tion and to understand the cross-resistance to other commonly used insecticides.

1. Sharma, V. P., *Curr. Sci*., 1998, **75**, 1127–1140.
2. Subbarao, Sarala K., Vasantha, K. and Sharma, V. P., in *Biosystematics of Haematophagous Insects*, Oxford University Press, 1988, pp. 25–37.
3. Kar, I. *et al*., *J. Med. Entomol*., 1999, **36**, 595–600.
4. Subbarao, S. K., Vasantha, K., Raghavendra, K., Sharma, V. P. and Sharma, G. K., *J. Am. Mosq. Control Assoc*., 1988, **4**, 29–33.
5. Subbarao, S. K. *et al*., *Trans. R. Soc. Trop. Med. Hyg*., 1992, **86**, 613–614.
6. Adak, T., Kaur, S. and Singh, O. P., *ibid*, 1999, **93**, 573–577.
7. Kaur, S., Singh, O. P. and Adak, T., *J. Parasitol*., 2000, **86**, 1345–1348.
8. Subbarao, S. K., Vasantha, K. and Sharma, V. P., *Med. Vet. Entomol*., 1988, **2**, 219–223.
9. Raghavendra, K., Vasantha, K., Subbarao, S. K., Pillai, M. K. and Sharma, V. P., *J. Am. Mosq. Control Assoc*., 1991, **7.** 255–259.
10. Raghavendra, K., Subbarao, S. K., Vasantha, K., Pillai, M. K. and Sharma, V. P., *J. Med. Entomol*., 1992, **29**, 183–187.
11. Rahman, J., Roy, M. L. and Singh, N. N., *Indian J. Malariol*., 1959, **12**, 125–130.
12. Pal, R., World Health Organization, Geneva, Mimeographed Report WHO/MAL/482.65, 1965.
13. Patel, T. B., Ramachandra Rao, T., Halgeri, A. V. and Deobhankar, R. B., *Indian J. Malariol*., 1958, **12**, 367–370.
14. Sharma, M. I. D. and Samnotra, K. G., *Bull. Nat. Soc. India Mal. Mosq. Dis*., 1962, **10**, 151–157.
15. Rajagopal, R., *Indian J. Med. Res*., 1977, **66**, 27–28.
16. World Health Organization, Geneva, WHO/CDS/CPC/MAL 98.12, 1998, (unpublished document).
17. Finney, D. J., *Probit Analysis*, Cambridge University Press, Cambridge, 1971, 3rd edn.
18. Green, C. A. and Hunt, R. H., *Genetica*, 1980, **51**, 187–195.
19. Vulule, J. M., Beach, R. F., Atieli, F. K., Mount, D. L., Roberts, J. M. and Mwangi, R. W., *Med. Vet. Entomol*., 1994, **8**, 71–75.
20. Brodgon, W. G. and Barber, A. M., *Pestic. Biochem. Physiol*., 1990, **37**, 130–139.
21. Kasap, H., Kasap, M., Alptekin, D., Luleyap, U. and Herath, P. R. J., *Bull. WHO*, 2000, **78**, 687–692.
22. Wang, J., *J. Am. Mosq. Control Assoc*., 1999, **15**, 308–311; 2000, **16**, 9–12.
23. Hargreaves, K., Koekemoer, L. L., Brooke, B. D., Hunt, R. H., Mthembu, J. and Coetzee, M., *Med. Vet. Entomol*., 2000, **14**, 181–189.
24. Mittal, P. K., Adak, T., Singh, O. P., Raghavendra, K. and Subbarao, S. K., *Curr. Sci.*, 2002, **82**, 185–188.
25. Ansari, M. A., Sharma, V. P., Razdan, R. K. and Mittal, P. K., *Indian J. Malariol*., 1990, **27**, 1–13.
26. Chandre, F., Darriet, F., Manguin, S., Brengues, C., Carnevale, P. and Guillet, P., *J. Am. Mosq. Control Assoc*., 1999, **15,** 53–59.
27. Raghavendra, K., Ph D thesis, Delhi University, Delhi, p 177.
28. Mueller-Beilschmidt Doria, *J. Pestic. Reform*, 1990, **10,** 34–38.
29. Armes, N. J., Jadav, D. R. and De Souza, K. R., *Bull. Entomol. Res*., 1996, **86**, 499–574.

ACKNOWLEDGEMENTS. We thank Dr R. S. Yadav, MRC Field Station, Nadiad and Mr B. D. Patel, Surat for help during field visit and providing data on insecticide application in study villages. Thanks are due to Mr Krishan Gopal, Mr B. M. Sharma and Mr Uday Prakash for technical assistance.

Received 15 November 2001; revised accepted 26 December 2001

Once-a-month injection of norethisterone enanthate and estradiol valerate combination suppresses pituitary FSH/LH secretion and testicular function in man

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The efficacy of 200 mg of norethisterone enanthate (NET-EN) and 2 mg of estradiol valerate (E-VAL) combination to suppress FSH/LH secretion and testicular function in adult volunteer men has been examined. The volunteer men received a single intra muscular injection of the hormone combination on days 1, 25, 50 and 75 of the study. On days 100, 125, 150 and 175 they received an injection of NET-EN alone. Within three days of first injection serum testosterone (T) became undetectable (indicates suppression in LH secretion) and FSH level was reduced by 85%. Maximal suppression in FSH and T levels lasted for 15 days, the levels returning to normal by 25–30 days of a single injection. The subjects were given every alternate day starting from day 30 of treatment an oral T supplement (40 mg T undeconate tablet). Seminal ejaculates obtained once in 15–20 days were analysed for sperm counts and motility. Sperm counts (expressed as million per ml or ejaculatory volume) showed significant drop in all treated men by day 54 (> 85%, $P < 0.05$) and reached acute oligo/azoospermia by day 110 and beyond. Motile sperm count showed drastic reduction from day 50 onwards (from 60.5 ± 13.7 mill/ml or 93.7 ± 23.7 mill/ejaculate before treatment to 1.5 ± 0.65 mill/ml or 2.4 ± 1.3 mill/ejaculate). Fertility index (FI), a product of motile sperm count and motility score, providing a measure of the fertilizing potential of sperm, was reduced from a pretreatment control of 268 ± 55.1 to 4.85 ± 2.7 by day 54 of treatment. Administration of NET-EN alone from the 5th injection onwards was adequate to maintain blockade in sperm production till the end of the study. No signs of hepatotoxicity or change in lipid profile were observable as a consequence of treatment. The potential uses of such a convenient regimen in suppressing testicular function appear many and need to be exploited.

A variety of steroidal compounds have been tested for their efficacy to block pituitary gonadotrophin secretion

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and testicular function in man. Most of these have been dependent on giving at fairly frequent intervals large doses of testosterone or its analogues with or without progestogens. Alternately, LHRH agonists have been used therapeutically to achieve this end. Since concern has been expressed as to the advisability of exposing normal men to large quantities of testosterone (T) for protracted periods and LHRH agonists/antagonists are particularly expensive in the Indian context, we attempted to develop a drug combination which will avoid using T altogether to suppress gonadotrophin secretion. The current study is based on our recent observation that it is possible to suppress gonadotrophin secretion and testicular function by injecting adult male monkeys (*Macaca radiata*), once a month, with a combination of norethisterone enanthate (NET-EN) and estradiol valerate (E-VAL)[1]. The study also showed that a combination of NET-EN and E-VAL is far superior to NET-EN alone in rapid block of testicular function. Inclusion of minimal T supplementation (to maintain accessory gland function in adult males) did not in any way accentuate or reverse the intended effect, i.e. blockade of spermatogenesis[1]. The E-VAL and NET-EN combination has been extensively tested in women for its ability to suppress pituitary function and for its contraceptive efficacy, safety and non-toxicity[2]. It was therefore felt appropriate to test the efficacy of such a combination to suppress pituitary and testicular function in man also. One of the major deterrents of such a combination drug therapy is the inclusion of oestrogen in it. However, the observation that the ratio of E-VAL : NET-EN found effective in the male monkey (0.011) is ten times less than that employed in women (0.10) made it all the more attractive to test this regimen in normal men. Results of an exploratory study are described hereunder. A brief presentation of this work was made at the International Congress of Hormonal Steroids held in June 1998 at Quebec City, Canada.

Four proven fertile, sexually active, healthy human male volunteers in the age group of 25–35 years were recruited for this study. The study was approved by the Ethics Committee for Safe Human Experimentation of the M.S. Ramaiah Medical Teaching Hospital, Bangalore. Each of the volunteers provided signed letters of informed consent.

Blood samples and seminal ejaculates of prospective volunteers, who were otherwise in good health as verified by physical examination, were taken to determine the normalcy of the haemogram, serum biochemistry, sperm counts and motility. All the four volunteers recruited received on day 1 NET-EN (Noristerat, Schering, 200 mg/ml) plus E-VAL (2.0 mg/0.2 ml Progynon, Schering), both in oily base-mixed and injected intra-muscular. The dose used was not arbitrary, but was arrived at based on our earlier experience with monkeys[1] as well as the dose recommended for use in suppressing pituitary gonadotrophin secretion in women. As preliminary study in man indicated that the pituitary suppressive effect of a single injection does not last beyond 25 days (serum FSH and T levels tended to return to normalcy by day-30), repeat injections were given regularly to all four volunteers once in 25 days. The experiment lasted for a period of 180 days and a total of 7 injections were given. During the last 4 injections (on days 100, 125, 150 and 175) E-VAL was excluded from the regimen and NET-EN alone was administered. Oral supplementation of testosterone [testosterone undeconate (TU) 40 mg, NUViR, Organon] was given every alternate night starting from day-30 of treatment.

Blood samples were collected at specified time intervals for the assay of hormones (serum FSH and T) and determination of haemogram and serum biochemistry. The methods adopted for this as well as determining sperm counts and motility score of sperm obtained from seminal ejaculates (collected by masturbation) have been described in detail in an earlier study[3].

A marked drop (> 80%) in both serum FSH (day-0: 5.39 ± 1.4 mIU/ml vs day-3: 0.89 ± 0.2 mIU/ml, $P < 0.01$) and T (day-0: 5.33 ± 1.4 ng/ml vs day-3: non-detectable) levels could be observed within 3 days of a single injection of NET-EN + E-VAL combination. The suppression in these two hormone levels continued till day-15 and by day-25 their levels (FSH: 4.0 ± 1 mIU/ml; T: 2.0–2.8 ng/ml) tended to return to normalcy. Hence injections were given once in 25 days. With the initiation of oral T supplementation from day-30 onwards, the serum T levels were maintained near normalcy and ranged between 0.65 and 7.5 ng/ml, depending upon individuals and time of sampling.

By day-54 of treatment all four volunteers showed marked and reproducible drop in total and motile sperm counts when expressed both as millions per ml or per total ejaculate volume (Figure 1). The progressive motility score (computed on a 0–5 scale) also dropped from a normal of 4.5 ± 0.28 before initiation of treatment to 1.0 or less by day-100 and beyond. An idea about the fertilizing potential of sperm was obtained by calculating fertility index, a product of motile sperm counts (millions/ml) and motility score (on a score of 0–5). The calculated fertility index dropped from a pre-treatment value of 268 ± 55.1 for the four volunteers (range from 168 to 384) to < 0.2–12.6 by day-54 of treatment, and to < 0.2–5.0 by day-105 of treatment (Figure 1).

A variety of parameters (haemoglobin, WBC, serum urea, creatinine, total bilirubin, total protein, SGOT, SGPT, alkaline phosphatase, total cholesterol, HDL, VLDL and LDL) were analysed in the blood/serum samples collected at the beginning (pre-treatment) and end (at day-180) of the study to determine if repeated injections of the steroid combination affected liver and kidney function in any way. Since values for all the parameters at both times were within normal range, it appeared the treatment has had no deleterious effect on clinical biochemistry (data not included).

Since no supplemental T was given during the first three weeks, all four volunteers complained of loss of libido and sexual drive during this period. However, with the initiation of oral T supplementation, libido and sexual drive were regained. No other clinical problems were observable, excepting in volunteer #3 who showed during routine clinical examination on day-75, signs of gynaecomastia. This appeared to be only transient as it regressed upon withdrawal of oestrogen from the drug combination. It was this observation that prompted our eliminating E-VAL from the steroid combination for all volunteers from fifth injection onwards.

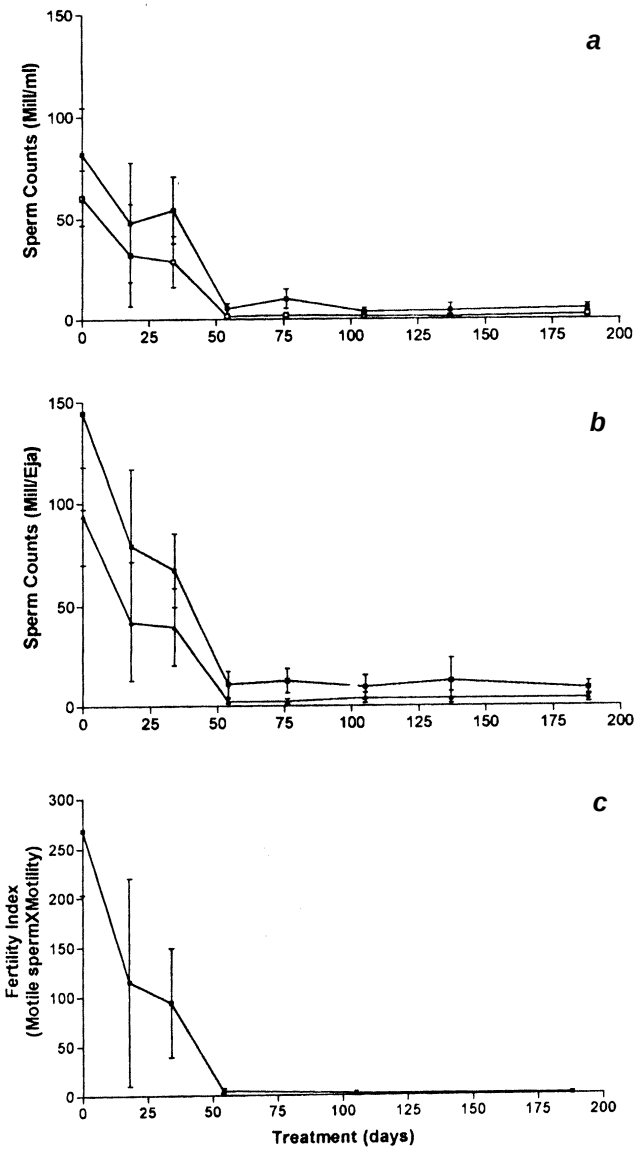

Figure 1. Effect of NET-EN + E-VAL treatment on sperm production and fertility index. ***a***, Upper line represents total (+) and lower line represents motile (□) sperm concentration in millions per ml; ***b***, Upper line represents total (+) and lower line represents motile (▲) sperm concentration in millions per ejaculate; and ***c***, Fertility index [motile sperm count (millions/ml) × motility score (on a scale of 0–5)]. Each point represents a mean ± SEM of four volunteers. For dosage and treatment frequency, see text.

The efficacy of long-acting T derivatives given alone[4,5] or in combination with progestational compounds such as medroxyprogesterone acetate (MPA) and levonorgestrel[6–9], oestrogen[10], and an anti androgen-like cyproterone acetate[11,12] to suppress testicular function (in particular spermatogenesis) has been reported. The dose of T given in man has been relatively high, ranging from 20 to 80 mg/day when given orally, to 100–600 mg/week when given as intra-muscular injection. Although the progestational activity of NET appears to be markedly less than that of MPA, its ability to inhibit gonadotrophin secretion seems to be much higher than that of MPA[13]. The use of potent and specific aromatase inhibitors in both the male monkey and man has shown that oestrogen rather than testosterone could be the true hypothalmo-pituitary feedback regulator of gonadotrophins in the male primate[14,15]. The results of the current investigation show that the human male responds to the NET-EN + E-VAL combination essentially very similar to the male monkey[1]. In this study the efficacy of E-VAL and NET-EN combination in the ratio 0.01, has been tested in man to block pituitary as well as testicular function. Based on our earlier study in monkeys wherein testicular function was carefully assessed following treatment with NET-EN + E-Val[1], it would appear the primary cause of azoo to acute oligospermia observed in the male volunteer is the result of blocking intratesticular testosterone production due to suppression of pituitary LH and FSH secretion.

Being an exploratory study, use of many variables in the current investigation was unavoidable. Despite this, the fact that the results obtained here carefully match those recorded in a much larger well-controlled monkey study[1] is highly encouraging. The recent study by Kamischke *et al.*[16] carried out in seven human volunteers to check the efficacy of a single injection of NET-EN, to bring about marked suppression in pituitary gonadotrophin secretion and serum T levels, lends credence to our thinking that NET-EN can be developed as a male contraceptive. This group has also recently evaluated the contraceptive efficacy of TU when given alone as an intra-muscular injection at a dose of 1000 mg once in 6 weeks, and when given along with NET-EN at a dose of 200 mg once every 6 weeks. The results indicated that TU alone is only 50% effective in bringing about azoospermia compared to a > 90% efficacy exhibited by the combination[17]. The supplemental dose of TU that we have used in the current study is very small (40 mg tablet every alternate day) and it is unlikely that it can bring about suppression in pituitary gonadotrophin levels and spermatogenesis. From our current human study as well as the earlier monkey study[1] it appears that addition of very small amounts of oestrogen to the NET-EN regimen facilitates, particularly during the first 1–5 injections, rapid and profound reduction in gonadotrophin and T-levels. Once blockade in spermatogenesis is achieved (by 60–100 days), continuation in arrest of spermatogenesis could be maintained

by injecting NET-EN alone. Though we have used an E-VAL : NET-EN ratio of 0.01 in the current study, it is felt based on the monkey study that it should be possible to reduce this ratio further without compromising on efficacy. Preliminary data indicate that such a combination drug therapy is indeed useful in correcting precocious puberty exhibited by some category of pre-pubertal boys (Prasanna Kumar *et al*., unpublished observations). Its use in replacing medical orchidectomy resorted to during some types of prostate hypertrophy needs to be verified.

In the current exploratory study we initiated supplemental T therapy from day-30 of treatment to ensure maintenance of libido and accessory gland function. Several variations are possible. The earlier monkey study had shown that T could be included along with NET-EN + E-VAL monthly injection without compromising on efficacy of the combination drug to block spermatogenesis[1]. From the recent observations of Rao *et al*.[18], it appears advantageous to substitute E-VAL + T with 7-alpha-methyl nor testosterone (MENT). This compound, currently under extensive testing by the Population Council, New York, is reported to be aromatized and as such can act as a weak oestrogen[19]. Though the compound is reported to have 10 × potency of T in maintaining accessory gland function, it is not a stimulator of prostate gland, not being acted upon by 5-alpha reductase. Though we have used a monthly injection schedule in the current study, it may be possible to supplant this with an implant capable of releasing the drugs NET-EN + E-VAL + T or NET-EN + MENT at a steady rate over a 3–6-month period. In the monkey study, using an Alzet mini pump to deliver NET-EN + E-VAL on a continuing basis, it was observed that significant reduction (computed on a monthly basis – once a month injection of NET-EN + E-VAL from 70 : 0.75 mg to 20 : 0.375 mg using Alzet mini pump) in the effective dose regimen could be achieved[1].

Although all four volunteers during the pre-treatment period exhibited 80% motile sperm and a motility score (progressive motility) of 4–5, there was a significant drop in both these parameters with the initiation of treatment. The fertility index was reduced by 99% (from a mean of 268 to < 3) by 100 days of treatment. In a previous monkey study by comparing calculated fertility index with data from actual fertility tests carried out, we have observed that a 10-fold drop in fertility index correlates with setting in of infertility[20]. We therefore believe that assessing sperm functionality may give us a clearer idea about the possible contraceptive efficacy of the drug combination. In the current short-term pilot study, as two out of four volunteers did show episodes of azoospermia, we believe continuous azoospermia can be achieved in all volunteer men by manipulating the dose and frequency of drug administration.

The toxicology of NET-EN and E-VAL has been extensively studied. Whereas bio-availability of NET-EN in dogs after an intra-muscular injection is 45%, it is about 20% in monkeys and 100% in humans[2]. Apparently, NET-EN and E-VAL become completely available in humans after intra-muscular injection of the depot form. This study also suggests that no pharmaco-kinetic interaction occurs between these two compounds when administered together as an oily solution[2]. Like in the monkey study, we did not observe any effects of treatment on serum lipid profile even in man.

In conclusion, this pilot study has demonstrated the usefulness of once-a-month injection of NET-EN and E-VAL combination to suppress pituitary gonadotrophin secretion and as such, testicular function like testosterone production and spermatogenesis in man. In addition to varied therapeutic uses of this combination, like in regulating precocious puberty, hypersexuality and some types of prostatic hypertrophy, it may be possible to develop a viable male contraceptive using this steroidal combination. It may be pertinent to point out here that there already exists a female monthly injection contraceptive made up of E-VAL + NET-EN mixed in the ratio of 0.1 (Mesagyna, Schering). Use of an appropriate drug-delivery system like once-a-month injectable or long-term implant of the drug, could ensure an easier acceptability and compliance on the part of men to this mode of contraception.

1. Shetty, G. *et al*., *Contraception*, 1997, **56**, 257–265.
2. Seibert, B. and Gunzel, P., *ibid*, 1994, **49**, 303–333.
3. Moudgal, N. R. *et al*., *Hum. Reprod*., 1997a, **12**, 457–463.
4. Handlesman, D. J., Conway, A. J. and Bylan, L. M., *J. Clin. Endocrinol. Metab*., 1992, **75**, 1326–1332.
5. Wallace, E. M., Aitken, R. J. and Wu, F. C., *Int. J. Androl.*, 1992, **15**, 416–424.
6. Frick, J. *et al*., *ibid*, 1982, **5**, 246–252.
7. Wu, F. C. and Aitken, R. J., *Fertil. Steril.*, 1989, **57**, 691–698.
8. World Health Organization, *ibid*, 1993, **60**, 1062–1068.
9. Bebb, R. A. *et al*., *J. Clin. Endocrinol. Metab*., 1996, **81**, 757–762.
10. Lobl, T. J. *et al*., *Contraception*, 1983, **27**, 383–389.
11. Roy, S., Abstr., Proceedings of 10th International Congress of Endocrinology, San Francisco, USA, 1996, S16/2, p. 32.
12. Meriggiola, M. C. *et al*., *Fertil. Steril*., 1997, **68**, 844–850.
13. Edgren, R. A., Jones, R. C. and Peterson, D. L., *ibid*, 1967, **18**, 238–256.
14. Trunet, P. F. *et al*., *J. Clin. Endocrinol. Metab*., 1993, **77**, 319–323.
15. Shetty, G., *et al*., *Steroids*, 1998, **63**, 414–420.
16. Kamischke, A., Diebacker, J. and Nieschlag, E., *Clin. Endocrinol.*, 2000, **53**, 351–358.
17. Kamischke, A., Venherm, S., Ploger, D., Von Eckardstein, S. and Nieschlag, E., *J. Clin. Endocrinol. Metab*., 2001, **86**, 303–309.
18. Rao, A. J., Sundaram, K. and Hardy, M. P., Paper presented at the Gordon Research Conference, Plymouth, USA, 1998.
19. Sriraman, V. and Rao, A. J., *Current Status in Fertility Regulation – Indigenous and Modern Approaches* (eds Chowdhury, S. R., Gupta, G. M. and Kamboj, V. P.), CDRI, Lucknow, 2001, pp. 163–178.
20. Moudgal, N. R. *et al*., *Endocrinology*, 1997b, **138**, 3065–3068.

ACKNOWLEDGEMENT. This study was supported by the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore.

Received 28 June 2001; revised accepted 18 December 2001