant investigation of sexual activity in 51 women who were double-blind and randomly assigned to placebo, sequential, or combination-type oral contraceptives (Fig. 3). In the placebo group the observation of interest is a transient fall in coital frequency in the mid-luteal phase. Surprisingly, this depression in coital activity was abolished by both contraceptive



(After Michael, 1971; Udry & Morris, 1970)

Fig. 3.

Coital frequency, by cycle day, in a group of women randomly assigned placebo or oral contraceptive medication (after Udry & Morris). In the placebo group, a depression in activity during the mid-luteal phase is observed. This is abolished in the contraceptive users; there was no difference between combinations of sequential agents. For comparison, a curve of rhesus coital activity during the cycle is shown.

preparations. It is to be noted that with one medication, progestational exposure was continuous from menstrual cycle day five for 20 days. With the sequential preparation, no progestational steroids were given until the 20th day of the cycle. That *both* these agents should abolish the normal mid-luteal depression is interesting in itself; in addition these data cast substantial doubt on the role of both endogenous progesterone and administered progestational steroids on human coital frequency. The progestational agent was a 19-norsteroid; it would be interesting to know if a 17-acetoxyprogesterin exerted the same influence. A comparison with the sexual activity of the macaque is shown in the figure.

Thus, while the meager clinical evidence available is at variance with the clear-cut effect of progestins in non-human primates, the difficulty in obtaining valid information in the human cannot be underestimated. Richter (1967) has demonstrated the profound importance of extraneous factors. In this study, oral contraceptives were administered to a large population of women, and the colour of the tableted medication was randomly changed every six months. With each change in the appearance of the medication, a sharp decrease in the libido index resulted, and this phenomenon could be reproduced four times over a two-year observation period. In sum, one may infer that the

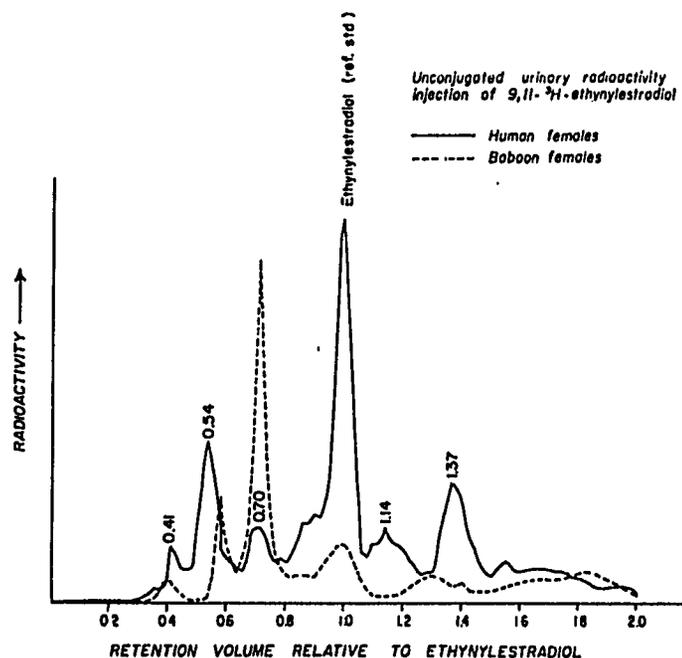


Fig. 4.  
Separation by chromatography on Sephadex LH-20 of radioactivity in baboon and human urine after administration of tracer doses of labelled ethynylestradiol. Analysis of the fraction excreted as free (*i. e.* unconjugated) steroid.

**Table 1.**  
Metabolic fate of some oestrogens and progestins in various primates.

Species	Steroid administered	Excretion		Comment
		Per cent in urine (days)	Per cent in faeces (days)	
<i>Homo sap.</i> <sup>1,2)</sup>	Oestrone, Oestradiol	50-80 (4-6)	15-20 (4-6)	MCR 1000-1400 L/d; plasma chiefly as E <sub>1</sub> -SO <sub>4</sub>
<i>Pan satyrus</i> <sup>3)</sup>	Oestrone	72 (3)	-	Little urinary E <sub>3</sub>
<i>M. mulatta</i> <sup>4)</sup>	Oestradiol	48-100 (5)	5 (4)	At first E <sub>1</sub> , later epiE <sub>3</sub> important
<i>Papio sp.</i> <sup>5,6)</sup>	"	50-70 (3)	-	Mostly E <sub>1</sub>
<i>Homo sap.</i> <sup>7,8)</sup>	Ethinylloestradiol	23-60	9 (5)	MCR = 1350 ± 220 L/d; EE <sub>2</sub> -SO <sub>4</sub> chief plasma constituent
<i>Papio sp.</i> <sup>6)</sup>	"	77-83 (6)	-	
<i>Homo sap.</i> <sup>7,8)</sup>	Mestranol	10-52 (5-8)	-	MCR = 1740 ± 390 L/d; EE <sub>2</sub> -SO <sub>4</sub> chief plasma constituent
<i>Homo sap.</i> <sup>9,10)</sup>	Progesterone	50-60 (5-7)	10	MCR = 1740 ± 140 L/d; mostly pregnanediols in urine
<i>P. satyrus</i> <sup>11)</sup>	"	33-50 (3)	-	Mostly urinary pregnanediols
<i>Papio sp.</i> <sup>5,12)</sup>	"	33-41 (4)	14-17 (4)	Urinary androsterone important. Pregnanediols insignificant
<i>M. mulatta</i> <sup>13,14)</sup>	"	28-46 (9)	41-57 (9)	"
<i>M. nemestrina</i> <sup>15)</sup>	"	44-60 (6)	17 (5)	"
<i>M. mulatta</i> <sup>16)</sup>	Chlormadinone acetate	35-36 (7)	26-28 (7)	
<i>M. mulatta</i> <sup>16)</sup>	Megestrol acetate	14-33 (6)	36-44 (6)	
<i>Papio sp.</i> <sup>16)</sup>	" "	33-43 (5)	0.4-2.8 (5)	
<i>Homo sap.</i> <sup>17)</sup>	Trengestone	50 in ♀, 10 in ♂		20α and β reduction; in human only 20α; in rat, 16α-hydroxylation only

influence of oestrogens and progestins on the level of human sexual activity is minimal, certainly far less important than a basal level of androgens in the female, and that environmental, psychosocial, and other factors are probably of overriding importance.

## II. Metabolism of steroids in non-human primates

The fate of oestrogenic and progestational steroids has been examined to some extent in several species (Table 1). With respect to the *natural* oestrogens (oestrone and oestradiol), data are available for rhesus, baboon, and chimpanzee. In all three, more than half of an intravenous tracer dose appears in the urine within four to six days. On a semilog plot, urinary excretion appears to be almost linear. Approximately 18% of administered oestrogenic radioactivity appears in the faeces in man; data are not available for the other species. The urinary metabolites are mostly glucuronides in all species tested. However, the pattern of individual urinary metabolites differs considerably. In man, approximately 20% of radioactivity is oestrone, 20% oestriol, 20% 2-hydroxyoestrone, and 5% 2-methoxyoestrone. The gorilla appears to be the only species which, like man, has a substantial ability to 16-hydroxylate, forming urinary oestriol. The increase in urinary oestriol during pregnancy in the gorilla is similar to that in man; it is not seen in pregnancy in the other primates which have been examined. In the baboon, the urinary radioactivity

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### Notes to Table 1.

- <sup>1)</sup> Longcope G., Layne D. S. & Tait J. F.: J. clin. Invest. 47 (1968) 93.
- <sup>2)</sup> Ruder H. J., Loriaux L. & Lipsitt M. B.: J. clin. Invest. 51 (1972) 1020.
- <sup>3)</sup> Jirku H. & Layne D. S.: Steroids 5 (1965) 37.
- <sup>4)</sup> Laumas K.: Gen. comp. Endocr. 2 (1969) 141.
- <sup>5)</sup> Kulkarni B. D., Kammer C. S. & Goldzieher J. W.: Gen. comp. Endocr. 14 (1968) 68.
- <sup>6)</sup> Kulkarni B. D.: J. Endocr. 48 (1970) 91.
- <sup>7)</sup> Goldzieher J. W. & Kraemer D. C.: Acta endocr. (Kbh.) Suppl. 166; 71 (1972) 389.
- <sup>8)</sup> Bird C. E. & Clark A. F.: J. clin. Endocr. 36 (1973) 296.
- <sup>9)</sup> Klopffer et al.: J. Endocr. 12 (1955) 209.
- <sup>10)</sup> Lin T. J., Billiar R. B. & Little B.: J. clin. Endocr. 35 (1972) 879.
- <sup>11)</sup> Romanoff L. P., Grace M. P., Sugarman E. M. & Pincus G.: Gen. comp. Endocr. 3 (1963) 649.
- <sup>12)</sup> Goldzieher J. W. & Axelrod L. R.: Gen. comp. Endocr. 13 (1969) 201.
- <sup>13)</sup> Reddy S. V., Balin H. & Nes W. R.: Steroids 17 (1971) 493.
- <sup>14)</sup> Plant T. M., James V. & Michael R. P.: J. Endocr. 51 (1971) 751.
- <sup>15)</sup> Jeffery J.: J. Endocr. 34 (1966) 387.
- <sup>16)</sup> Unpublished data.
- <sup>17)</sup> Knuppen R. & Breuer H.: Excerpta Med. Int. Congr. Series 210 (1970), p. 234, abstract no. 505.

is mostly oestrone; oestriol is a very minor constituent. Similarly, judging from the oestrogen excretion in normal ovulatory cycles in chimpanzees, oestriol appears to be a minor metabolite. The rhesus shows an unusual pattern. In the first 24 hours after administration of radioactive oestrogen the major metabolite is oestrone, but thereafter there is a gradual increase in epioestriol, which after several days constitutes 33 to 50 % of the urinary metabolites.

A few estimates of plasma clearance of radioactive oestrone or oestradiol have been carried out. In man, the biphasic curve of plasma radioactivity disappearance yields two half-lives of 20 and 70 minutes. In rhesus, oestrone yields half-lives of 8½ and 34 minutes; approximately the same has been found for oestradiol. Total plasma radioactivity from oestradiol has an estimated 90-minute half-life for the slower component in baboons.

*Ethynloestradiol* and its 3-methyl ether, *mestranol*, are widely used in clinical contraceptive preparations, and their metabolism is of major interest. Very few data are available in non-human primates; these were summarized by Goldzieher & Kraemer (1972). In man, 25 to 50 % of ethynloestradiol radioactivity appears in the urine within 5 days, and a semilog plot gives a nearly linear relationship. Approximately half of the radioactivity appears

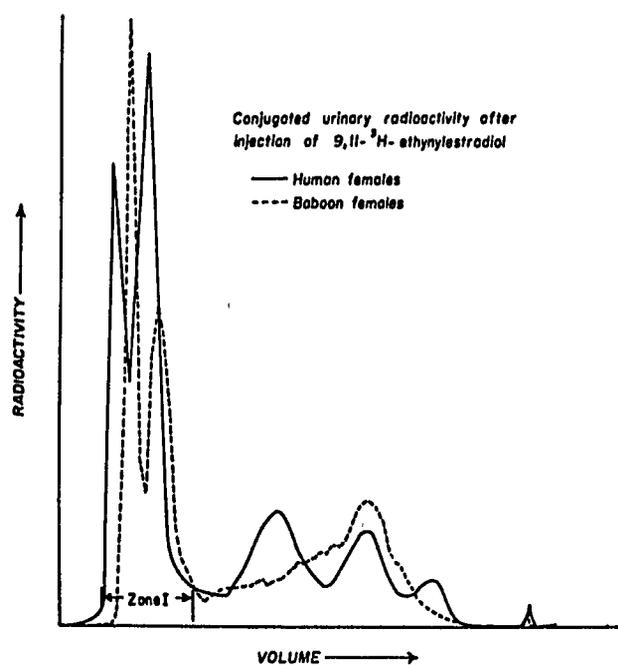


Fig. 5.

Chromatographic separation of radioactivity in baboon and human urine after administration of tracer doses of labelled ethynloestradiol. Fraction excreted as conjugated radioactivity.

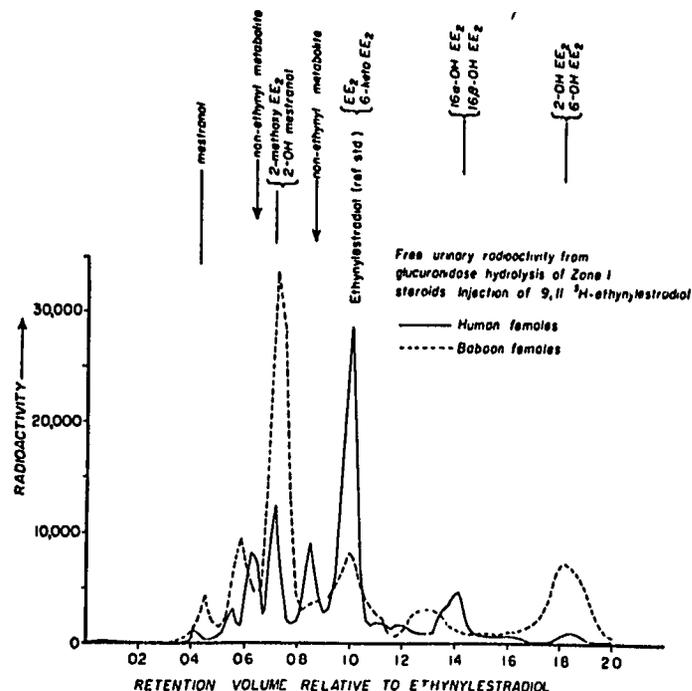


Fig. 6.

Sephadex LH-20 chromatography of radioactivity in baboon and human urine after administration of tracer doses of ethinyloestradiol. The major peak seen in Fig. 5 has been hydrolyzed with glucuronidase, purified, re-extracted, and chromatographed in the system used for free steroids. The location of relevant marker compounds in this system is indicated at the top of the figure.

during the first day. In two baboons, *Kulkarni* (1970) reported that 77 to 83 % of the radioactivity appeared in the urine within six days, suggesting a slightly faster turnover than in man. The biological half-life, as judged by urinary studies, ranged from 20 to 44 hours. In man as in the baboon, most of the urinary metabolites are glucuronides, with 10-15 % as sulphates. However, there appear to be qualitative differences in the relative proportion of various conjugates and metabolites, as judged by chromatographic separation. Fig. 4 shows the pattern of unconjugated urinary radioactivity after ethinyloestradiol administration to several normal adult human females, compared to a urine collection from a female baboon. Fig. 5 shows the pattern of conjugated radioactivity. The major peak is almost exclusively glucuronide. When this is hydrolyzed and re-chromatographed, certain differences are observed between baboon and human (Fig. 6).

Mestranol has been studied only in man and the baboon. In man, from 10 to 40 % of injected radioactivity appears in the urine in five to eight days.

The biological half-life, as estimated from urinary excretion, has ranged from 44 to 71 hours. Urinary metabolites in man seem to consist chiefly of ethynyl-oestradiol and the metabolites of that substance. Mestranol apparently does not appear as such in the urine. About 18% of continuously infused mestranol is converted to plasma ethynyl-oestradiol (*Bird & Clark 1973*). This compares quite favourably with the physiological conversion of oestradiol to oestrone. The metabolic clearance rate of mestranol (*Bird & Clark 1973*) has been shown to be higher than that of ethynyl-oestradiol in man ( $1700 \pm 400$  L/d, compared to  $1350 \pm 220$  L/d). Our studies in man indicated a much lower initial plasma level of mestranol radioactivity, but a disappearance rate about the same as for ethynyl-oestradiol. In the baboon, the plasma levels following the administration of mestranol were also considerably lower than those after ethynyl-oestradiol; the disappearance rate could not be determined satisfactorily. There seems to be some discrepancy in the accumulated data: urinary excretion of mestranol radioactivity seems to be slower than that of ethynyl-oestradiol in both species; plasma radioactivity levels with mestranol are considerably lower in both baboon and man than for ethynyl-oestradiol. On the other hand, the metabolic clearance rate for mestranol is higher than that of ethynyl-oestradiol in man. Possibly, this represents rapid sequestration of the mestranol in peripheral tissues, such as fat, followed by further metabolism and excretion.

These preliminary findings of differences in the urinary metabolite pattern between baboon and man are not surprising, since conjugation of other substances is also known to vary among the non-human primates. The conjugation of sulphadimethoxine (*Williams 1967*) is an example (Table 2).

Table 2\*.  
Conjugation of sulphadimethoxine in various species.

Species	% of drug excreted in 24 h	% composition of 24-h excretion	
		N <sup>1</sup> -glucuronide	N <sup>4</sup> -acetyl
Man	25	70	21
Baboon	42	72	16
Rhesus monkey	42	70	21
Capuchin monkey	8	48	15
Squirrel monkey	9	51	37
Rat	9	7	46
Rabbit	43	0	94
Dog	23	19	0

\* From *Williams (1967)*.

The metabolism of ethinyloestradiol has been studied in blood as well. *Bird & Clark (1973)* have shown that the metabolic clearance rate of ethynyl-oestradiol in man ( $1350 \pm 220$  L/d) is not significantly different from that of oestradiol itself. Moreover, the major circulating metabolite of ethinyloestradiol is the sulphate, a situation which corresponds closely to the physiological relationship between endogenous oestradiol and plasma oestrone sulphate. The conversion rate of  $EE_2$  to  $EE_2$  sulphate is similar to that of oestrone to oestrone sulphate. In baboons, we have found that levels of radioactivity in the blood after administration of labelled ethynyl-oestradiol are considerably lower than those following oestradiol administration. This may well be related to the more rapid turnover of this substance in the baboon, as shown in the total amount excreted in the urine.

Far more substantial differences are seen in the metabolism of *progesterone* among various non-human primate species. In man, 50 to 60 % of administered progesterone radioactivity appears in the urine, approximately half of it during the first day. In the rhesus monkey, only 26 to 48 % appears in the urine over seven days; on the other hand, 40 to 60 % of the total radioactivity appears in the faeces, compared to 10 % in the baboon and 17 % in the pig-tailed monkey. Of particular interest is the pattern of urinary metabolites. In man, reduction of the  $\Delta^4$ -3-keto-configuration and reduction of the 20-ketone to a 20-hydroxyl group form the major routes of metabolism, with 6-hydroxylation and 16-hydroxylation each accounting for less than 5 % of the metabolites. On the other hand, the major human urinary metabolite,  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol is insignificant in most of the non-human primate species. Androsterone is a major metabolite in rhesus, pigtail, and baboon. In the chimpanzee, urinary pregnanediol is present. Whereas the plasma progesterone levels in man and chimpanzee are quite similar in the follicular and luteal phases of the menstrual cycle, urinary pregnanediol in the chimpanzee luteal phase rarely exceeds 2 mg per day, while values of several times this magnitude are normally encountered in man. This suggests that alternate pathways of metabolism may be relatively more important in the chimpanzee. In *Saimiri*, neither pregnanediol nor androsterone can be detected in significant quantities even in the pregnant state.

Whether these significant differences in metabolic pathways extend to synthetic progestational substances as well, is unknown. Studies of such compounds in non-human primates are virtually non-existent; their metabolism and properties in man and other species have been summarized by *Tausk (1972)* and *Fotherby & James (1972)*.

It may be seen that our knowledge of the metabolism of both natural and synthetic steroids is extremely limited in non-human primates. However, the available information indicates relatively minor qualitative differences between man and the Old World monkeys, except for the predominance of 16-

hydroxylation in the metabolism of oestrogens in man and gorilla, and the importance of side-chain cleavage in the metabolism of progesterone by the monkeys. This stands in sharp contrast to comparisons of man to the dog or rat.

### III. Metabolic effects of steroids

The effects of oestrogens and of oestrogen-progestin combinations on various parameters of lipid metabolism in man are well recognized. Their possible relevance to the problem of atherogenesis has been the subject of extensive clinical investigation and debate. The enormous volume of research using small laboratory animals is beyond the scope of this presentation. Much less has been done to study the effect of these sex hormones on lipid metabolism in the non-human primate than might be anticipated even though some baseline data have been accumulated (*Kritchewsky 1970; Wilson 1970; de la Pena et al. 1972*). We are currently studying the interaction of high-cholesterol diets, physical inactivity, and various oestrogens on blood lipids and lipoproteins as well as on the vascular system of baboons. In another study, both beagle dogs and baboons are being given cyclic treatment with the two synthetic oestrogens used in contraceptive formulations, at first by themselves, and subsequently with the addition of representatives of the various classes of progestins used in these formulations. These studies will be compared with similar clinical

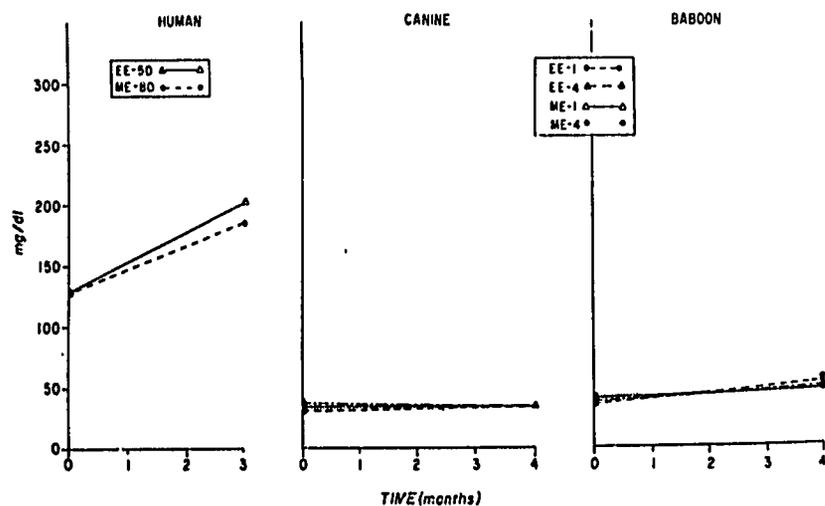


Fig. 7.

Concentration of plasma triglycerides in three species treated in cyclic fashion with ethnyloestradiol or mestranol. In the human, the legend indicates a dosage of 50  $\mu\text{g/day}$  of ethnyloestradiol, or 80  $\mu\text{g/day}$  of mestranol. In the animal experiments, the dosages are 1 or 4  $\mu\text{g/K/day}$  of either oestrogen, given for 3 weeks of a 4-week treatment cycle.

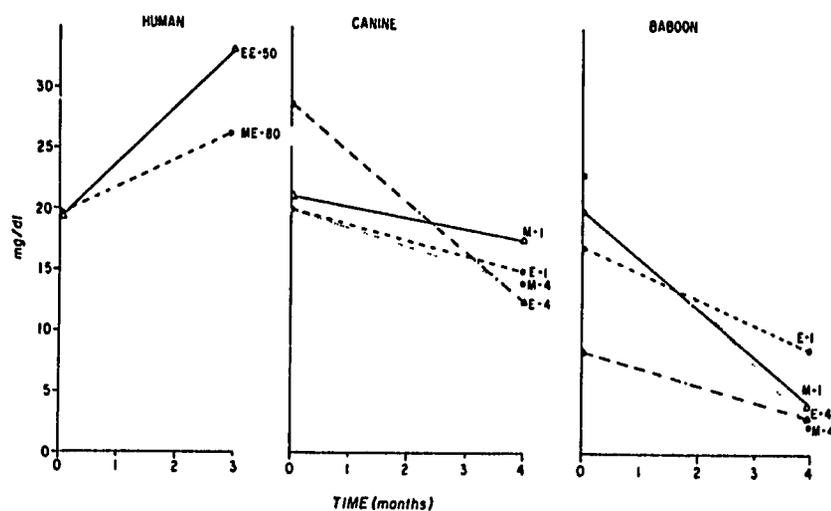


Fig. 8.  
Concentration of phospholipids in the chylomicron fraction of plasma lipoproteins in three species treated with ethinyloestradiol (EE) or mestranol (ME).

investigations. While this work is still in the early stages, some of the species differences, for example in ultracentrifugally separated plasma lipoprotein fractions, are striking. All three species show a slight tendency for an increase in cholesterol concentrations in the various density fractions. However, there is a marked difference between human triglyceride response and that of dogs and baboons (Fig 7). A considerable rise of triglyceride in the chylomicron,  $\alpha_2$ -lipoprotein and  $\beta$ -lipoprotein fractions is evident in man, but no such changes are observed in the two other species. The phospholipids show a similar disparity (Fig. 8): in women, there is a tendency for the phospholipid level in the chylomicron and  $\alpha_2$ -lipoprotein fractions to rise; in the  $\beta$ -lipoprotein frac-

Table 3.  
Effects of oestrogens and pregnancy on baboon serum proteins.

Group	N	Total serum protein gm %	Albumin gm %	Ceruloplasmin $\Delta A_{520}$
Normal males	5	6.7 $\pm$ 0.8	3.0 $\pm$ 0.4	0.33 $\pm$ 0.07
Normal females	5	7.1 $\pm$ 0.4	3.2 $\pm$ 0.3	0.43 $\pm$ 0.15
Amenorrhoeic females	4	7.0 $\pm$ 0.4	3.6 $\pm$ 0.5	0.41 $\pm$ 0.16
Pregnant females	4	6.3 $\pm$ 0.4	2.3 $\pm$ 0.3	0.92 $\pm$ 0.07
Oestrogen-treated females	5	5.6 $\pm$ 0.6	1.9 $\pm$ 0.4	1.08 $\pm$ 0.03

tion they do not appear to change within three months of oestrogen exposure. In the other two species, however, there is a profound fall in the phospholipids in all three density fractions.

Depending on the parameter selected, the two animal species may or may not be suitable models for the study of human lipid metabolism. Since the exact relationship of the various plasma lipid and lipoprotein constituents to the process of atherogenesis is unclear, it is impossible to identify the relevant parameters and thus to judge the adequacy of either species as a model in this context. In the sense that neither animal species responds to oestrogen administration in exactly the same way as man, the interpretation of experiments both in beagles and baboons, and their relationship to human atherogenesis, must be undertaken with the greatest of caution.

The levels of various plasma proteins are altered by oestrogen therapy, presumably from changes in hepatic biosynthesis. Albumin synthesis is depressed in human subjects on combination-type oral contraceptives (*Honger & Rossing* 1969), and a lowering of total plasma protein has also been observed by us in baboons and dogs treated with synthetic oestrogens. A considerable portion of this decrease is attributable to a drop in albumin (Table 3). A number of important plasma proteins, such as ceruloplasmin, haptoglobin, plasminogen, thyroxine-binding globulin, and corticosteroid-binding globulin are altered by oestrogen treatment in humans (*Seal & Doe* 1969). Studies by *Barbosa et al.* (1971) in several species of macaques demonstrated that these animals do not show an elevation of CGB during pregnancy or upon administration of oestrogens; thus they do not serve as adequate models for studies of adrenal steroid metabolism under these conditions. The green monkey does respond in the same manner as man, and our collaborative studies with *Seal* (unpublished) show that the response of baboon CBG, TBG and ceruloplasmin is similar to the human (Tables 3 & 4). The comparative response of human, baboon and dog to synthetic oestrogen administration on total plasma cortisol level (as an indicator of CBG) is shown in Fig. 9.

*Table 4.*  
Effects of oestrogens and pregnancy on baboon hormones and binding proteins.

Group	N	Cortisol $\mu^0/0$	CBG $\mu^0/0$ F bound	Thyroxine $\mu g^0/0$	TBG $\mu g^0/0$ T <sub>4</sub> bound
Normal males	5	16 ± 2.4	27 ± 4	6.0 ± 1.1	9 ± 4
Normal females	5	26 ± 9.9	31 ± 3	5.8 ± 1.6	15 ± 4
Amenorrhoeic females	4	26 ± 10.4	29 ± 1.4	6.0 ± 2.7	16 ± 8
Pregnant females	4	31 ± 7.4	40 ± 3.6	8.8 ± 4.9	18 ± 3
Oestrogen-treated females	5	73 ± 18	77 ± 16	14 ± 2.1	40 ± 10

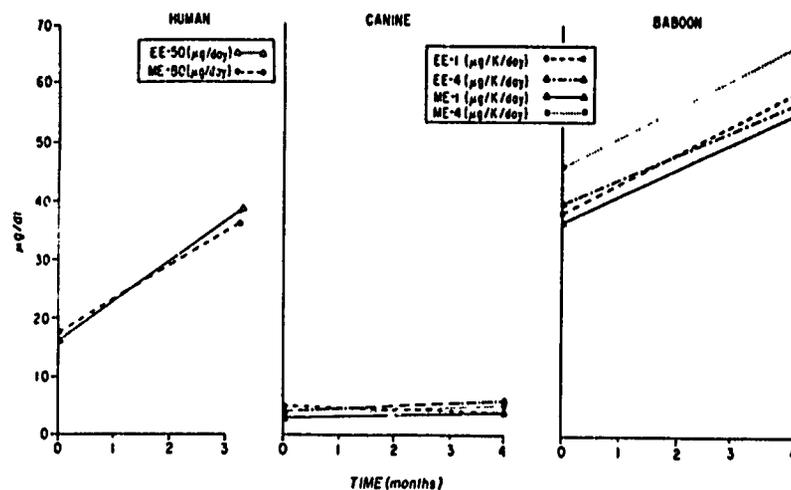


Fig. 9.  
Effect of ethynyl oestrogens on total plasma cortisol level in three species.

#### IV. Relevance of toxicity results from non-human primates to humans

In a previous publication (Goldzieher & Kraemer 1972) we summarized the available information on tumorigenesis in dogs and monkeys receiving oral contraceptive steroids singly and in combination. An updating of FDA-supported studies on mestranol, anagestone, ethynlerone, and chloroethynyl norgestrel has recently been presented (Wazeter *et al.* 1973). Dogs and monkeys have been maintained on cyclic medication at 10 to 50 times the human dose for intervals ranging from 194 to 242 weeks. The study has been summarized by the investigators in these words: "Because positive results (with regard to mammary tumours) have been obtained in dogs and *negative results have been obtained in monkeys thus far*, most of this report will deal with dogs." A number of the dogs on medium or high doses of the combination agents also developed diabetes, but no monkeys were so affected.

Prospective studies on the incidence of mammary tumours in human subjects receiving steroid preparations for contraceptive purposes have not yet appeared in the literature. The long time interval between certain oncogenic stimuli and the appearance of tumours in man, which is alleged to apply also to the oestrogens as potential tumorigens, probably makes such studies premature. However, the only unequivocal cases of oestrogen-induced breast malignancy in the human (male transvestites) showed an induction period of

less than 6 years. The available information at this time is in the form of clinical studies carried out by the retrospective case-control approach. The controversy regarding the validity of this epidemiological technique (*Feinstein 1973*) is beyond the scope of this presentation. In any event, *Vessey et al.* (1972) have reported findings consistent with the monkey experiments: their data "do not suggest that the use of oral contraceptives is related in any way to the risk of breast cancer, but provide some evidence that the preparations may actually protect against benign breast disease. This protective effect is largely confined to women who have . . . used them altogether for more than two years. Such women appear to have only about 25 % as great a risk of being admitted to a hospital for a breast biopsy as women who have never used oral contraceptives at all."

Toxicity studies with respect to blood coagulation and other parameters of interest in relation to contraceptive steroid preparations, as well as studies of prostaglandin toxicity in non-human primates and man will be discussed by others.

A few substances of possible contraceptive interest, such as methallibure, colcemid and ergocornine, have been examined in rhesus and in man, but the toxicity of these agents is such as to make the data of academic interest only. The same is true of  $\alpha$ -chlorohydrin (3-chloro-1,2-propanediol), which is an effective antifertility compound in the male rat, hamster, guinea pig, ram and rhesus monkey. Toxicity studies in male rhesus monkeys showed that the difference between the effective level (25 mg/kg/day) and the toxic level (30 mg/kg/day) was too narrow to warrant testing in man (*Kirton et al.* 1970). Toxic levels produced severe bone marrow depression and central nervous system disturbances. Administered orally to an adult male baboon at 35 mg/kg/day for 107 days, chlorohydrin caused a drop in the bone marrow erythrocyte series and in corresponding peripheral blood elements. There was no significant change in the semen profile during treatment (unpublished data).

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## DISCUSSION

*Prasad:* Dr. Goldzieher, you pointed out in your presentation that you can get from sub-human primate studies data which are similar to those obtained from laboratory rodents. In what way do you think that the primates are better models than the laboratory rodents?

*Goldzieher:* I think what needs to be determined here is really the *sequence* of the experiments that need to be done. A great deal of time can be lost in carrying out experiments on an animal in which the metabolism or the efficacy of a compound turns out to be irrelevant to man. Once some promising compound has been identified, its useful activity should be validated in a primate, on the premise that this is less likely to yield irrelevant data, on the average. The next item should be a search for a suitable experimental animal, which might be simpler and more economical to use, but which still has the desired responsiveness. Once you can demonstrate that that which is seen in the primate is also seen in a small, more convenient animal, then one has added confidence in going ahead and making the investment in time and effort to study the compound further. There are some things which I do not think can be done in the lower animals. A study of the menstrual cycle and effects thereupon has to be done in the primate. Problems of placentation probably have to be done in the primate, and one must select the species very carefully. To study steroid metabolism, one must look among non-human primates as well as the usual laboratory animals to find out which one will suit the purpose. For certain studies of conjugation, some of the sub-human primates are not good at all. On the other hand, in terms of steroid metabolism studies, the dog may be very bad or the rat may be quite good. The sub-human primate has to be included in looking for a valid model for the human.

*Diczfalussy:* I would like to ask whether you have any explanation to offer as to the differences in triglyceride levels in baboons, on the one hand, and in humans, on the other hand, when treated with ethinyloestradiol and mestranol.

*Goldzieher:* Triglyceride levels are notoriously susceptible to dietary fat content and also to exercise. We currently have underway some studies in baboons as to the effect of high fat and high cholesterol diets on exactly this question, but I regret that I do not have any answers at the moment. Certainly the dietary differences would be a most important factor.

*Chaudhury:* Can you envisage, in years to come, toxicity testing requirements in each species identified with certain parameters only where the human responses can be replicated – rather than doing everything in one or two or three species?

*Goldzieher:* I would certainly agree with the notion that different species might be used to test different parameters. It seems quite clear that no single species will serve all purposes. On the other hand, the use of various species introduces the complexity of the peculiarities associated with each species; thus the problems multiply and interpretation might become exponentially more difficult.

*Overbeck:* One of the main problems when trying to change the ratio of progestational compounds and oestrogens is the occurrence of breakthrough bleedings in women. Is it possible to use primates to predict what will happen in the human with such preparations?

*Goldzieher:* I do not think that the rhesus or the baboon could be used, because their menstrual bleeding is minimal, and these would really be the only animals which could be usable on a practical basis. For menstrual phenomena I would say that there is probably no substitute for the human female.

*Lipsett:* Why should we study the pharmacology of steroids in any experimental animal, except for intrinsic interest? If we are really interested in what is happening in man, we can synthesize radioactive steroids and give them to man.

*Goldzieher:* If we are going to look at the side effects of these compounds in large doses, there is a limitation to what we can do in the human being, whereas there is no problem at all in giving many multiples of the ordinary dose to the primate. As another example, the question of drug interaction is one which is difficult to study in humans, because it is a little hard to come by liver tissue and things of that kind; in many pharmacological experiments a sub-human primate, whose metabolic pathways are similar to those of man, is most useful. Finally, if we look at the problem which Dr. Galal raised before, what about these steroids in populations which are not well nourished, or which smoke cigarettes, or which have a variety of hepatic or renal diseases? Sometimes one can provide a much cleaner model in a specifically-deprived sub-human primate than one can find in a population of people who have a host of parasites, various nutritional deficiencies and so forth, making it almost impossible to unravel the relative roles of the nutritional deficiencies and the diseases on the question of interest.

*Fotherby:* In general I agree with Dr. Lipsett. In our own work I think that about 80% of our metabolism studies have been carried out in humans and very little in experimental animals. The difficulty until recently was that one had to use labelled steroids and it is not possible to give repeated doses of radioactive steroids to humans. However, the development of new sensitive techniques for measuring the synthetic compounds has now overcome this difficulty. In our own work we have studied the tissue distribution of a number of the synthetic compounds in humans, but of course, there are certain tissues which you cannot get at laparotomy and which one would very much like to investigate, so that one has to resort to animal studies. The other rationale for studying metabolism in animals is in order to try to correlate metabolism with toxicological effect.

*Mishell:* Dr. Goldzieher, I was very interested in seeing your slides showing the lack of parallelism in corticosteroid binding globulin between the human and the dog as well as the baboon. Are you implying that these studies negate other studies in species where there is a lack of parallelism of corticosteroid binding globulin? For example, are toxicologic effects given by high doses of steroids on the adrenal in certain animals meaningless because there is not a similar metabolism or effect on the protein as there is in the human?

*Goldzieher:* Not entirely. What I intend to say is this: if the dosage of corticosteroids in a given experiment is so high that the corticosteroid binding capacity is overwhelmed, then it really does not make any difference, but if one is studying physiological adrenal steroid dynamics, and particularly how it might be influenced by oestrogens or pregnancy, then one must have an animal which has corticosteroid binding globulin whose synthesis in the liver responds to oestrogens. I am not saying that the effects of adrenal steroids or the nature of adrenal steroid metabolism cannot be studied in this species, but I am saying that the adrenal steroid physiology, as it is

influenced by oestrogens, cannot be studied in species whose CBG does not respond to oestrogen.

*Shearman:* Dr. Goldzieher, you mentioned the importance of diet on, for example, the triglycerides. Are there any other elements in the toxicologic screening for which dietary control is important? How long does it take after you receive an animal for dietary stabilisation to be reflected by stability in the parameters you are measuring?

My second question relates to the possible mediation of some undesirable effects by prolactin. We heard this morning of the difference in response of rats to oestrogens and of dogs to some progestins and not to others. Dr. Berliner mentioned that galactorrhoea is often seen in women with medroxyprogesterone acetate, but of course, it is often seen in women who were taking other steroids and equally frequently seen in women who have taken no steroids at all (*Shearman & Smith 1972*). Is the non-human primate, particularly the chimpanzee, a suitable model to look at pituitary mediation of the impact of progestins and oestrogens on things other than LH and LSH, such as prolactin and such as growth hormone, for extrapolation to the human?

*Goldzieher:* I am not familiar with prolactin studies which would give you the answer that you want. From what little is known about growth hormone in rhesus I think there are some reasonably good parallels to man. With respect to diet, it is difficult to answer because animals are routinely kept in quarantine for 3-6 months until their cycles stabilize and so we have no data before 3 months. An interval of 2-3 months is a reasonable one for an animal to come into dietary balance, provided it is treated medically first, to get rid of intestinal parasites and other diseases. Incidentally, the baboon is particularly convenient because it is the one available laboratory sub-human primate which does not go into a summer anovulatory period. Therefore, for breeding for reproductive purposes, you can use it all year round, whereas a rhesus colony shuts down in part during the summer period.

*Bryson:* There is talk about adrenal steroid hormones possibly antagonizing androgen and oestrogen and creating a type of biological population control under conditions of stress, such as crowding, by antagonizing the action at the receptor side. This work has been proposed in rats and the non-primate type of research organism. Do you have anything to say about whether this would be an applicable model in non-human primate research?

*Goldzieher:* Certainly, the observation from prisons and concentration camps regarding amenorrhoea suggest no medical adverse effects. This is a spontaneous antifertility mechanism which certainly deserves exploration. It has, undoubtedly, a most complex mechanism which probably would require the sub-human primate as an animal model, if indeed any animal could serve that purpose.

*Laputt:* Adrenal corticosteroids do not antagonize the binding of oestrogen or progesterone to any receptor protein.

*Bryson:* I was actually addressing myself to the theory of *Christian et al.* (1965) about that, which refers not to a direct pharmacological antagonism of the steroids to the sex hormones but to their effects, that there is competitive inhibition at the receptor site, if I recall correctly.

*Laputt:* Whatever the antagonism reported, it is not at the level of the steroid protein receptor.

*Briggs:* I was interested to see the relatively high production of 2-hydroxylated oestrogens from ethynylloestradiol in the studies with baboons. These compounds are said to be inhibitors of catechol-O-methyl transferase (*Ball et al.* 1972), which may be involved in the aetiology of depression in humans (*Cohn et al.* 1970). I was wondering if you thought this has any relevance to behavioural studies in sub-human primates treated with synthetic oestrogens.

*Goldzieher:* 2-Hydroxylation is also very important in the human. The reason it is generally not seen is that these compounds are very unstable in the urine. Without very substantial precautions, this material simply turns into pigments and gums and gets lost. The reason that we are seeing 2-hydroxylated and 2-methoxylated compounds is a matter of methodological improvements. Now then, to extrapolate actions at the O-methyl transferase level into the realm of animal behaviour or human psychological disturbances is a little further than I would care to reach.

*Prasad:* What are the recent developments in newer methods, sensitive methods like radioimmunoassays, for measurements of compounds circulating in the blood as well as of their metabolites?

*Goldzieher:* There are two classes of technological improvement. One is the recognition that all methods for processing or isolating these compounds have to be done at neutral pH. Therefore the older procedures, using acids and bases, are not acceptable. Nor can one indiscriminately expose minute quantities of these steroids to the atmosphere, as for example in thin layer chromatography. The other class of improvement is radioimmunoassay, which is well on its way. Several groups now have antibody to ethynylloestradiol. There are problems with antibody to mestranol, because mestranol is hydrolysed in the body to ethynylloestradiol. Thus one is likely to get antibodies to both mestranol and ethynylloestradiol. Certainly, radioimmunoassay should make investigation of blood levels, half-lives, etc. far more practical and far more feasible in large numbers of patients.

*Diczfalusy:* A certain proportion of women develop hypertension on contraceptive steroids, others may show signs of diminished carbohydrate tolerance. Do you see something similar in the baboon or any other non-human primate, or would you suggest a suitable animal for screening compounds for these side effects?

*Goldzieher:* The problem is that one must identify the mechanism for the final effect, namely the diminution of carbohydrate tolerance or the elevation of the blood pressure, before one can talk about an animal model. I did illustrate that mestranol reacts the same way in rhesus monkey as in man with respect to the insulin tolerance test. *Beck* (1969) has shown that progesterone has similar effects in rhesus monkeys and in the human with respect to carbohydrate tolerance. One really should look at insulin, glucagon and growth hormone dynamics to establish whether or not the animal model is suitable. The situation is complicated in hypertension, because although a lot of changes have been demonstrated in the renin-angiotensin-aldosterone mechanism, these occur equally in women who do or do not develop hypertension. Until we can better identify that factor which is highly correlated with the development of hypertension, I do not think we can specify an animal model.

*Lipsell:* Unfortunately, in the rat, renin substrate activity goes up uniformly, but the rat does not develop hypertension. In women, renin substrate activity increases in response to ordinary doses of contraceptives, but only some women become hypertensive.

*Hodge:* Renin substrate and activity rise in women on the pill, but renin concentration usually falls, presumably by feedback inhibition (*Skinner et al. 1969*). Plasma aldosterone levels also rise (*Beckerhoff et al. 1973*). I agree the mechanism of hypertension in women on the pill is obscure, but one possible cause might be found in those women whose renin concentration does not fall.

*Westerholm:* What about blood clotting studies in the baboon? Have you done any of these?

*Goldzieher:* No, we have not investigated the coagulation mechanism at all in the baboon, for two reasons. First of all, the methodology is highly personal, and certain laboratories get nice results with a technique and others do not. The second reason is that no matter how many haematologists I have asked, none of them are able to tell me that any coagulation test correlates with or is predictive of the development of thromboembolic disease.

*Prasad:* Dr. Goldzieher, now that you have said that no one species of primate is an ideal model, for different studies that need to be carried out you have to use different species, do you think that with reference to the macaque and to the baboon there are any specific areas of research in the female in the context of development of new contraceptives?

*Goldzieher:* I think that is far too broad a question for me to embark on now. I would emphasize once again that a knowledge of the peculiarities of each species would be very helpful. For example, if the uterus must be approached by way of a transcervical approach, the baboon cervix is much more amenable than the tortuous passage of the rhesus. Similarly, if one is involved in some agent which is going to affect placentation or placental function, the baboon has a haemochorial placenta, the rhesus does not. The Sukhumi Symposium (*Diczfalusy & Standley 1972*) specifically addressed itself to questions of this type.

*Kirton:* Two comments with regard to Dr. Prasad's question of the relevance of different species of primates as models: anatomical differences preclude use of some species with highly convoluted cervix for studies involving mechanical devices, and in general non-human primates seem to be especially sensitive to alteration of the reproductive cycle by stress. Abortion and corpus luteum regression may be induced in some species by stressful situations, so a great degree of care is required when dealing with these phenomena.

*Tuchmann-Duplessis:* Did people use guinea pigs for this type of studies and what would be the value of this species?

*Goldzieher:* Our only experience with the guinea pig has been on the male side in studying methods for reversible vasectomy. We were forced to use the guinea pig instead of the primate for reasons of grant-agency economy, and this choice turned out to be disastrous. Otherwise, I cannot make any contribution to the potentials of the guinea pig.

*Tuchmann-Duplessis:* I mentioned the guinea pig because the duration of pregnancy is long and the placentation is rather closely related to that of humans.

*Prasad:* In the Sukhumi Symposium (*Diczfalusy & Standley 1972*), when we were discussing the question of implantation, it was pointed out that the hamster could be as good a model as the guinea pig, if not better.

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