Comparative effects of progesterone, norgestrel, norethisterone and tamoxifen on the abnormal uterus of the anovulatory rat

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Progesterone therapy results in partial reversibility of histological abnormalities of the rat uterus exposed to constant oestrogen stimulation and is associated with a decrease in nuclear oestrogen receptor content, which may underlie the tissue response to hormone treatment [White, Moore, Elder & Lim (1982) *Biochem. J.* **202**, 535–41]. The synthetic progestins norgestrel and norethisterone used in this study were as effective as progesterone in decreasing the content of nuclear oestrogen receptor. However, only norgestrel had an ameliorative effect on epithelial hyperplasia and metaplasia. The non-steroidal anti-oestrogen tamoxifen caused a significant decrease in both nuclear and cytosol oestrogen receptor content without any change in luminal epithelial hyperplasia and metaplasia. Each progestin caused an increase, whereas tamoxifen caused a decrease, in the proportion of nuclear oestrogen receptors that were unoccupied. Each compound caused a decrease in the content of cytosol progesterone receptor. The effectiveness of compounds used as oestrogen antagonists is discussed with reference to their mode of action.

Constant unopposed oestrogen stimulation induces hyperplasia of the human endometrium (King & Whitehead, 1980) and has been associated with the development of endometrial cancer (Hammond *et al.*, 1979). In the rat, neonatal administration of testosterone propionate renders the adult female anovulatory and hence exposed to constant oestrogenic stimulation unopposed by progesterone. Such oestrogenic stimulation of the uterine luminal epithelium results in hyperplasia and metaplasia (White *et al.*, 1981); after treatment with progesterone the cells of the luminal epithelium have a cuboidal appearance (White *et al.*, 1982), suggesting antagonism of oestrogen stimulation (Hsueh *et al.*, 1979). The content of nuclear oestrogen and total progesterone receptor was decreased after progesterone treatment (White *et al.*, 1982), consistent with the effects of progesterone in the normal uterus (Coulson & Pavlik, 1977).

Synthetic progestins are used to counteract the effects of unopposed oestrogen stimulation (King & Whitehead, 1980); their effects on the uterus are related to their antagonism of oestrogen stimulation and their inherent mitogenic activity as progestins (Martin, 1980). In the present study we have used two synthetic progestins derived from 19-nortestosterone, norgestrel and norethisterone, that are respectively potent and weak progestogenic agents (Edgren *et al.*, 1968), although only norethisterone

has any inherent oestrogenic activity (McPherson *et al.*, 1974). The non-steroidal anti-oestrogen tamoxifen has been used in comparison to investigate the effects of the three compounds on tissue histology and sex hormone receptor concentrations.

Experimental

Production of anovulatory rats and progestin therapy

Testosterone propionate (Sigma Chemical Co., Poole, Dorset, U.K.) was administered to 5 day-old female pups (1.2 mg subcutaneous injection in oil), which were then returned to the lactating mother. At 120–140 days animals showing a persistent oestrous-like vaginal histology were selected for therapy. Animals received (a) progesterone (pregn-4-ene-3,20-dione; Sigma Chemical Co.) for 3 and 6 days at a dose of 5 mg/day, (b) norgestrel [(+)-13 β -ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one; Schering A.G., Berlin/Bergkamen, Germany] or (c) norethisterone acetate (17 α -ethynyl-17 β -hydroxy-3-oxo-oestr-4-ene 17 β -acetate; Sigma Chemical Co.) each for 6 days, at a dose of 1 mg/day. Tamoxifen (ICI 46474) was administered on 6 consecutive days at a dose of 500 μ g/day. All steroids were administered subcutaneously in propylene glycol (0.1 ml); animals

were killed within 24–25 h of the last injection of progestin or tamoxifen.

Oestradiol administration

At 24 h after the final injection of progestin, sub-groups of animals within each treatment group were injected with 2.5 µg of oestradiol-17β [oestra-1,3,5(10)-triene-3,17-diol; Sigma Chemical Co.] intraperitoneally in 0.2 ml of ethanol/0.9% NaCl (1:1, v/v) 1 h before being killed.

Histological examination of tissue

The preparation of tissue for sectioning was as previously described (White *et al.*, 1981). Ehrlich's haematoxylin and eosin were used to examine the microscopic structure of the tissue. Examination of the ovaries from each animal confirmed the anovulatory status.

Measurement of oestrogen receptors

Nuclear receptor. Specific nuclear receptor was measured in triplicate 0.2 ml samples. A saturating concentration (15 nM) of radioactive oestradiol [2,3,5,7-³H]oestradiol-17β; sp. radioactivity 87–99 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.] was used with and without a 200-fold excess of diethylstilboestrol, to measure oestrogen

nuclear receptors. Incubations were for 1 h at 30°C (to measure total receptor) and 4°C (to measure unoccupied receptor) (White *et al.*, 1981).

Cytosol receptor. Specific cytosol receptor was measured in duplicate 0.2 ml samples at 4°C for 18–24 h; for oestrogen receptor 5 nM [³H]oestradiol was used in the presence or absence of a 200-fold excess of diethylstilboestrol. Progestin receptor was measured by using a saturating concentration (20 nM) of [³H]progesterone with unlabelled progesterone as competitor and cortisol to minimize binding to corticosteroid-binding globulin (White *et al.*, 1981). Macromolecular-bound radioactivity in the cytosol fraction was separated from free steroid by chromatography on columns of Sephadex LH-20 [Pharmacia (G.B.) Ltd., Hounslow, Middx., U.K.] (Thrower *et al.*, 1976).

Results

The content of nuclear oestrogen receptor measured at 30°C (i.e. total receptor) was significantly decreased after 6 days of treatment with progestins or tamoxifen compared with the untreated group. Treatment with progesterone for 3 days was without effect (Table 1). Nuclear oestrogen receptors that were complexed with oestradiol at

Table 1. Nuclear oestrogen receptor content after hormone therapy

The Table shows the effect of progestin and tamoxifen therapy on the content of uterine nuclear oestrogen receptors of anovulatory rats (treated neonatally with testosterone propionate). Adult anovulatory rats in persistent vaginal oestrus were selected by examination of vaginal smears; each animal was used as a single experiment. Norgestrel (1 mg), norethisterone (1 mg) and tamoxifen (0.5 mg) were injected subcutaneously on six consecutive days; progesterone (5 mg) was injected subcutaneously for either 3 or 6 consecutive days. Animals were killed 24 h after the final injection; tissue was prepared and fractionated and nuclear oestrogen receptor was measured as described in the Experimental section. Each value is the mean ± s.d. for the number of estimations indicated in parentheses. Results from animals not treated with progestin and tamoxifen are presented for comparison (10 separate estimations; three to five animals/estimation). The ratios (b/a) and (c/a) × 100 are means ± s.d. obtained from the values in each separate experiment. Statistical analysis was by two-tailed Student's *t* test. *, Significantly different from 'none' (*P* < 0.05); **, significantly different from 'none' (*P* < 0.02); ***, significantly different from 'none' (*P* < 0.025); †, significantly different from 'none' (*P* < 0.005); ††, significantly different from 'none' (*P* < 0.001).

Treatment	Receptor content (pmol/g)				
	(a) Total	(b) Unoccupied	(b/a) × 100	(c) Occupied	(c/a) × 100
None	2.91 ±0.95 (10)	1.96 ±0.30 (10)	60.86 ±16.52 (10)	1.37 ±0.72 (8)	35.13 ±18.44 (8)
Progesterone (3 days)	2.63 ±0.42 (4)	2.52 ±0.83 (4)	94.75* ±25.39 (4)	0.38*** ±0.43 (3)	15.66 ±17.78 (3)
Progesterone (6 days)	1.84** ±0.75 (9)	1.77 ±0.38 (9)	93.12** ±31.03 (9)	0.65* ±0.36 (5)	27.20 ±11.07 (5)
Norgestrel	1.37†† ±0.72 (11)	1.33 ±0.56 (11)	114.22† ±47.10 (11)	0.56** ±0.22 (4)	39.00 ±12.27 (4)
Norethisterone	1.30†† ±0.61 (8)	1.72 ±0.87 (4)	154.66†† ±39.17 (4)	1.51 ±0.15 (4)	100 (4)
Tamoxifen	2.00* ±0.54 (5)	0.65†† ±0.22 (5)	36.49* ±19.94 (5)	1.56 ±1.00 (5)	63.6 ±19.6 (5)

EXPLANATION OF PLATE 1

Histology of uteri (a) with no progesterone therapy, (b) after 3 days of progesterone therapy, and (c) after 6 days of progesterone therapy

Longitudinal sections (magnification $\times 55$) of uteri from anovulatory adult female rats (neonatally treated with testosterone propionate) are shown.

EXPLANATION OF PLATE 2

Histology of uteri (a) after 6 days of norgestrel therapy, (b) after 6 days of norethisterone therapy, and (c) after 6 days of tamoxifen therapy

Longitudinal sections (magnification $\times 55$) of uteri from anovulatory adult female rats (neonatally treated with testosterone propionate) are shown.

4°C (unoccupied receptor) were also detectable (Table 1); the content of unoccupied receptors was unchanged after progestin therapy, but was significantly decreased after tamoxifen therapy compared with the untreated group. The proportion that the unoccupied component represented of the total receptor content was significantly increased after treatment with each progestin in contrast with treatment with tamoxifen, when a corresponding decrease occurred.

Oestradiol administration to animals that had received 3 to 6 days of progesterone therapy resulted in a significant increase in total nuclear oestrogen receptor content (cf. Tables 1a and 2a). Animals that had not previously been treated with progesterone showed no increase in nuclear oestrogen receptor content after oestradiol administration (Tables 1a and 2a; White *et al.*, 1981). In the norethisterone-treatment group, oestradiol administration resulted in an increase in total nuclear oestrogen receptor content; there was no significant change in total nuclear oestrogen receptor content after oestradiol administration to the norgestrel-treatment group.

The content of unoccupied receptors in the progesterone-treatment group was increased after oestradiol administration such that this component again represented the major proportion of the nuclear receptor. Oestradiol administration to the norgestrel-treatment group resulted in a decrease in the content of unoccupied receptors; after oestradiol administration, unoccupied receptors were not detectable in the norethisterone-treatment group.

The content cytosol receptor was significantly decreased after progesterone or norethisterone therapy; after treatment with norgestrel there was no significant change in cytosol oestrogen receptor content (Table 3). After treatment with tamoxifen the content of cytosol oestrogen receptor was decreased in four cases to values below the limit of detection of the assay. A single injection of

oestradiol resulted in a significant decrease in cytosol receptor content in both the untreated and progestin treatment groups. The cytosol progestin receptor content was significantly lower in the uteri of anovulatory rats compared with normal rats during dioestrous (White *et al.*, 1981). After treatment of anovulatory rats with progestins or tamoxifen there was a significant decrease in cytosol receptor content (Table 3).

The uterine luminal epithelium of the anovulatory rat was hyperplastic throughout each horn with intermittent foci of metaplasia that were apparently gland-associated (Plate 1a; White *et al.*, 1981). After 3 or 6 days of progesterone therapy the uterine luminal epithelium was cuboidal in appearance with no histological evidence of proliferative activity (Plates 1b and 1c; White *et al.*, 1982). The histological appearance after norgestrel treatment was similar to the progesterone-treated groups (Plate 2a); the luminal epithelium was a mixture of cuboidal and columnar cells, whereas the number and size of the stromal glands was increased. The hyperplasia and metaplasia of the luminal epithelium persisted after treatment with norethisterone and tamoxifen (Plates 2b and 2c), despite the effects of these compounds on receptor concentrations.

Discussion

The properties of progesterone, norgestrel, norethisterone and tamoxifen as oestrogen antagonists in the abnormal uterus of the anovulatory rat, under constant oestrogenic stimulation, have been compared. Each of the compounds caused significant decreases in the content of nuclear oestrogen receptors and therefore were effective oestrogen antagonists (Katzenellenbogen *et al.*, 1979). Progesterone and norgestrel selectivity caused a decrease in the occupied nuclear receptor, which may be explained by the recent reports of a progesterone-

Table 2. *Effect of oestradiol administration to progestin-treated rats*

Animals were selected and treated with progestins as described in the legend to Table 1. Each group was injected with oestradiol [2.5 µg in 0.2 ml of ethanol/0.9% NaCl (1:1, v/v)] intraperitoneally 1 h before killing. Tissue preparation fractionation and measurement of nuclear receptor was as described in the Experimental section. Statistical analysis was by two-tailed Student's *t* test; comparisons are made with the corresponding value before oestradiol administration (compare with Table 1). *, Statistically different from 'no oestradiol' ($P < 0.001$). Results are means $\pm$ s.d. for the number of estimations indicated in parentheses.

Nuclear receptor content (pmol/g)

Treatment	(a)	(b)			
	Total	Unoccupied	(b/a) $\times$ 100	(c)	(c/a) $\times$ 100
None	3.83 $\pm$ 1.05 (7)	0.86* $\pm$ 0.26 (4)	25.21 $\pm$ 12.13 (4)	3.03 $\pm$ 1.59 (4)	74.78 $\pm$ 12.13 (7)
Progesterone (3 days)	5.76* $\pm$ 1.14 (5)	4.90 $\pm$ 2.87 (5)	89.95 $\pm$ 51.76 (5)	3.96, 3.34	58, 71
Progesterone (6 days)	7.56* $\pm$ 1.52 (5)	6.09* $\pm$ 1.68 (5)	84.00 $\pm$ 16.95 (5)	1.72 $\pm$ 1.43 (3)	21.94 $\pm$ 13.79 (3)
Norgestrel	1.61 $\pm$ 0.33 (4)	0.47* $\pm$ 0.26 (4)	26.00 $\pm$ 12.75 (4)	1.23 $\pm$ 0.21 (4)	74.00 $\pm$ 12.75 (4)
Norethisterone	2.98* $\pm$ 0.51 (4)	—	—	—	—

Table 3. *Cytosol oestrogen and progesterin receptor after hormone therapy*

Animals were selected for therapy as described in the legend to Table 1; receptor analysis was as described in the Experimental section. (a) Cytosol oestrogen receptor was measured before or 1 h after the injection of 2.5 µg of oestradiol. Statistical analysis was by two-tailed Student's *t* test. *, Significantly different from 'no treatment' ($P < 0.05$); **, significantly different from 'no treatment' ($P < 0.005$); †, significantly different from 'before oestradiol' ($P < 0.001$). (b) Progesterin receptor content was compared in each treatment group with the 'no treatment' group regardless of oestradiol injection, as this did not influence progesterin receptor determination (White *et al.*, 1981). Statistical analysis was by two-tailed Student's *t* test. *, Significantly different from 'no treatment' ($P < 0.005$); **, significantly different from 'no treatment' ($P < 0.001$). Abbreviation: ND, not detectable. Results are means $\pm$ s.d. for the number of estimations shown in parentheses.

	Receptor content (pmol/g)					
	No treatment	Progesterone (3 days)	Progesterone (6 days)	Norgestrel	Norethisterone	Tamoxifen
(a) Oestrogen						
Before oestradiol- administration	6.37 $\pm$ 2.84 (18)	3.96** $\pm$ 0.65 (4)	4.21* $\pm$ 2.18 (10)	6.23 $\pm$ 3.58 (11)	2.89** $\pm$ 2.50 (8)	ND (5)
At 1 h after 2.5 µg of oestradiol	0.30† $\pm$ 0.19 (7)	0.82† $\pm$ 0.11 (5)	0.60† $\pm$ 0.49 (5)	0.60† $\pm$ 0.38 (4)	ND (4)	—
(b) Progesterone						
	4.67 $\pm$ 2.21 (11)	0.41 $\pm$ 0.22** (7)	1.34 $\pm$ 0.59** (15)	0.47 $\pm$ 0.23** (16)	2.04 $\pm$ 0.76* (8)	1.98 $\pm$ 0.85* (5)

induced nuclear activity that influences the metabolism of this nuclear receptor component (Okulicz *et al.*, 1981; MacDonald *et al.*, 1982). Despite the ability of norethisterone to decrease total nuclear oestrogen receptor content there was no consistent effect on either the occupied or unoccupied receptor component (Table 1). The functional significance of this observation remains to be established, but it is noteworthy that, based on histological evidence (Plates 1 and 2), norethisterone was the least effective oestrogen antagonist of the progestins used. Therefore the relative efficacy of progestins used as oestrogen antagonists may in part reside in their ability to influence the concentration of occupied nuclear oestrogen receptors.

In contrast with progesterone and norgestrel, tamoxifen caused a significant decrease in the unoccupied nuclear oestrogen receptor content. The biological half-life of tamoxifen may be important in this respect as its relatively low clearance rate (Fromson *et al.*, 1973) could result in residual concentrations *in vivo* that serve to maintain nuclear oestrogen receptors in the occupied form. The observation that tamoxifen causes a decrease in total nuclear receptor content contrasts with that of the effects of the short-term use of non-steroidal anti-oestrogens (Katzenellenbogen *et al.*, 1979; Jordan *et al.*, 1977), which promote an increase in nuclear oestrogen receptor content. We have previously reported, however, that oestrogen receptors (in the uteri of anovulatory rats) fail to accumulate in the nucleus after oestradiol administration (White *et al.*, 1981); the same may be true after tamoxifen

treatment. It has been suggested that non-steroidal anti-oestrogens are antagonistic due to their depletion of cytosol receptor, thus decreasing the tissue sensitivity to further oestrogen stimulation (Katzenellenbogen *et al.*, 1979). The mechanism of achieving such cytosol receptor depletion may involve impairment of receptor synthesis (Clark *et al.*, 1974) or may be due to continual translocation of cytosol receptor into the nucleus by excess ligand (Jordan *et al.*, 1978). Our observations of a depletion of both cytosol and nuclear oestrogen receptor after long-term tamoxifen treatment may result from a combination of effects. Tamoxifen may cause continuous translocation (hence depletion) of cytosol receptor, which, owing to the inability of anti-oestrogens to activate oestrogen receptors (DeBoer *et al.*, 1981), causes incomplete stimulation of receptor synthesis. This would ultimately cause a decrease in nuclear oestrogen receptor content as a result of the metabolism of receptor molecules being recycled between the nuclear and cytoplasmic compartments.

The uterus of the anovulatory rat is maximally stimulated by oestrogen based on the failure of exogenous oestradiol to increase the nuclear content of receptor despite depletion of the cytosolic component (White *et al.*, 1981). Progesterone and norethisterone therapy, apparently by decreasing the nuclear oestrogen receptor content and hence increasing the number of available nuclear acceptor sites for oestrogen receptors (Thrower & Lim, 1981), facilitated an increase in nuclear oestrogen receptor in response to subsequent oestradiol

administration. Norgestrel therapy did not facilitate such an increase in nuclear oestrogen receptor in response to subsequent oestradiol administration. Differences between the progestins may be explained by the duration of their abilities to induce the nuclear enzyme activity, thought to be acid phosphatase (MacDonald *et al.*, 1982), that regulates nuclear oestrogen receptor content. Oestrogen receptors translocated to the nucleus after oestradiol administration may be inactivated by the residual enzyme activity induced by norgestrel treatment.

Antagonism of oestrogen stimulation by progesterone may also occur at the level of the cytosol receptor (Hsueh *et al.*, 1976; Coulson & Pavlik, 1977). Progesterone and norethisterone were effective in decreasing cytosol oestrogen receptor content and differed from norgestrel, which did not cause any significant change. In the present study progesterone was shown to cause a significant decrease in cytosol oestrogen receptor content; in a previous report we observed a decrease that was not statistically significant, perhaps because of an inadequate sample size (White *et al.*, 1982). Although there were differences between the progestins with respect to their effects on cytosol oestrogen receptor content, they were equally effective in decreasing cytosol progesterin receptor, which is oestrogen-regulated (Feil *et al.*, 1972). The mechanism of progesterone and norethisterone antagonism of oestrogen stimulation involves the regulation of receptor content in both cellular compartments, whereas norgestrel apparently selectively affects the nuclear component of the oestrogen receptor.

The change in nuclear oestrogen receptor content, due to antagonism after progestin and tamoxifen treatment, did not completely correlate with the histological changes. Progesterone and norgestrel were effective oestrogen antagonists in terms of their effect on nuclear oestrogen receptor and tissue histology; in contrast, the antagonistic properties of norethisterone and tamoxifen on receptor content did not manifest themselves in any histological change. Tamoxifen is a partial agonist of oestrogen action (Jordan *et al.*, 1978), and norethisterone is known to have oestrogenic properties (McPherson *et al.*, 1974; Marr *et al.*, 1980), which may therefore explain their maintenance of the occupied nuclear receptor component and apparent inability to change the abnormal tissue histology. Recent evidence suggests that anti-oestrogens differentially stimulated cell division in the separate cell types of the rat uterus (Mukku *et al.*, 1981). Similarly differential stimulation of isocitrate dehydrogenase and oestradiol dehydrogenase by progestins occurs in the epithelium and stroma of the human endometrium (King *et al.*, 1981). An investigation of the anti-oestrogenic effects of progestins and tamoxi-

fen on the separated cell types, obtained from the oestrogen-stimulated uterus, will be necessary in order to explain the differences between these compounds. The consistent observation of a decrease in nuclear oestrogen receptor content after treatment with oestrogen antagonists suggests a common pathway in the action of these compounds. However, a more detailed understanding of the mechanism of action of such oestrogen antagonists will facilitate their discriminatory selection to treat the effect of unopposed oestrogen.

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References

- Clark, J. H., Peck, E. J., Jr. & Anderson, J. N. (1974) *Nature (London)* **251**, 446–448
- Coulson, P. B. & Pavlik, E. J. (1977) *J. Steroid Biochem.* **8**, 205–212
- DeBoer, W., Notides, A. C., Katzenellenbogen, B. S., Hayes, J. R. & Katzenellenbogen, J. A. (1981) *Endocrinology* **108**, 206–212
- Edgren, R. A., Jones, R. C., Clancy, D. P. & Nagra, C. L. (1968) *J. Reprod. Fertil. Suppl.* **5**, 13–45
- Feil, P. D., Glasser, S. R., Toft, D. O. & O'Malley, B. W. (1972) *Endocrinology* **91**, 738–746
- Fromson, J. H., Pearson, S. & Bramah, S. (1973) *Xenobiotica* **3**, 693–710
- Hammond, C. B., Jelovsek, F. R., Lee, K. L., Creasman, W. T. & Parker, R. T. (1979) *Am. J. Obstet. Gynecol.* **133**, 537–547
- Hsueh, A. J. W., Peck, E. J., Jr. & Clark, J. H. (1976) *Endocrinology* **98**, 438–444
- Hsueh, A. J. W., Erickson, G. F. & Lu, K. H. (1979) *Biol. Reprod.* **21**, 793–800
- Jordan, V. C., Dix, C. J., Rowsby, L. & Prestwich, G. (1977) *Mol. Cell. Endocrinol.* **7**, 177–192
- Jordan, V. C., Rowsby, L., Dix, C. J. & Prestwich, G. (1978) *J. Endocrinol.* **78**, 71–81
- Katzenellenbogen, B. S., Bhakoo, H. S., Ferguson, E. R., Lan, N. C., Tatee, T., Tsai, T. S. & Katzenellenbogen, J. A. (1979) *Recent Prog. Horm. Res.* **35**, 259–300
- King, R. J. B. & Whitehead, M. I. (1980) in *Perspectives in Steroid Receptor Research* (Bresciani, F., ed.), pp. 259–271, Raven Press, New York
- King, R. J. B., Lane, G., Siddle, N., Taylor, R. W., Townsend, P. T. & Whitehead, M. I. (1981) *J. Steroid Biochem.* **15**, 175–182
- MacDonald, R. G., Okulicz, W. C. & Leavitt, W. W. (1982) *Biochem. Biophys. Res. Commun.* **104**, 570–576
- Marr, W., Elder, M. G. & Lim, L. (1980) *Biochem. J.* **190**, 563–570
- Martin, L. (1980) in *Estrogens in the Environment* (Mclachlan G., ed.), pp. 103–130, Elsevier/North-Holland, Amsterdam
- McPherson, J. C., Costoff, A., Eldridge, J. C. & Mahesh, V. B. (1974) *Fertil. Steril.* **25**, 1063–1070

- Mukku, V. R., Kirkland, J. L., Hardie, M. & Stancel, G. M. (1981) *Endocrinology* **109**, 1005–1010
- Okulicz, W. C., MacDonald, R. G. & Leavitt, W. W. (1981) *Endocrinology* **110**, 2273–2275
- Thrower, S. & Lim, L. (1981) *Biochem. J.* **198**, 385–389
- Thrower, S., Hall, C., Lim, L. & Davison, A. N. (1976) *Biochem. J.* **160**, 271–280
- White, J. O., Moore, P. A., Elder, M. G. & Lim, L. (1981) *Biochem. J.* **196**, 567–565
- White, J. O., Moore, P. A., Elder, M. G. & Lim, L. (1982) *Biochem. J.* **202**, 535–541