Clinical Endocrinology (1979) **10**, 29-38.

PLASMA LEVELS OF NORETHINDRONE AFTER SINGLE ORAL DOSE ADMINISTRATION OF NORETHINDRONE AND LYNESTRENOL

V. ODLIND, E. WEINER, A. VICTOR AND E. D. B. JOHANSSON

Department of Obstetrics and Gynaecology, University Hospital, S-750 14 Uppsala, Sweden

(*Received 31 January 1978; revised 6 June 1978; accepted 16 June 1978*)

SUMMARY

Single oral doses of 0.3 mg, 0.5 mg and 5 mg Norethindrone (NET) and 0.5 mg and 5 mg Lynestrenol (lyn) were given to five women. Lynestrenol is probably metabolized through NET and exerts its main biological activity as NET. Plasma concentrations of NET were determined by a radioimmunoassay at different intervals after administration of the tablets. Peak concentrations of NET were found within two hours after intake of each tablet. The plasma half life of NET after NET and lyn administration for the period 8-24 h was 8-11 h. No significant difference was found between the half life of NET after the NET tablets and after the lyn tablets. When 5 mg NET was given the plasma half life of NET for the period 24-72 h was around 10 h and this was significantly shorter than the half-life of NET after 5 mg lyn, which was 16½ h. The systemic availability of the drugs was estimated by calculating and comparing the areas under the plasma concentration versus time curve (AUC). 0-24 h. The AUC 0.24 after 0.3 mg NET was almost identical to the AUC 0-24 after 0.5 mg lyn. The AUC 0-24 after 0.5 mg NET was significantly larger than after 0.5 mg lyn. No difference was found between the AUC 0-24 after 5 mg lyn and 5 mg NET. This study supports the concept of a conversion from lyn to NET. It also shows that there were only minor pharmacokinetic differences between the drugs when all samples were measured as NET.

Norethindrone (NET) is a synthetic gestagen commonly used as a contraceptive alone or in combination with oestrogens. It is a derivative of 19-nortestosterone as is lynestrenol (lyn). Lyn is widely used throughout Europe in oral contraceptives. Chemically lyn differs from NET only in the absence of an oxo group at position 3. Lyn is, according to several investigators, metabolized through NET (i.e. Kayyab *et al.*, 1968a, b; Mazaheri *et al.*, 1970) and it has been shown that it does not bind to the progesterone receptor whereas NET binds strongly (Briggs, 1975). It is thus probable that lyn exerts its main biological effect after being metabolized to NET.

Correspondence: Dr V. Odlind, Department of Obstetrics and Gynaecology, University Hospital, S-750 14 Uppsala, Sweden

0300-0664/79/0100-0029$02.00

This study was performed to compare plasma levels of NET after oral administration of different single doses of NET and lyn using a radioimmunoassay for determination of NET.

MATERIAL AND METHODS

Five healthy, regularly menstruating women, 23-37 years old, participated in the study. The tablets used were NET 0.3 mg (Mini Pe®, Astra Syntex), NET 0.5 mg (gift from Astra Syntex) and NET 5 mg (gift from Astra Syntex) and lynestrenol 0.5 mg (Exlutena®, Organon) and lynestrenol 5 mg (Orgametil®, Erco) taken in this order. All five participants took each dose letting one week elapse between every tablet. All tablets were taken before breakfast. The tablets were taken independently of the day of the menstrual cycle.

Blood samples were collected immediately before and 1, 2, 4, 6, 8, 12, 24, 36, 48, 60 and 72 h after each tablet. Blood samples were also collected 84 and 96 h after intake of the 5 mg tablets. The blood was collected from an antecubital vein into heparinized tubes. The samples were centrifuged and the plasma was withdrawn and frozen at −20°C until assayed.

The plasma levels of NET were determined by a radioimmunoassay according to Nygren *et al.* (1974) with certain modifications (Weiner & Johansson, 1975). As tracer 6-7-^{3}H norethindrone (obtained from New England Nuclear Corp) with a specific activity of 40 Ci/nmole was used. It was diluted to 1.492 ng/ml in absolute ethanol. The solution used in the assay contained 99 pg tritiated steroid per 100 μl 0.1% gelatin phosphate buffered saline solution (PBS). The antiserum was diluted in 0.1% gelatin PBS solution. A dilution of 1 : 2500 was found to bind approximately 45% of the ^{3}H-NET.

All points on the standard curve (0.025-5.0 ng/ml) were significantly different ($P < 0.001$). The first point of the standard curve (0.025 ng) was significantly different from zero ($P < 0.001$, *t*-test paired data). When assaying different amounts of plasma samples with no NET or lyn added values below 0.05 ng/ml were constantly obtained. Therefore 0.05 ng/ml was considered the practical detection limit of the method. Extraction volumes varied between 0.2 and 1.0 ml. No correction was made for procedural losses, neither was the plasma blank subtracted.

No cross reaction for the naturally occurring steroids was found. Lyn showed a cross reaction of 13%.

The accuracy of the method was tested by adding known amounts of NET (0.25, 1.0 and 5 ng/ml) to a pool of male human plasma. The mean value of the 0.25 ng/ml plasma pool was 0.3 ng/ml (SD 0.049, $n = 18$). The 1 ng/ml plasma pool had a mean value of 0.95 ng/ml (SD 0.12, $n = 20$) and the 5 ng/ml pool had a mean of 4.96 ng/ml (SD 0.45, $n = 16$).

The within assay precision was estimated from duplicate determination of unknown samples run in the same assay and the coefficient of variation (CV) was calculated according to Snedecor & Cochran (1967). The CV varied between 13.45% and 10.85% as seen in Table 1. The between assay precision was estimated from duplicate determinations of unknown samples run in different assays. The between assay CV varied between 14% and 18% as seen in Table 2.

RESULTS

The plasma levels of NET after an oral single dose of 5 mg and 0.5 mg NET and lyn, respectively, and of 0.3 mg NET are shown in Figs 1-5. These figures reveal great interindividual

Table 1. Within assay precision of the NET radioimmunoassay. The coefficient of variation (CV) of duplicate determinations of unknown samples run in the same assay

Range ng/ml	CV %	*n*
0.05–1.0	11.53	25
1.1–2.0	13.45	28
2.1–5.0	11.76	21
5.1–20.0	10.85	40

Table 2. Between assay precision of the NET radioimmunoassay. The coefficient of variation (CV) of duplicate determinations of unknown samples run in different assays

Range ng/ml	CV %	*n*
0.05–1.0	18.0	27
1.1–2.0	17.2	26
2.1–5.0	15.7	22
5.1–20.0	14.0	14

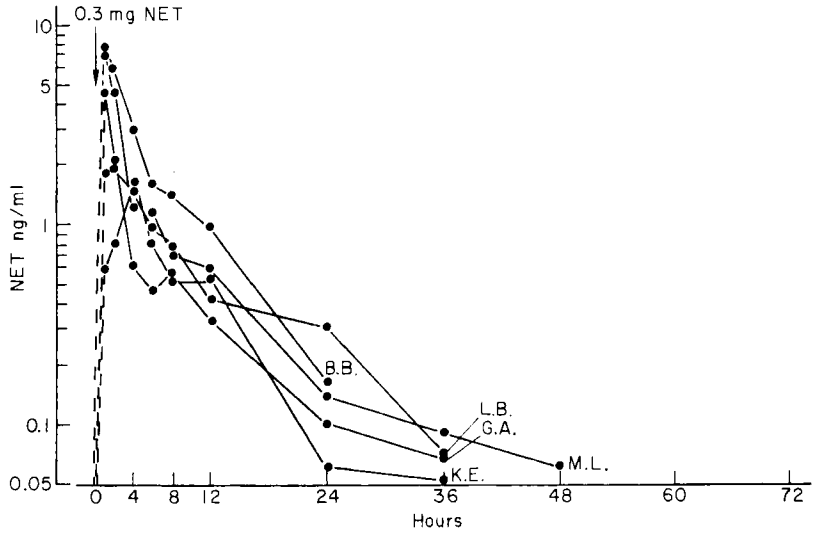

Fig. 1. Plasma levels of norethindrone in five women after a single oral dose of 0.3 mg NET.

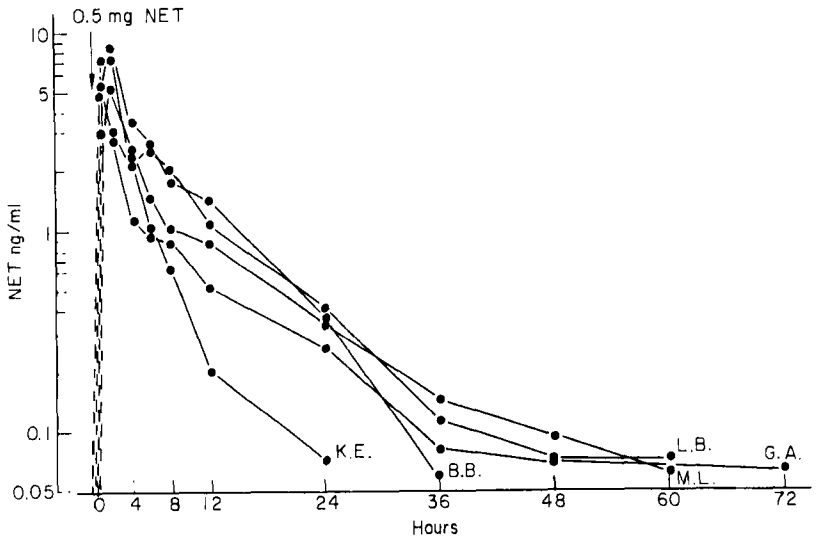

Fig. 2. Plasma levels of norethindrone in five women after a single oral dose of 0.5 mg NET.

differences in all five participants. The mean levels of NET after a single dose administration of the different doses are plotted in Fig. 6.

A good correspondence between the plasma levels of NET was found after equivalent doses of NET and lyn. After the 5 mg tablets an initial peak occurred within 2 h. The mean value of the peak was 23 ng/ml and there was no significant difference between the preparations. With the 0.5 mg tablets the plasma concentrations of NET were significantly higher after NET administration than after lyn administration at 1 and 2 h. The mean peak value of NET amounted to 5.5 ng/ml after the 0.5 mg NET tablet and to 2.76 ng/ml after the

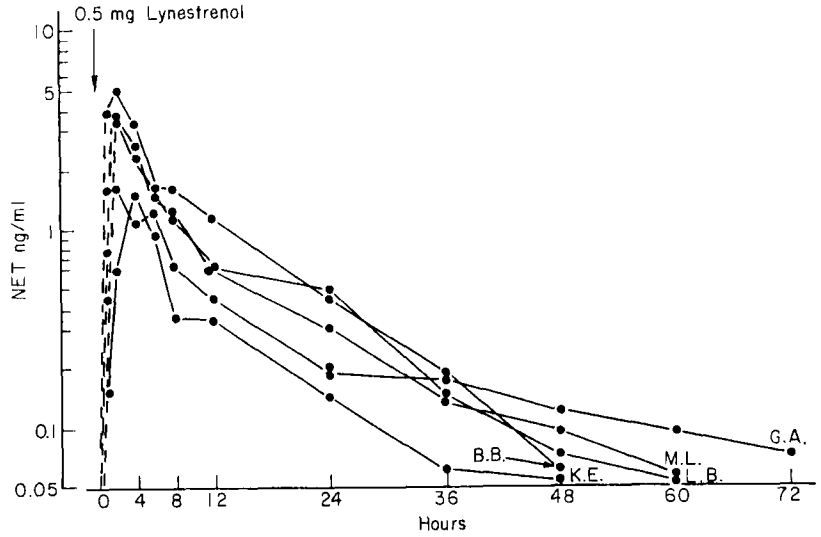

Fig. 3. Plasma levels of norethindrone in five women after a single oral dose of 0.5 mg lynestrenol.

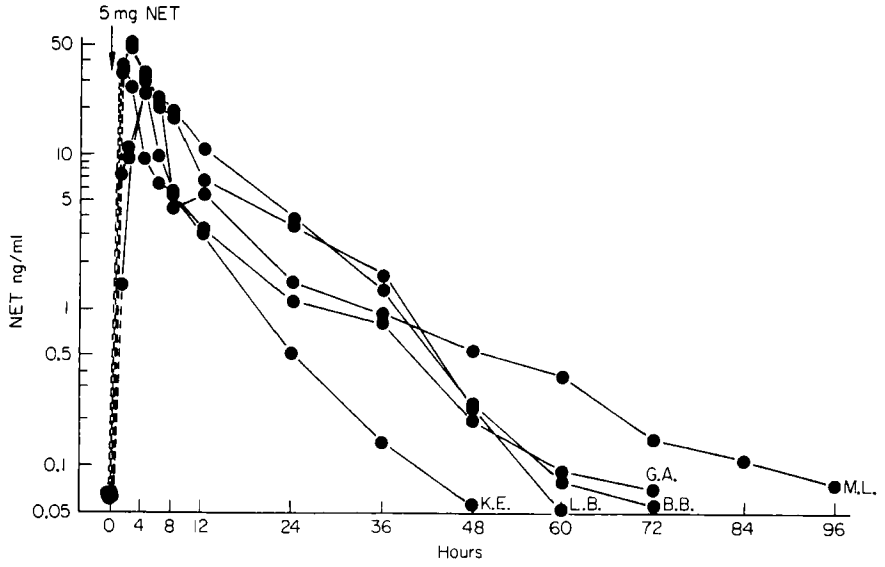

Fig. 4. Plasma levels of norethindrone in five women after a single oral dose of 5 mg NET.

0.5 mg lyn tablet ($P < 0.01$). After the 0.3 mg NET tablets there was a peak after 1 h amounting to a mean of 4.36 ng/ml. NET was detectable in plasma still 36 h after oral administration of the low-dose formulations of NET and lyn. Thereafter the NET concentrations were very near or below the detection limit of the assay. After 5 mg NET most subjects had very low concentrations of NET after 72 h whereas NET was still detectable 84 h after intake of 5 mg lyn.

During the phase of elimination there was a similar declination of the plasma levels during the first 36 h. After 48 h there were slightly higher NET concentrations in plasma after lyn

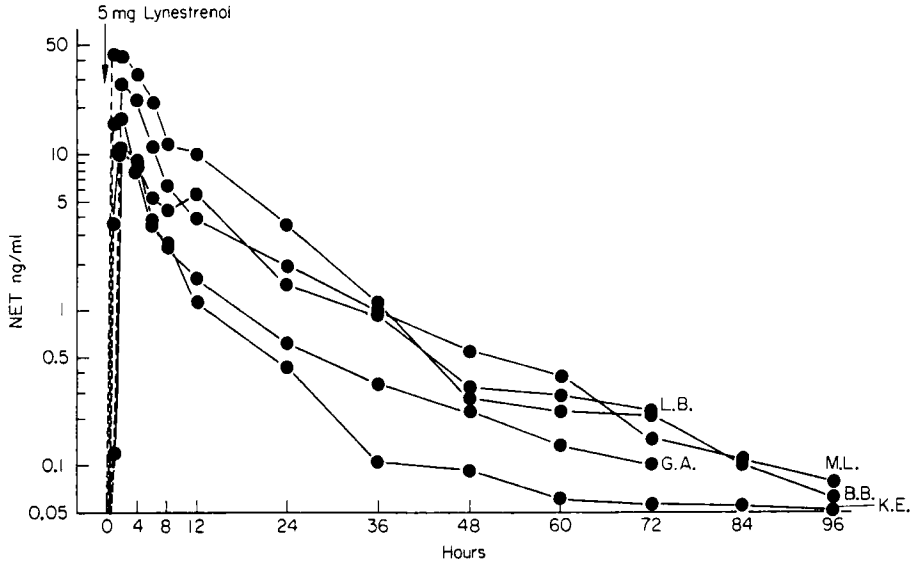

Fig. 5. Plasma levels of norethindrone in five women after a single oral dose of 5 mg lynestrenol.

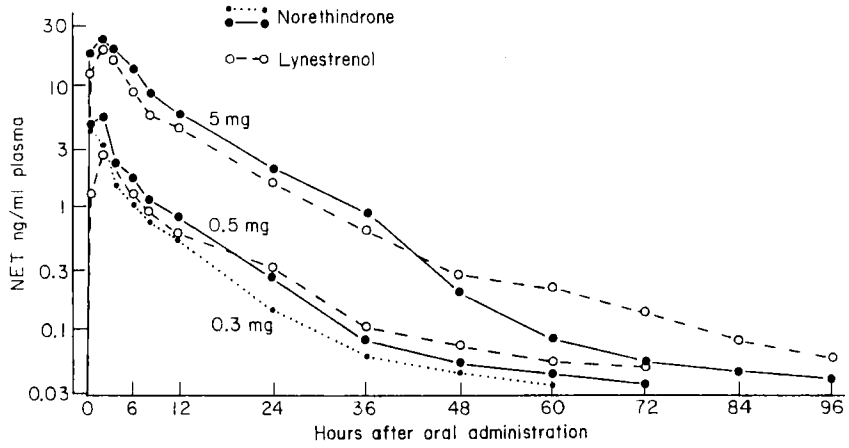

Fig. 6. Mean plasma concentrations of NET in five women after oral administration of 5 mg and 0.5 mg NET and lyn and 0.3 mg NET. (Mean of 5w).

Table 3. Area under the NET plasma concentration vs time curve. ng/ml/24 h after oral administration of various doses of NET and lyn

Dose	Mean (n = 5)	SD	Range
0.3 mg NET	22.1	10.9	13.2-39.1
0.5 mg NET	33.5	13.8	18.8-50.2
0.5 mg lyn	22.0	10.6	10.6-37.3
5 mg NET	204.5	88.4	118.4-317.9
5 mg lyn	160.6	121.0	68.6-364.9

Table 4. Plasma half life of NET 8-24 h after oral administration of various doses of NET and lyn to five subjects

	Subjects						
Dose	ML	BB	GA	LB	KE	mean	SD
0.3 mg NET	8.6	5.0	6.6	14.4	4.9	7.9	3.9
0.5 mg NET	10.3	6.9	9.7	7.0	5.5	7.9	2.0
0.5 mg lyn	8.6	8.9	9.4	15.1	11.6	10.7	2.7
5 mg NET	9.2	8.2	7.4	9.2	4.9	7.7	1.8
5 mg lyn	9.7	8.9	8.2	8.9	6.6	8.4	1.7

administration. However, there were no statistically significant differences between the mean plasma levels of NET after the two gestagens at any point.

The area under the plasma concentration versus time curve (AUC) after different doses were calculated for the period 0 to 24 h for each preparation and subject to compare the relative availabilities. The mean AUCs 0-24 are shown in Table 3. These calculations show that the mean AUC 0-24 after 0.5 mg lyn was 66% of the mean AUC 0-24 after 0.5 mg NET. This difference was statistically significant ($P < 0.01$). After the 5 mg doses the mean AUC 0-24 for lyn was 79% of that for NET. This difference was not statistically significant.

Table 5. Plasma half life of NET 24-72 h after oral administration of 5 mg NET or 5 mg lyn to five subjects

	Subjects						
Dose	ML	BB	GA	LB	KE	mean	SD
5 mg NET	10.8	7.6	10.8	6.2	14.4	9.9	3.2
5 mg lyn	14.4	11.6	18.9	17.8	18.9	16.3	3.2

The plasma half life was also calculated at different intervals by a linear regression analysis as seen in Tables 4 and 5. The mean plasma half life during the interval 8-24 h varied between 7.9 and 10.7 h. There was a good correspondence between the calculated mean half lives of NET after 0.3 mg, 0.5 mg and 5 mg NET—7.9 h, 7.9 h and 7.7 h, respectively. There were no significant differences between the plasma half lives of lyn and NET during this interval. It is hazardous to draw conclusions about the plasma half life during the interval 24-72 h after the lower doses (0.3-0.5 mg tablets). The measured values after 36 h were close to or below the practical detection limit of the assay. Therefore, only the calculations of the plasma half life after the 5 mg tablets are presented for the period 24-72 h (Table 5). The mean plasma half life of NET after lyn administration during this interval was 16.3 h which is significantly longer than that after NET administration which was 9.9 h ($P < 0.05$).

DISCUSSION

According to several investigators lyn is metabolized in the same way as NET, conversion into NET being the first metabolic step. This conversion was first suggested by Okada *et al.* (1964). Other investigators have reported great similarities between the metabolites of the two compounds suggesting a conversion of lyn to NET and a similar pathway of metabolism of the two steroids (Kamyab *et al.*, 1968a; Kamyab *et al.*, 1968b; Littleton *et al.*, 1968). Mazaheri *et al.* (1970) reported evidence of an *in vitro* metabolism of lyn to NET. Hümpel *et al.*, using a thin layer chromatography, found that only 10% of the ^{14}C activity extracted from plasma from patients who had been given ^{14}C-lynestrenol behaved chromatographically like lynestrenol and 80% behaved like NET (Hümpel *et al.*, 1977). It has, however, been pointed out by Fotherby (1974) that NET may not be an obligatory intermediate in lyn metabolism.

Briggs (1975) showed that lyn only weakly binds to the uterine progesterone receptor

site whereas NET binds strongly, this being an indirect evidence of a conversion of lyn to NET before lyn can exert its gestagenic activity.

After oral administration of lyn and Net in this study the plasma concentrations of NET and the time of elimination were very much alike. This further strengthens the idea of a conversion of lyn to NET. If the measured values of NET after lyn administration were due only to the known crossreaction of 13% with lyn, they would be expected to be much lower than after NET administration.

In this study we found peak plasma concentrations of NET within 2 h after oral administration of lyn and NET. The peak levels varied considerably between individuals as did the concentrations at all times studied. No correlation was found between the logarithmic values of the ingested dose per kilo body weight and the peak plasma levels. Such a correlation after oral administration of NET was reported by Nygren *et al.* (1974) for three subjects. Interindividual variations of the plasma concentrations after oral administration of gestagens have been reported by other investigators (Weiner *et al.*, 1976; Pasqualini *et al.*, 1977; Stanczyk *et al.*, 1975). This probably reflects differences in drug absorption, distribution and metabolism among individuals.

Both 0.3 mg NET and 0.5 mg lyn are commercially available as low dose gestagen contraceptives. The relative availability of NET as reflected by the AUC 0-24 was very similar for these two preparations. This finding agrees with the clinical experience that they have a similar contraceptive efficacy.

The AUC 0-24 of NET after 0.5 mg NET was significantly larger than the AUC 0-24 of NET after 0.5 mg lyn. This seems to be due to the higher peak level found after 0.5 mg NET. There was no difference in AUC 0-24 of NET after the 5 mg doses of NET and lyn. This lack of difference is difficult to explain but could be due to too infrequent sampling during the first hours and its is possible that a higher peak plasma concentration of NET after 5 mg NET has been accidentally omitted.

The plasma half lives found in this study agree quite well with those reported by Warren and Fotherby (1974) who found the half life of NET to be 5 h during the period 8-24 h after oral administration of 1 mg NET. They are also in agreement with results by Pasqualini *et al.* (1977) who calculated the half life of NET to be 6½ h. Previous results from our laboratory by Nygren *et al.* (1974) showed a half life of about 3 h during the first 8-10 h and during the next two days the half life was calculated to 11½ h. During the period 24-72 h lyn was found in our study to have a longer half life than NET (9.9-16.3 h, respectively) possibly reflecting a somewhat delayed metabolism of lyn. This difference in half life between the two gestagens seems to have no clinical importance when low doses are used, since the NET concentrations in plasma after the lower doses of both lyn and NET were very low after 24 h and hardly detectable after 36 h. When higher doses are used the longer half life of lyn may give rise to an accumulation. After administration of 2.5 mg lynestrenol and 50 μg ethinyloestradiol Hümpel *et al.* calculated the half life of radioimmunoassayable NET to be 3-4 h during the first 12 h and 16.5 h during the period 24 to 72 h (Hümpel *et al.*, 1977). These authors also showed that after a single oral dose of 2.5 mg ^{14}C-lynestrenol together with 50 μg ethinyloestradiol, lynestrenol or its metabolites, recognized as plasma radioactivity, was detectable to a considerable degree after 120 h, indicating an accumulation of the drug or its metabolites.

The plasma half lives of NET and lyn have been calculated by other authors using radioactively labelled NET and lyn. Kamyab *et al.* (1968a, b) using (4-^{14}C)-lyn and (4-^{14}C)-NET compared the compounds and calculated their biological half lives to be 26.5 and 19 h,

respectively. Even longer half life–60-70 h–was reported by Mills *et al.* (1976) using ^{3}H-NET. Van der Molen *et al.* (1969), using radioactively labelled lyn, found that the half life of lyn and metabolites was about 40 h.

When radioactively labelled steroids are used the native substance as well as unconjugated and conjugated metabolites are determined in the assay.

This may explain the long half lives reported when such methods have been used. In a radioimmunoassay the conjugated metabolites are not measured since they are lost in the extraction procedure. In our assay diethyl ether was used for extraction and it is known to extract only unchanged-free and protein bound steroids (Boilert *et al.*, 1973). This explains the shorter half lives found when radioimmunoassays have been used.

In conclusion this study has given further support to the opinion that lyn is metabolized through NET. No significant differences in the half lifes of the two gestagens were found when the low-dose formulations were administered. When higher doses were given the late half life of NET (e.g. 24-72 h) after lyn administration appeared to be longer than after NET administration. The pharmacokinetic differences found in this study seem only to have clinical importance when high doses of the gestagens are used and hardly in the doses used in contraceptive pills.

ACKNOWLEDGEMENT

This study was supported by the Ford Foundation, the Swedish Medical Research Council (Grant No. 03495), the International Committee for Contraception Research of the Population Council and Astra Syntex AB, Södertälje, Sweden.

REFERENCES

BOILERT, B., EDQVIST, L.-E., JOHANSSON, E.D.B., LINDBERG, P. & MARTINSSON, K. (1973) The influence of conjugated estrogen in radioimmunoassays using different antibodies against estradiol-17β. *Steroids*, **22**, 891-894.

BRIGGS, M.H. (1975) Contraceptive steroid binding to the human uterine progesterone receptors. *Current Medical Research and Opinion*, **3**, 95-98.

FOTHERBY, K. (1974) Metabolism of synthetic steroids by animals and man. *Acta Endocrinologica (Kbh.)*, Supplement 185, 119-142.

HÜMPEL, M., WENDT, H., DOGS, G., WEISS, CHR., RIETZ, S. & SPECK, U. (1977) Intraindividual comparison of pharmacokinetic parameters of d-norgestrel, lynestrenol and cyproterone acetate in 6 women. *Contraception*, **16**, 199-215.

KAMYAB, S., FOTHERBY, K. & KLOPPER, A.I. (1968a) Metabolism of (4-^{14}C)-lynestrenol in man. *Journal of Endocrinology*, **42**, 337-343.

KAMYAB, S., FOTHERBY, K. & KLOPPER, A. (1968b) Metabolism of (4-^{14}C)-norethisterone in women. *Journal of Endocrinology*, **41**, 263-272.

LITTLETON, P., FOTHERBY, K. & DENNIS, K.J. (1968) Metabolism of (^{14}C)-norgestrel in man. *Journal of Endocrinology*, **42**, 591-598.

MAZAHERI, A., FOTHERBY, K. & CHAPMAN, J.R. (1970) Metabolism of lynestrenol to norethisterone by liver homogenate. *Journal of Endocrinology*, **47**, 251-252.

MILLS, T.M., LIN, T.J., BRASELTON, W.E., ELLEGOOD, J.O. & MAHESH, V.B. (1976) Metabolism of oral contraceptive drugs. The formation and disappearance of metabolites of norethindrone and mestranol after intravenous and oral administration. *American Journal of Obstetrics and Gynecology*, **126**, 987-992.

VAN DER MOLEN, H.I., HART, P.G. & WIJMENGA, H.G. (1969) Studies with 4-^{14}C-lynestrenol in normal and lactating women. *Acta Endocrinologica (Kbh)*, **61**, 255-274.

NYGREN, K.G., LINDBERG, P., MARTINSSON, K., BOSU, W.T.K. & JOHANSSON, E.D.B. (1974)

Radioimmunoassay of norethindrone: Peripheral plasma levels after oral administration to humans and rhesus monkey. *Contraception*, **9**, 265-278.

OKADA, H., AMATSU, M., ISHIHARA, S. & TOKUDA, G. (1964) Conversion of some progestins to oestrogens. *Acta Endocrinologica (Kbh)*, **46**, 31-36.

PASQUALINI, J.R., CASTELLET, R., PORTOIS, M.C., HILL, J.L. & KINCL, F.A. (1977) Plasma concentration of ethinyl estradiol and norethindrone after oral administration to women. *Journal of Reproduction and Fertility*, **49**, 189-193.

STANCZYK, F., HIROI, M., GOEBELSMANN, U., BRENNER, P.F., LUMKIN, M.E. & MISHELL, D.R., JR. (1975) Radioimmunoassay of serum d-norgestrel in women following oral and intravaginal administration. *Contraception*, **12**, 279-298.

SNEDECOR, G.W. & COCHRAN, W.G. (1967) *Statistical Methods*, 6th ed. The Iowa State University Press. Ames, Iowa.

WARREN, L.J. & FOTHERBY, K. (1974) Radioimmunoassay of synthetic progestogens, norethisterone and norgestrel. *Journal of Endocrinology*, **62**, 605-618.

WEINER, E. & JOHANSSON, E.D.B. (1975) Plasma levels of norethindrone after i.m. injection of 200 mg norethindrone enanthate. *Contraception*, **11**, 419-425.

WEINER, E., VICTOR, A. & JOHANSSON, E.D.B. (1976) Plasma levels of d-norgestrel after oral administration. *Contraception*, **14**, 563-570.