administered to the *Camellia sinensis* plant as previously described.[3] The plant was harvested after five weeks and α-spinasterol (II) was isolated from the non-saponifiable lipid in the usual manner.[3] The carrier α-spinasterol was added and the sterol crystallized to constant specific activity and $^3H/^{14}C$ ratio 2·67, atomic ratio (a.r.) 2·44 : 5. Oxidation[4] of the biosynthetic α-spinasterol yielded 2-ethyl-3-methylbutanoic acid which was isolated as the *p*-bromophenacylester derivative (III) and purified by *tlc* on silica gel. The $^3H/^{14}C$ ratio obtained was 2·74 (a.r. 0·51 : 1).

These results indicate that only 50% of the expected tritium migrates to the C-25 position and the other 50% is eliminated, probably during the formation of the intermediate C-24-methylene derivative.

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1. Akhtar, M., Hunt, P. F. and Pravez, M. A., *Biochem. J.*, 1967, **103**, 616.
2. Raab, K. H., DeSouza, N. J. and Nes, W. R., *Biochim. Biophys. Acta*, 1968, **152**, 742.
3. Sharma, R. K., *Phytochemistry*, 1970, **9**, 565.
4. Conner, R. L., Mallorey, F. B., Landrey, J. R. and Iyengar, C. W. L., *J. Biol. Chem.*, 1969, **244**, 2325.

ANTIESTROGENICITY OF NORGESTREL

NORGESTREL (*dl*-17 α-ethinyl-13 β-ethyl-17 β-hydroxygon-4-en-3-one), a synthetic progestogen has been reported to be a highly potent estrogen antagonist at the same time being completely free from estrogenicity.[1] Continuous daily oral administration of this steroid in micro doses prevents pregnancy in women and rats.[2,3] The antiestrogenic property has been considered to be a crucial factor in contraceptive *modus operandi* of the progestogen.[3]

The present communication is concerned with the assessment of antiestrogenic potency of norgestrel in terms of its effect on vascular permeability of the rat uterus.

Colony-bread albino rats (150–200 gm.) of the Institute with regular estrus cycle (4–5 days) were used. Norgestrel (0·3 μg./rat, oral), estrone (1 μg./rat, i.m.) and progesterone (0·25, 0·50, 1·00 and 2·00 mg./rat, i.m.) were given in olive oil. The vascular permeability of the uterus was determined by the method of Cecil *et al.*[4] with the modification that intact diestrus animals were used instead of ovariectomized ones.

After a single dose of norgestrel the vascular permeability of the uterus registered a marked fall between 3 and 9 hr. (*vs.* control $P < 0·01$, Table I), but returned to normal at 12 hr.

TABLE I
Effect of norgestrel on vascular permeability of the rat uterus

Control	Hours after norgestrel administration			
	3	6	9	12
6·41 ±0·65(9)*	1·00 ±0·26(6)	3·02 ±0·41(9)	2·13 ±0·2)(9)	7 60 ±1·06 (9)

* Mean ± S E. with number of animals in parentheses; vascular permeability expressed as μg. trypan blue/100 mg. uterine tissue.

Assessment of graded doses of progesterone (3 hr. after injection) showed that the inhibition of uterine permeability caused by 0·5 mg. dose was equivalent to the effect seen after administration of 0·3 μg. norgestrel (Table II).

TABLE II
Effect of graded doses of progesterone on vascular permeability of the rat uterus

Treatment	μg. trypan blue/100 mg. uterine tissue
Control ..	6·41 ±0·65 (9)*
Progesterone—	
0·25 mg. ..	3·25 ±0·23 (4)
0·50 mg. ..	2·11 ±0·22 (5)
1·00 mg. ..	1·35 ±0·39 (5)
2·00 mg. ..	0·51 ±0·03 (4)

* Mean ± S.E. with number of animals in parentheses.

In other words, the antipermeability effect of the latter was about 1666 times as high as that of progesterone. Further, it was evident that 2 mg. progesterone reduced the permeability to about 1/15th of the normal value within 3 hr. When assayed by the mouse vaginal cornification method norgestrel was found to be about 730 times more active than progesterone.[1]

Administration of norgestrel to rats either concurrently or 3 hr. after estrone (i.e., 3 hr. prior to sacrifice) not only prevented the

characteristic estrogen-induced rise in vascular permeability[4] (*vs.* control, $P < 0{\cdot}01$), but also reduced it drastically (*vs.* control or estrone, $P < 0{\cdot}01$, Table III). However, norgestrel

TABLE III

Effect of norgestrel on uterine vascular permeability of rats pretreated with estrone

Treatment			μg. trypan blue/100 mg. uterine tissue
Control	..		6·41 ± 0·65 (*9*)
Estrone	..	(6 hr.)	9·00 ± 0·87 (*8*)*
Estrone + Norgestrel		(6 hr.) (3 hr.)	1·96 ± 0·21 (*5*)
Estrone + Norgestrel		(6 hr.) (6 hr.)	3·22 ± 0·17 (*6*)

* Mean ± S.E. with number of animals in parentheses.

given 3 hr. before sacrifice to estrogenised animals seemed to exert a more profound antipermeability effect than when administered concurrently with estrone ($P < 0{\cdot}01$). Thus a dose of 0·3 μg. norgestrel was sufficient to nullify the stimulatory effect of 1 μg. estrone, and at the same time to exert its intrinsic inhibitory influence on the uterine vascular permeability.

It thus appears that the potent antipermeability effect of norgestrel is crucially involved in its contraceptive *modus operandi*, conceivably by depriving the uterus of some of the essential substrates and co-factors which may be needed for growth, differentiation and nidation of the blastocyst, as well as for the sustenance of pregnancy.

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AMIYA B. KAR.

1. Edgren, R. A., Jones, R. C., Clancy, D. P. and Nagra, C. L., *J. Reprod. Fertl.*, 1968, Suppl. 5, p. 13.
2. Foss, G. L., Svendsen, E. K., Fotherby, N. and Richards, D. J., *Brit. Med. J.*, 1968, **2**, 489.
3. Sen, A. (Late), Sanwal, P. C., Srivastava, A. K., Pande, J. K., Dasgupta, P. R. and Kar, A. B., *Contraception*, 1970 (submitted for publication).
4. Cecil, H. E., Hannum, J. A., Jr. and Bitman, J., *Am. J. Physiol.*, 1966, **211**, 1099.

FORMATION OF SUPERNUMERARY SURFACE LAYERS IN RADIATED AMOEBAE

A SPECTACULAR peeling off of the surface layer has been observed from the radiated soil amœba—*Acanthamœba* sp. This process of denuding of the surface layer has been observed in a variety of dosages employed. The most significant effect, however, has been seen in the irradiated amœbæ, in the 3rd subculture of 125,000, 150,000 and 175,000 rads and in the 2nd subculture of 200,000 rads. For irradiation upto 1,500 rads, a Picker's Army Field X-ray unit was used at 70 KVP; 4 mA; 555 rads/min. with 0·25 mm. Al-filter. A water-cooled Muller's MG-150 X-ray machine was used for the doses 2,000 rads and above, operated at 80 KVP; 9 mA; 4,000 rads/min. (for further details *see* Chatterjee[1]).

The denudated surface layers were arranged concentrically around the amœbæ (Fig. 1). After peeling off of the surface layers amœbæ never remained naked because continuously new surfaces were formed within the cell body to replace the previously shredded off limiting membrane. Layer after layer of new surface materials were being continuously formed and periodically thrown out. They appeared as concentric layers surrounding the amœbæ, filling up quite a portion encircling the cell body. Such amœbæ were immobilised and firmly attached to the glass surface. In the following few subcultures the incidence of peeling off of the surface layers became infrequent and finally disappeared in the onward subcultures.

At lower dosages, *viz.*, between 50–15,000 rads, no detachment of the surface materials as distinct layers was visible. From the 3rd subculture, localised areas were found where supernumerary depositions of membrane were noticed inside the cell (Fig. 2). These areas of isolated patches of extra surface membrane might be present in some particular region of the cell. These could be thrown out and discarded. This phenomenon of production of extra surface material was continued for 6th and 7th subcultures. The size of the peeled off surface became gradually smaller before their final disappearance in the further culture. Often the ejected surface areas appeared as stiff rod-like bodies projecting out from the cell having a dense mass (Fig. 3). Their length varied from 3–15 mμ or even more. Sometimes, the lifted surface material from the cytoplasm were fragmented into a number of still smaller