Journal of Steroid Biochemistry, 1977, Vol 8, pp 965–969 Pergamon Press Printed in Great Britain

STIMULATION OF EARLY PROTEIN SYNTHESIS IN THE UTERUS OF THE OVARIECTOMIZED RAT, BY CONTINUOUS INFUSION OF [^{3}H]-ESTRADIOL-17β *IN VIVO*—II: RELATIONSHIP WITH INFUSION RATE AND TISSUE CONCENTRATION

R. DE HERTOGH, I. VANDERHEYDEN and E. EKKA
Endocrinology and Nutrition Unit, University of Louvain, School of Medicine, U.C.L. 54.29, av. E. Mounier 53, 1200 Brussels, Belgium

(*Received* 29 *December* 1976)

SUMMARY

Intravenous infusion with [^{3}*H*]-estradiol-17β ($E_2{}^3H$) at different rates were performed in ovariectomized rats in order to obtain in the uteri varying levels of $E_2{}^3H$ which remained constant after 30 min of infusion. The relative increase of estrogen induced protein (I.P.) synthesis was measured at 90 min of infusion, and correlated with the uterine level of $E_2{}^3H$ obtained at 30 min. A highly significant and linear correlation was obtained between I.P. synthesis and uterine levels below the tissue specific binding capacity. $E_2{}^3H$ in excess of the specific binding capacity was rapidly lost from the uterus and did not induce a further significant increase in I.P. synthesis. Below the tissue binding capacity, I.P. synthesis was stimulated sub-maximally. Under these conditions, an increase in the infusion rate could elicit a secondary (although more limited) increase in I.P. synthesis. The successive stimulations of I.P. synthesis were additive until the overall % increase equivalent to the maximal stimulation, was reached. This observation shows that the uterus is capable to respond to estrogen stimulation by increasing I.P. synthesis to a limited extent, which will be termed the I.P. synthesizing potentiality. The latter corresponds closely to the tissue binding capacity and is available either for one or for several successive stimulations, depending on the level of estradiol obtained in the uterus. That part of the I.P. synthesizing potentiality which has been used does then enter a refractory period during which a similar level of estradiol does not further stimulate the I.P synthesis

INTRODUCTION

The synthesis of specific proteins (induced proteins or I.P.) is an early event resulting from the action of estradiol in the rat uterus [1, 2]. This synthesis requires the transcription of new mRNA [3] which might be directly stimulated by the hormone–receptor complex in the uterus. Alternatively, the I.P.-mRNA activity might be regulated at the translational level by a specific inhibitor which in turn is blocked by the nuclear estradiol–receptor complex [4]. The synthesis of I.P. is proportional to the amount of nuclear bound estradiol, as was shown in *in vitro* experiments [5]. However, both by *in vitro* and *in vivo* experiments, it was shown that the rate of increase of the I.P. synthesizing capacity, which was stimulated by the hormone, leveled off rapidly, notwithstanding the further increase of estradiol uptake within the nucleus [5]. I.P. synthesis then decreased and eventually reached a refractory state lasting for about 40 h [4, 6].

In a previous work [7], we showed that this time sequence of I.P. synthesis was not modified by the maintenance of a steady level of estradiol in the uterus, and that the decrease in I.P. synthesis occurred in spite of the presence of residual I.P. synthesizing potentiality, after a submaximal stimulation with estradiol. This observation stressed the importance of the degree of the first "impact"* of the hormone and of a graded response of I.P. synthesis. In the present work, we studied the relationship between the tissue level attained after $E_2{}^3H$ administration and the maximal I.P. response observed after 90 min of infusion.

MATERIALS AND METHODS

Experimental procedures have been described in detail in the preceding report [7]. All I.P. responses were measured after 90 min of infusion with $E_2{}^3H$ (time of maximal I.P. response with our procedure). $E_2{}^3H$ infusions were always done by sequential high rate-short term (8 min) and low rate-long term infusions.

RESULTS

1. *$E_2{}^3H$ levels in the uterus at 1/2, 1 1/2 and 4 hours of infusion, and I.P. response at 90 minutes in function of the infusion rates*

Figure 1 shows that the $E_2{}^3H$ levels in the uterus at 1/2, 1 1/2 and 4 h increased in a parallel way with

* See footnote on p. 961 in the preceding paper.

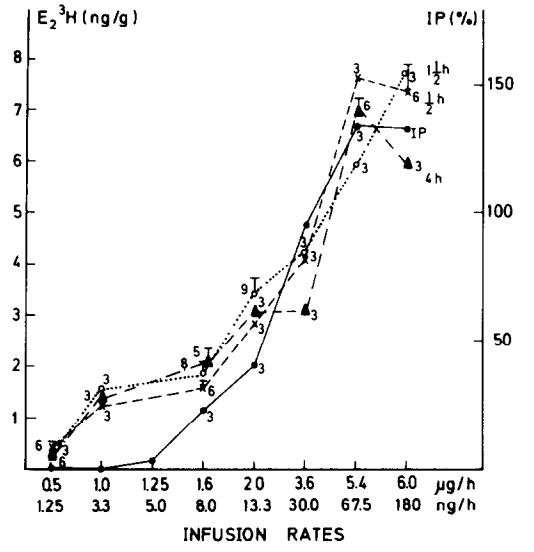

Fig. 1. Adult female rats 10–12 days postovariectomy, were infused during 8 min at a high rate (upper figures on the abscissa) and up to the indicated time at a lower rate (lower figures on the abscissa). The curves indicate the relationship between $E_2{}^3H$ infusion rates and uterine $E_2{}^3H$ levels (in ng/g wet weight) at 1/2 h (× – ×), 1 h 1/2 (O---O) and 4 h (▲-·-▲) of infusion and the I.P. response at 90 minutes (●——●). Each point of the $E_2{}^3H$ levels is the mean ±S.E.M. of the indicated number of experiments.

increasing infusion rates I.P. response was not detectable at $E_2{}^3H$ level below 1 ng/g. Between 1 and 2 ng/g, I.P. response became detectable and its increase was then parallel to the increase in the uterus $E_2{}^3H$ level. Figure 2 shows the same results obtained with higher infusion rates. $E_2{}^3H$ levels at 1/2, 1 1/2 and 4 h again increased in a parallel way up to an infusion rate of 10.8 µg/h + 500 ng/h. For higher rates of infusion, $E_2{}^3H$ levels increased more steeply at 1/2 h than at 1 1/2 h, and remained constant at 4 h. The leveling off of the $E_2{}^3H$ concentration in the uterus at 4 h is analysed on Fig. 3, where $E_2{}^3H$ levels in uterus and plasma at 4 h are plotted against the long term infusion rates, which determine the steady level achieved at that time. It is shown that the plasma levels increased linearly with increasing infusion rates, confirming the constant metabolic clearance rate of the hormone over a wide range of infusion rates, as reported previously [8]. $E_2{}^3H$ levels in the uterus showed a typical saturation curve, comparable to that described previously in adult and in immature rats [9]. The plasma concentration at half saturation of the tissue (1.2×10^{-10} M) was also similar to the previous results [9]. The saturating level in the uterus (10–11 ng/g wet weight) was similar to the saturating level reported for immature rats [9]. However, in the present experiments the long term

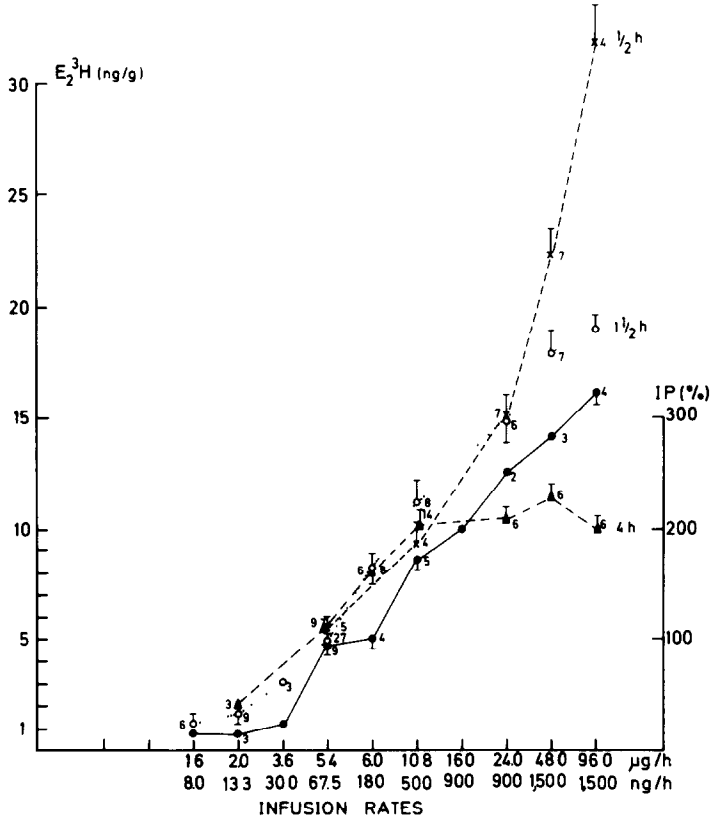

Fig. 2. Same data as in Fig. 1 obtained in adult female rats, 21–28 days postovariectomy. Higher infusion rates were realized in this group of animals. Legends see Fig. 1

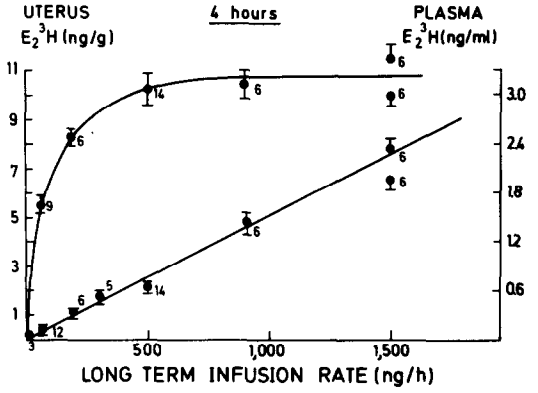

Fig. 3. $E_2{}^3H$ levels in the uterus (ng/g wet weight) and in the plasma (ng/ml) at 4 hours of infusion, in function of the long term infusion rates. The experiments were performed 21–28 days postovariectomy. Each point is the mean ± S.E.M of the number of animals indicated. The increase in $E_2{}^3H$ level in the plasma shows a linear correlation with the long-term infustion rate. ($r = 0.992$).

infusion was preceded by a short term (8 minutes)—high rate infusion to speed up equilibrium as discussed earlier. Hence, the amount of estradiol given to the animal in this short term-high rate infusion was sufficient to induce water imbibition by 4 h. To evaluate this effect, whole uteri of rats, infused during either 1/2 or 4 h, were compared on a weight basis for similar rates of infusion. The results (Table 1) show that the water imbibition at 4 h was already maximal (about 36%) at an infusion rate of 10.8 μg/h + 500 ng/h, and was not increased further at an infusion rate of 96 μg/h + 1,500 ng/h. At 30 min there was no dose effect and the uterine weights were not significantly increased above those of control animals, confirming the absence of significant water imbibition at this early time [10].

Taking into account the observed weight gain at 4 h, it may be estimated that the saturating level in the uterus (Fig. 3) was underestimated by approximately 36%. The true saturating concentration is then likely to be closer to 15 ng/g, if we refer to the uterine weight at 30 min, before water imbibition occurs. However, the existence of the saturation process is not questioned, since the correcting factor is similar both at 500 and at 1,500 ng/h infusion rates.

2. *Correlation between $E_2{}^3H$ level in the uterus at 30 minutes, and maximal I.P. response at 90 minutes*

In the preceding report [7], the importance of the degree of the initial "impact" of estradiol with the tissue on the maximal I.P. response at 90 min was shown. We then correlated the I.P. response at 90 min with the $E_2{}^3H$ level in the uterus at 30 min. Taking into account a saturating level of 15 ng/g wet weight (corrected for water imbibition), the correlation was done separately for I.P. responses obtained below and above this level. $E_2{}^3H$ Levels at 30 min were taken from Fig. 1 and the mean values correlated with individual I.P. responses obtained at 90 min for the corresponding rates of infusions. Figure 4. shows a linear and highly significant correlation ($P < 0.001$) between I.P. responses and $E_2{}^3H$ concentration at 30 min, below the saturating level. Above this level, no significant increase of I.P. was seen, the maximal response being of the order of 300%.

3. *Quantitative aspect of I.P. synthesis response (I.P. synthesizing potentiality)*

In our previous report [7], we pointed out that I.P. synthesis decreased after 90 min despite the presence of residual I.P. synthesizing potentiality. The experiments described on Fig. 5 tentatively quantitate this observation. In these experiments, the I.P. response was stimulated sequentially by repeating at given intervals a high rate-short term infusion, in course of a long term-low rate infusion. When the first stimulus was maximal (Fig. 5A), giving a 300% I.P. response, a second stimulus was not able to elicit any further response. When the first stimulus was submaximal (Fig. 5B, C, E), a second or even a third stimulus were able to induce I.P. responses, until the additive effects of all stimuli totalled the maximal response of 300%. Hence, the effect of a subsequent stimulus (for instance 96 μg/h for 8 min) could either be zero or maximal, depending on the residual I.P. synthesizing potentiality left over by preceding stimuli.

Table 1. Uterine weights (mean ± S) in course of long term infusion with estradiol-2, 4, 6, 7 ^3H

Infusion rates (short term (8 minutes)-high rate + long term-low rate.	Long term infusion time 1/2 h	4 h	% increase
0.5 μg/h + 1.25 ng/h (controls)	49.7 ± 7.9 $n = 6$	48 ± 9.1 $n = 6$	0
10.8 μg/h + 500 ng/h	56.9 ± 3.4 $n = 8$	77.6 ± 14.7 $n = 13$	36
24 μg/h + 900 ng/h	52.3 ± 8.9 $n = 7$	64.5 ± 12.0 $n = 6$	23
48 μg/h + 1,500 ng/h	48.0 ± 11.4 $n = 4$	68.3 ± 4.9 $n = 3$	42
96 μg/h + 1,500 ng/h	54.8 ± 5 3 $n = 8$	79.0 ± 5.3 $n = 3$	44

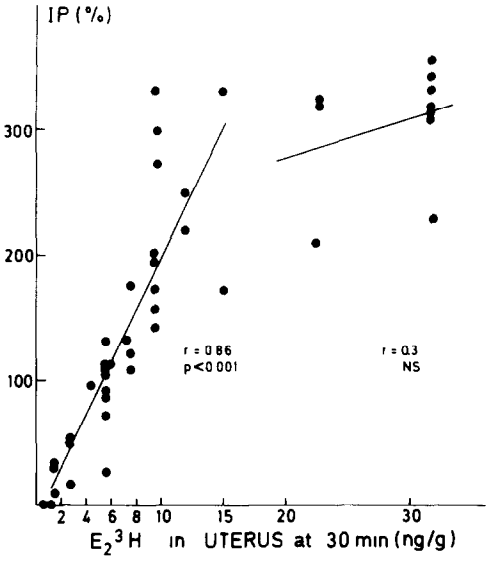

Fig 4 Relationship between I.P. response at 90 min and $E_2{}^3H$ level in the uterus (ng/g wet weight) at 30 min of $E_2{}^3H$ infusion. $E_2{}^3H$ levels at 30 min are the means taken from Fig. 1 and 2 where the dispersions are indicated The points are the individual I.P. responses at 90 min for all experiments performed at the corresponding infusion rates. Linear correlation between the mean tissue level at 30 min and the individual I.P. responses are indicated separately for tissue levels below saturation (up to 15 ng/g) and above saturation (see text for details). Correlation coefficient and statistical significance are indicated.

DISCUSSION

The dose-dependent response of I.P. synthesis, following estradiol stimulation *in vitro* or *in vivo*, has been documented by several reports [5, 11, 12] and further confirmed by the present experiments. Indeed, the I.P. response, maximal at 90 min, increased with increasing rates of infusion of $E_2{}^3H$.

With the two step infusion method used in the present work, it was further shown that the I.P. response was linearly correlated with the tissue level of the hormone at equilibrium, up to the saturation level. The latter was previously defined as the tissue specific binding capacity [9]. Its value (10^{-8} mol/Kg) is similar to the concentration of uterine receptors [13].

Estradiol taken up *in vivo* is rapidly translocated into the nucleus, and an equilibrium appears to establish between cytosol and nuclear fraction, the larger part of the hormone–receptor complex being located in the latter [13]. This situation is obtained either by injection [13] or in course of continuous infusion of the hormone [14]. Hence, the tissue concentration of $E_2{}^3H$ is likely to represent mostly nuclear bound hormone (estradiol contamination from circulating plasma or even from extracellular fluid would be minimal as evidenced from Fig. 3). The correlation between I.P. response and tissue concentration below the saturating level then fits with a similar correlation between nuclear bound estradiol and I.P. response obtained *in vitro* [5].

For the highest rates of infusion, levels of estradiol in excess of the tissue specific binding capacity were obtained at 30 min, but they had disappeared at 4 h. Although subcellular repartition of estradiol has not been analyzed in the present work, it appears that the amount of estradiol in excess over the binding capacity, was indeed loosely bound and rapidly disappeared from the tissue. This fraction of the tissue estradiol was not able to further significantly increase the I.P. response.

In our previous report [7], it was shown that the I.P. response was not maintained despite the persistance of a constant level of estradiol in the uterus. Alternatively, it was shown that the I.P. response was dependent on the degree of the first "impact" of the hormone with the tissue; the maintenance of the tissue level of the hormone was not critical to the induction of the I.P. response. This degree of impact, which gives an I.P. response directly proportional to its importance, might then be related to the nuclear level of the hormone receptor complex which is reached after estradiol administration.

The significance of the degree of "impact" is further stressed by the I.P. synthesizing "potentiality", which

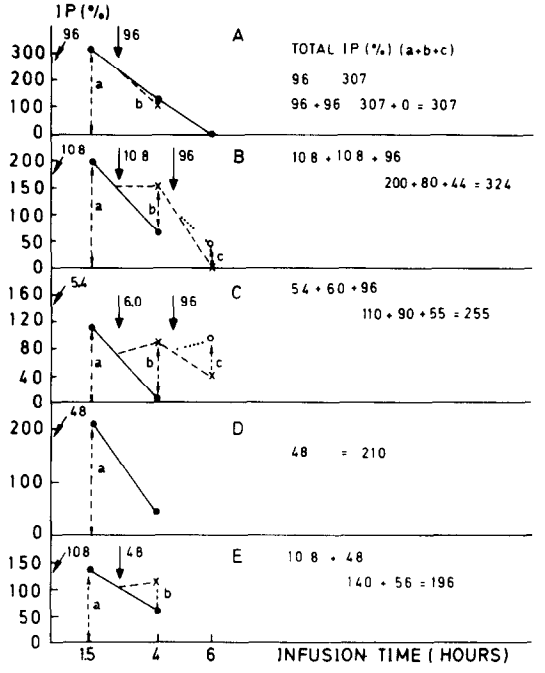

Fig 5. Time sequence of I.P. response in constant infusion experiments, where short term (8 min)-high rate infusions were done in repetition. I.P. response was measured 90 min after each short term infusion. The time sequences of I P response are indicated after the first (●——●, the second (×——×) and the third (○···○) short term-high rate infusion. The arrows and the corresponding figures indicate the times and the rates of the short term infusions. The corresponding long term-low rates infusions which follow the short term ones are the same as in Fig. 1 and 2 a, b and c are the increments of I.P. responses above basal level of corresponding infusion time On the right, the sum of the increments are computed for each experiment. Adult female rats ovariectomized for 21–28 days, were used.

might be defined as the total capacity of response of the tissue. When all this potentiality has been used up by a maximally stimulating impact, the tissue enters a refractory period of 40 h [6]. However, a weaker "impact" will leave some I.P. synthesizing potentiality in the tissue. The latter will not be affected by the hormonal movements taking place below the hormonal level realized at the first "impact", but will elicit a supplementary I.P. response if a degree of "impact", greater than the first one, is realized.

What is really meant by degree of "impact" is at the present time only speculative. However, the close correlation between I.P. response and tissue level of $E_2{}^3H$ below saturation strongly suggests a critical role played by the cytosol receptor, as already put forward by Anderson *et al.* [15]. The cytosol receptor could be translocated to the nucleus according to a dynamic equilibrium depending on the estradiol level within the tissue [14, 16, 17]. The dynamic exchange of the hormone between the subcellular fractions would not further exhaust the residual (unused) cytosol receptor pool [7], although increasing the tissue level of the hormone would displace more cytosol receptor to the nucleus and induce a further increase of I.P. synthesis. The I.P. synthesizing potentiality would then be directly linked to the amount of "unused" receptor left in the cytosol. This scheme, however, appears too simplistic if one considers the delay existing between the replenishment of cytosol receptor (24 h) and the recurrence of the I.P. response (40 h) [6]. In addition, rapid resynthesis of receptor has been evoked by several authors [18, 19]. It should then be considered that the newly synthesized receptor remains in an inactive state for several hours, unless some other mechanism were responsible for the refractory state. Lack of uterine response to estradiol has also been described during the early development of the rat, despite the presence of specific receptors in the cytosol and their ability to be translocated to the nucleus under the influence of estrogens [20–22]. Hence, a nuclear factor may be responsible for the tissue refractoriness both in developing uteri and after estradiol stimulation. In the latter situation estradiol binding in nuclei has been shown to exist in multiple forms [13, 23], differing in solubility [24], exchangeability [25] and specificity [26]. The physiological significance of this complexity is at present ill understood [26] so also the role played by the I.P. response itself [4, 24].

The present data are in favor of a quantitative relationship between a limited capacity of response of the uterus (called I.P. synthesizing "potentiality" to avoid confusion with I.P. synthesis "capacity" [4] which implicates the presence of already formed active m-RNA) and the amount of specific binding of estradiol in the tissue as a whole. The total I.P. synthesizing potentiality is used up when tissue binding capacity has been saturated. Whether the secondary fractional or total refractoriness of the tissue is secondary to a lack of nuclear translocation of active cytosol receptors or to a nuclear "blockade" remains an open question.

Acknowledgement—The financial support of the Fonds de la Recherche Scientifique Médicale Belge, Grant No: 3.4533.75, is gratefully acknowledged.

REFERENCES

1. Notides A. and Gorski J.: *Proc. natn. Acad. Sci. U.S.A.* **56** (1966) 230–235.
2. Barnea A. and Gorski J.: *Biochemistry* **9** (1970) 1899–1904.
3. De Angelo A. B. and Gorski J.: *Proc. natn. Acad. Sci. U.S.A.* **66** (1970) 693–700.
4. De Angelo A. B. and Fujimoto G. I.: *Proc natn. Acad. Sci. U.S.A.* **70** (1973) 18–21.
5. Katzenellenbogen B. S. and Gorski J.. *J. biol. Chem.* **247** (1972) 1299–1305.
6. Katzenellenbogen B. S.: *Endocrinology* **96** (1975) 289–297.
7. Ekka E., Vanderheyden I. and De Hertogh R.: *J. steroid Biochem.* **8** (1977) 959–963.
8. De Hertogh R., Ekka E., Vanderheyden I. and Hoet J. J.: *Endocrinology* **87** (1970) 874–880.
9. De Hertogh R., Ekka E., Vanderheyden I. and Hoet J. J.: *Endocrinology* **88** (1971) 165–174.
10. Emmens C. W.: In *Methods in Hormone Research* (Edited by R. I. Dorfman). Academic Press, New York, Vol II (1962) p. 98.
11. Mayol R. F. *Molec. Cell. Endocr.* **2** (1975) 133–146.
12. Sömjen D., Kaye A. M. and Lindner H. R.: *Molec. Cell. Endocr.* **1** (1974) 77–88.
13. Gorski J. and Gannon F.: *Ann. rev. Physiol.* **38** (1976) 425–450.
14. De Hertogh R., Ekka E., Vanderheyden I. and Hoet J. J.: *J. steroid Biochem.* **4** (1973) 289–299.
15. Anderson J. N., Peck E. J. and Clark J. H.: *Endocrinology* **95** (1974) 174–178.
16. De Hertogh R., Ekka E., Vanderheyden I. and Hoet J. J.: *J. steroid Biochem.* **4** (1973) 301–311.
17. Williams D. and Gorski J.: *Proc. natn. Acad. Sci. U.S.A.* **69** (1972) 3464–3468.
18. Little M., Szendro P., Teran C., Hughes A. and Jungblut P. W.: *J. steroid Biochem.* **6** (1975) 493–500.
19. Cohen M. E. and Hamilton J. H.: *Proc. natn. Acad. Sci. U.S.A* **72** (1975) 4346–4350.
20. Sömjen D., Sömjen J., King R. J. B., Kaye A. M. and Lindner H. R.: *Biochem. J.* **136** (1973) 25–33.
21. King R. J. B., Sömjen J., Kaye A. M. and Lindner H. R.: *Molec. Cell. Endocr.* **1** (1974) 21–36.
22. Katzenellenbogen B. S. and Greger N. G.: *Molec. Cell Endocr.* **2** (1974) 31–42.
23. Ekka E. and De Hertogh R.: *J. steroid Biochem.* **7** (1975) 249–254.
24. Bauleiu E. E.: *Molec Cell. Biochem.* **7** (1975) 157–174.
25. De Hertogh R., Ekka E., Vanderheyden I and Hoet J. J.: *J. steroid Biochem.* **4** (1973) 313–320.
26. Yamamoto K. R. and Alberts B. M. *Ann. Rev. Biochem.* **45** (1976) 721–746.