A Comparison of the Binding of Epidermal Growth Factor to Cultured Granulosa and Luteal Cells*

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Epidermal growth factor (EGF) binds in a specific and saturable manner to both bovine granulosa ($K_d = 2.3 \times 10^{-10} M, n = 2.3 \times 10^6$ EGF molecules/cell) and luteal cells ($K_d = 7.0 \times 10^{-10} M, n = 1.65 \times 10^5$ EGF molecules/cell). In luteal cells, however, as opposed to granulosa cells, the binding of EGF does not promote a mitogenic response.

The spontaneous luteinization of cultured granulosa cells derived from large, preovulatory follicles is associated with an increased number of EGF receptor sites per cell and with a loss of responsiveness to EGF. Both EGF-responsive granulosa cells and unresponsive luteinizing cells showed a similar degree of internalization and lysosomal degradation of the bound EGF molecules as well as a specific loss of EGF receptor sites after exposure to EGF ("down regulation").

Preincubation with nonsaturating concentrations of EGF produced a degree of receptor loss which exceeded the percentage of receptors occupied at these concentrations. Nonetheless, even after an extended exposure to oversaturating concentrations of EGF (100 ng/ml), 15 to 25% of the receptor sites still remained available for EGF binding. This degree of receptor occupancy is sufficient to induce a maximal mitogenic response in granulosa cells. Exposure to either EGF or low density lipoprotein specifically affected the appropriate surface receptor sites, but neither molecule had any effect on the heterologous receptor sites.

Our results on the binding of EGF to granulosa and luteal cells, as well as to early and late passages of granulosa cells, indicate a lack of correlation between EGF-binding capacity and mitogenic activity. The loss of mitogenic response in luteinizing cells is not due to a defect in the internalization and degradation of the cell-bound EGF, or to a defect in the EGF-induced receptor modulation and recovery, or both.

In previous studies (1) it has been shown that cultured bovine granulosa cells, derived from medium-sized ovarian follicles, are highly responsive to the mitogenic stimulus provided by both the epidermal and fibroblast growth factors. In contrast, bovine luteal cells, which are derived from granulosa cells through the process of cytomorphosis, are no longer sensitive to EGF, although they do respond quite well to FGF (2).

In the present study we have looked at the quantitative and kinetic aspects of the binding of EGF to granulosa and luteal cells. Our results demonstrate that granulosa cells, as well as luteal cells, are capable of binding EGF in a highly specific manner. Thus, the lack of response of luteal cells to EGF cannot be explained on the basis of a loss of EGF receptor sites. Since the cytomorphosis of granulosa cells to luteal cells is associated with a loss of sensitivity to EGF but not of EGF receptor sites, the in vitro proliferative response of these cell types to EGF and PGF provides a direct way to study the relationship between mitogenicity, binding capacity, and the associated decreased concentration ("down regulation") of surface receptor sites for EGF.

EXPERIMENTAL PROCEDURES

Materials—Fibroblast growth factor (FGF) was purified from bovine pituitary glands (6) and brains (7) as previously described. Both pituitary and brain FGF yielded single bands on polyacrylamide gel electrophoresis at pH 4.5. No bands were observed at pH 8.5.

EGF was purified as described by Savage and Cohen (6, 9) from the submaxillary glands of adult male, Swiss Webster mice that had been given daily subcutaneous injections of testosterone propionate (1 mg per animal) for 8 days. The final preparation yielded a single band on polyacrylamide gel electrophoresis at pH 8.5 and, like the preparation of Savage and Cohen (8), three amino acids, lysine, alanine, and phenylalanine, were absent. The biological activity of EGF, as measured by the stimulation of the initiation of DNA synthesis in human foreskin fibroblasts, was equal to that of a reference preparation from Dr. S. Cohen, Vanderbilt University. Crystalline bovine serum albumin was obtained from Schwarz/Mann; Na$^{125}$I and $[^{3}H]$acetate anhydride from Amersham/Searle; tissue culture medium (Ham's F-12) and calf serum from Gibco; gentamicin was from Schering; tissue culture dishes from Falcon Plastics; chloramine-T, chloroquine, and 2,4-dinitrophenol from Sigma and concanavalin A (conA) from Miles-Yeda.

Iodination of EGF—The iodination of EGF was carried out as described by Carpenter and Cohen (4), using a molar ratio of protein to Na$^{125}$I of approximately 1:1. EGF (6 µg) was dissolved in 25 µl of 0.05 M phosphate buffer, pH 7.5, and added to 55 µl of 2 M phosphate buffer, pH 7.5, containing 2 µCi of carrier-free Na$^{125}$I. Chloramine-T (100 µg in 10 µl) was then added for 1 min at 4°C. The reaction

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was stopped by the addition of sodium metabisulfite (200 μg in 10 μl), potassium iodide (3 mg in 10 μl), and albumin (2.5 mg in 20 μl). The labeled protein was separated from unreacted Na125I by gel filtration through Sephadex G-10 with a phosphate-buffered saline (0.9% NaCl solution) containing 0.1% albumin. The labeled EGF was stored frozen in the presence of 0.1% albumin. The specific activity of the labeled EGF was 370,000 to 460,000 dpm/ng. Alternatively, the cell surface as described (13, 14).

Low density lipoprotein (LDL) was a generous gift from Drs. P. and C. Fielding (University of California, San Francisco). It was obtained from human plasma by differential ultracentrifugal flotation (12, 13), radioiodinated, and tested for specific binding to the labeled protein was separated from unreacted Na125I by gel filtration through Sephadex G-10 with a phosphate-buffered saline (pH 7.4) containing 0.1% albumin. To detach the cells, 2 ml of a 0.05% solution of trypsin in a calcium, magnesium-free phosphate-buffered saline was added to each dish, and the dishes were incubated at 37°C for 10 min. A 0.2-ml aliquot of the cell suspension was removed for cell counting (Coulter counter). The remaining contents of the dishes were transferred to counting vials, and the radioactivity measured with a Beckman 310 series γ counter. Nonspecific binding, as determined by measuring the binding in the presence of excess unlabeled EGF (5 μg/dish), amounted to less than 5% of the total label bound. All experiments were performed in duplicate or triplicate, and different determinations of 125I-EGF binding varied by less than 10% of the mean values.

**RESULTS**

**Binding Properties of 125I-EGF to Granulosa and Luteal Cells**

The time course of binding of 125I-EGF to bovine granulosa and luteal cells is shown in Fig. 1. At 37°C maximal binding was reached after 30 min for the granulosa cells and at 1 h for the luteal cells. The maximal amount of hormone specifically bound by a luteal cell was 8-fold higher than the amount bound by a granulosa cell. Upon continued incubation of luteal cells with the labeled hormone, the amount of cell-bound radioactivity decreased in 4 h to 62% of the initial maximal amount bound at 1 h. The same observation was made with granulosa cells; by 4 h the amount of cell-bound radioactivity was 55% of that observed at 30 min.

The effect of increasing concentrations of EGF on the binding of 125I-EGF to granulosa and luteal cells is shown in Fig. 2. EGF receptor sites on granulosa cells were saturated at EGF concentrations as low as 2 ng/ml (5.3 x 10-10 M), and half-maximal binding was obtained at 0.75 ng/ml (1.2 x 10-11 M). With luteal cells maximal binding was observed at 12 ng/ml (2.0 x 10-9 M) (Fig. 2). A Scatchard plot of these data is shown in Fig. 3. While for the granulosa cells the dissociation constant (Kd) was 2.4 x 10-9 M and the maximal binding was

![Fig. 1. Time course of 125I-EGF binding to granulosa and luteal cells.](http://www.jbc.org)
undergo cytomorphosis and luteinize spontaneously in cultures devoid of pituitary hormones. In contrast, granulosa proliferation by EGF. sites is, by itself, not sufficient for the induction of cell poration at concentrations as low as 0.2 rig/ml (3.3 X lo-* increase (lo- to 20-fold) in cell number and thymidine incor-
mitogen for granulosa cell cultures and gave a maximal increase in cell number (2). By contrast, EGF was a strong increase for granulosa cells. Thus, approximately 23,000 and iO5,000 EGF molecules respectively. although EGF did bind to luteal cells, it had no mitogenic cultures, it was observed, as previously reported (1, 21, that
binding (total binding minus nonspecific binding), determined after a 40-min incubation at 37°C, is expressed in picograms of 1015-P-EGF bound per 106 cells.

**Relationship Between EGF-binding Capacity and Mitogenicity**

When the binding of EGF to granulosa and luteal cell cultures was compared to its mitogenic effect on these cultures, it was observed, as previously reported (1, 2), that although EGF did bind to luteal cells, it had no mitogenic effect upon them, as indicated by the absence of a significant increase in cell number (2). By contrast, EGF was a strong mitogen for granulosa cell cultures and gave a maximal increase (10- to 20-fold) in cell number and thymidine incorporation at concentrations as low as 0.2 ng/ml (3.3 X 10-11 M) and a half-maximal effect at 15 pg/ml (2.5 X 10-12 M) (1). These results indicate that the presence of EGF receptor sites is, by itself, not sufficient for the induction of cell proliferation by EGF.

**Interaction of 125I-EGF with Granulosa Cells Maintained in Culture for Short and Long Terms**

Granulosa cells harvested from large, preovulatory follicles undergo cytomorphosis and luteinize spontaneously in cultures devoid of pituitary hormone. In contrast, granulosa cells obtained from small follicles fail to luteinize spontaneously in culture (18). Using granulosa cells maintained in tissue culture, one can study the dependency or loss of dependency on growth factors which could be developed during the process of cytomorphosis, i.e. when granulosa cells luteinize to give a population of luteal cells. The following experiments were undertaken to determine whether a long-term, luteinizing, culture of granulosa cells becomes unresponsive to EGF and whether changes in responsiveness are associated with changes in the EGF-binding capacity, internalization, and release and/or in the EGF-induced loss and recovery of surface receptors for EGF.

Mitogenic Activity and Binding Capacity Freshly isolated granulosa cells and late passages of cultured granulosa cells derived from 10-mm follicles were tested for sensitivity to EGF and for EGF-binding capacity. The results indicate that cells from a late passage undergo luteinization in vitro and, as with luteal cells, EGF no longer had a significant effect on either cell proliferation (Fig. 4) or thymidine incorporation. This change in sensitivity was associated with an EGF-binding capacity 6 times higher than the binding to cells freshly isolated (Fig. 5). Freshly isolated granulosa cells which are EGF-responsive show a saturation of EGF binding at 4 ng/ml, whereas cultures from late passages which no longer respond to EGF and show signs of terminal differentiation saturate at higher concentrations (10 ng/ml) (Fig. 5).

In the following experiments EGF-responsive cells were obtained from small follicles (3 to 5 mm) and cultured in the presence of PGF for 1 to 3 weeks (two to four cell passages). The nonresponsive cells were derived from big follicles (10 to 15 mm) and cultured (2 to 3 weeks) in the presence of PGF and then in its absence for at least 4 weeks before use in the experiments. These cells stopped proliferating, became considerably enlarged, and showed the morphology of luteal cells.
Release of Cell-bound \(^{125}\text{I}-\text{EGF}\) - To measure the release of cell-bound EGF, monolayers were incubated at 4°C with labeled EGF, washed extensively to remove the unbound hormone, and the amount of cell-bound radioactivity determined at various times after transferring to 37°C. The data (Fig. 6) indicate that with both EGF-responsive and nonresponsive granulosa cells the bound radioactivity decreased rapidly \((t_{1/2} = 60\text{ min})\) during the subsequent incubation at 37°C. As with other cell types \((4)\), 80 to 95% of the initial cell-bound radioactivity was not associated with the cells after 2 h incubation in these conditions. A 70 to 80% inhibition of this release was obtained in the presence of chloroquine (0.1 mM) \((19)\) or 2,4-dinitrophenol (0.3 mM) (Table I). This indicates that the loss of cell-bound radioactivity is dependent on the prior internalization and degradation of the EGF molecules, which require both the generation of a metabolic energy and the proteolytic activity of lysosomal enzymes \((19)\).

Loss of EGF Receptor Sites Due to Preincubation with EGF - The following experiments were undertaken to determine whether the exposure of ovarian cells to EGF is, as in other cell systems \((3-5)\), associated with a decreased capacity of the cells to rebind EGF and whether EGF-responsive and nonresponsive granulosa cells differ in the EGF-induced modulation of the concentration, or availability of its own surface receptor sites, or both. For this purpose cells were preincubated for 10 h with saturating and nonsaturating concentrations of unlabeled EGF, washed, incubated \((37°C)\) for 3 h to allow the membrane-bound EGF to degrade, and tested for their ability to rebind fresh \(^{125}\text{I}-\text{EGF}\). As shown in Fig. 7A, preincubation with physiological concentrations of EGF induced in both EGF-responsive and nonresponsive cells up to an 80% reduction in EGF-binding capacity. A significant decrease in binding was observed in cells that were preincubated with EGF concentrations as low as 0.1 ng/ml, and a 40 to 50% reduction in binding was obtained by preincubation with EGF at 0.5 ng/ml. With cells that respond to EGF these concentrations are enough to obtain a maximal mitogenic response. Saturating concentrations of EGF are therefore not required for the induction of both cell proliferation and the loss of surface receptor sites for EGF.

![Graph showing the effect of EGF concentration on binding to ovarian cells](image-url)
Fig. 7. Loss of EGF-binding capacity induced by preincubation with unlabeled EGF. A, ten hours preincubation with saturating and non-saturating concentrations of unlabeled EGF. B, preincubation for different time periods with unlabeled EGF. Cultures of granulosa cells that respond (△), or lost response (●) to EGF were incubated at 37°C with unlabeled EGF in F-12 medium containing 1% calf serum. The cells were then washed free of exposure (up to 24 h) to excess quantities of EGF (up to 100 ng/ml). It is of interest that with granulosa cells which do not luteinize in culture even less than 10% of the receptor sites for EGF have to be occupied in order to obtain a maximum mitogenic effect.

Recovery of EGF-binding Capacity. Cells that were first incubated with EGF to induce a 70 to 80% decrease in EGF-binding capacity were incubated for different time periods in the absence of EGF, and their ability to rebind fresh EGF was tested. The results (Fig. 8) indicate that both luteinizing and nonluteinizing granulosa cells are capable of gaining the initial EGF-binding capacity within 12 to 24 h of incubation in the presence, but not in the absence, of serum.

Effect of LDL and ConA on EGF Receptors

Granulosa cells show a specific binding capacity for concanavalin A (ConA) and for low density lipoprotein (LDL) (at saturation, 2.8 x 10^7 ConA and 6 x 10^6 LDL molecules are bound per cell). We have therefore studied whether the modulation of EGF surface receptors can affect the binding of ConA or LDL molecules to their appropriate surface receptors and vice versa. The results (Table II) indicate that in cells that were preincubated (37°C, 12 h) with EGF (10 ng/ml), a 70 to 80% decrease in the EGF-binding capacity was not associated with a detectable change in the binding of either ConA or LDL molecules to the cell surface. Similarly, a 70% decrease in the binding of LDL was induced by preincubation (37°C, 24 h) with LDL (50 μg/ml), but this did not produce a change in the binding of EGF to these cells. The same experiment was not possible with ConA, since it was found that this lectin blocks the binding of EGF. In cells that were preincubated (37°C, 1 h) with 2.5 to 10 μg of ConA/ml and washed free of unbound ConA there was a 50 to 90% decrease in EGF-binding capacity.

Since various ConA-induced changes (cell agglutination, receptor redistribution, mitogenic stimulation) are temperature-dependent (11, 24), cells were preincubated with ConA either at 4°C or 37°C and their capacities to bind EGF were compared. The results (Fig. 9) indicate that at 4°C, in contrast to 37°C, there was no effect of preincubation with 2.5 μg of ConA/ml, and even at saturating concentrations of ConA (10 to 100 μg/ml) there was only 20 to 50% inhibition of EGF binding, although a similar number of ConA molecules was bound at both temperatures. This might indicate that factors other than a possible steric hindrance or binding to a common mannos-containing glycoprotein are involved in the ConA-induced inhibition of EGF binding.
These observations lead us to conclude that 1) binding studies whereas luteal cells which do not respond at all can even bind which proliferate in response to EGF bind 10 times more EGF molecules/cell than granulosa cells. than is needed to induce a maximal proliferative response, sensitivity to EGF but not to FGF (1, 2, 18).

Binding of EGF to Granulosa and Luteal Cells

FIG. 9. Effect of preincubation with concanavalin A on EGF binding. Confluent cultures of granulosa cells (7 mm, third passage) were washed three times with phosphate-buffered saline containing 0.1% albumin and incubated with various concentrations of conA in the same medium for 1 h at either 4°C (△) or 37°C (○). The cell monolayers were then washed free of unbound conA and incubated with 125I-EGF (5 ng/ml, 4°C, 75 min) to determine the specific binding of EGF.

TABLE II

Effect of preincubation with EGF or LDL on binding of 125I-EGF and 125I-LDL

Subconfluent cultures of granulosa cells (7 mm, third passage) were cultured for 36 h in F-12 medium containing 10% LDL-deficient serum. The medium was replaced with fresh medium containing 1% LDL-deficient serum, and the cells were incubated with or without unlabeled EGF (20 ng/ml, 12 h) or LDL (50 μg/ml, 24 h). Each plate was washed free of unbound LDL or EGF, incubated (2 h, 37°C), and washed again to permit complete degradation of the bound molecules. To determine the amount of radioactivity specifically bound to the cell surface, cells were then incubated with either 125I-EGF (5 ng/ml, 75 min, 4°C) or 125I-LDL (25 μg/ml, 2 h, 37°C), both in the presence and absence of an excess of unlabeled molecules. Cells were incubated with heparin (10 mg/ml, 1 h, 4°C) to determine the amount of 125I-LDL specifically bound to the cell surface and hence accessible for heparin release (13, 14). LDL uptake (heparin resistant radioactivity) was effected to the same extent by preincubation with unlabeled LDL.

We have shown in a previous study that the cytomorphosis of granulosa cells to luteal cells is associated with a loss of sensitivity to EGF but not to FGF (1, 2, 18).

Our present results on the binding of EGF to granulosa and luteal cells as well as to early and late, luteinizing, passages of granulosa cells indicate a lack of correlation between EGF-binding capacity and mitogenic activity. Granulosa cells which proliferate in response to EGF bind 10 times more EGF than is needed to induce a maximal proliferative response, whereas luteal cells which do not respond at all can even bind 4 to 6 times more EGF molecules/cell than granulosa cells. These observations lead us to conclude that 1) binding studies should be correlated with biological activity as expressed by the increase in cell number, since it is often implied that binding of a given agent will induce a biological effect. The example of EGF binding to granulosa cells versus that to luteal cells demonstrates that this is not always the case; 2) the injection of a 125I-labeled mitogen in vivo and its subsequent distribution in the body can lead to false conclusion regarding the locus of mitogenic activity. For example, with 125I-EGF the in vivo binding to granulosa cells will be barely detectable because of the high sensitivity of the cells, while luteal cells which do not respond to EGF will show, in comparison to granulosa cells, a detectable binding. This could lead to the wrong conclusion that luteal, and not granulosa, cells are the target cells for EGF.

We have shown that during the in vitro luteinization of granulosa cells the number of surface receptors for EGF is increased and, as with EGF-responsive cells, can be down regulated by the extracellular concentration of EGF. Our experiments on the binding of EGF clearly indicate that the loss of a mitogenic response to EGF in cells that undergo luteinization is not due to a defect in the internalization and release of cell-bound EGF and/or in the EGF-induced loss and recovery of surface receptor sites for EGF.

We have further shown that in both luteinizing and nonluteinizing granulosa cells the bound molecules are degraded and released into the medium subsequent to EGF binding and that the cells become incapable of rebinding the same amount of additional EGF molecules. The magnitude of this effect was dependent both on the concentration of EGF and on the duration of the exposure. A 40 to 60% decrease in EGF receptor sites was induced by the occupancy of only a small percentage of the receptors (25% and 10% occupancy in EGF-responsive and nonresponsive cells, respectively). On the other hand, even with very high concentrations of EGF (up to 100 ng/ml) and after an extended preincubation time, 15 to 25% of the initial EGF receptor sites remain available for EGF binding. This fraction is still higher than the percentage of EGF surface receptors that have to be occupied for a maximal mitogenic response (1). It therefore seems that, as receptor sites are lost, the capacity of the cells to further reduce the concentration of receptors is impaired, so that cells with fewer receptor sites become resistant to the hormone-induced decrease in their own surface receptor sites (6). We have also demonstrated that the EGF-induced receptor modulation was limited to EGF receptors, since conA or LDL receptors on the same cells were totally unaffected. Conversely, while exposure to LDL did affect the LDL receptor sites, it had no effect on the EGF receptor sites. Similar results on the effect of hormones upon heterologous receptors were obtained in other systems (3, 5).

There are various examples, both in vivo and in vitro, in which the exposure of a target cell to high levels of hormone results in a decreased sensitivity to the hormone, whereas the deprivation of a stimulating agent may result in an increased sensitivity to the introduction of this agent (3, 5). In both granulosa and luteal cells, surface receptors for EGF are subjected to modulation by EGF, and this might provide an initial locus for regulating the cellular sensitivity to EGF. The present results indicate, however, that in the ovarian cell system this type of receptor regulation cannot be a major factor in determining the target cell's sensitivity, since it exists in both EGF-responsive and nonresponsive cells. It is therefore necessary to look for other steps in the pathway within the target cell which might alter the cell's responsiveness to EGF. One possibility is to study whether EGF-binding
proteins are present in the cell’s cytosol or nucleus or whether EGF surface receptor sites are transferred into the cell when down regulation takes place. Changes in these possible sites of interaction with EGF, rather than changes in the cell surface receptors, could eventually lead to a loss of sensitivity to EGF without a noticeable change in the properties of the cell’s surface receptor sites.

The induction of cell division is accompanied by various changes in cellular physiology and properties of the cell surface (22, 23). It is, however, not clear which of the induced alterations are correlative and which are both necessary and sufficient for the G1-G0 transition. Our results indicate that the internalization and release of cell-bound EGF as well as the induced loss and recovery of EGF receptor sites are by themselves not sufficient to commit the cells to undergo cell division. This type of receptor modulation might, however, have a role in transmitting the mitogenic signal. In view of our results it is more likely that the inactivation of both the cell-bound EGF and its appropriate surface receptors simply serves as a control mechanism by which the cell can degrade and release the cell-bound EGF and regulate the concentration of its surface receptors, so that an excess of hormone can specifically decrease the number of its own functional receptor sites.

Note Added in Proof — ECF concentration as low as 15 pg/ml is enough to induce in granulosa cells a half-maximal mitogenic response (1). It is therefore possible that the loss of EGF responsiveness in luteinizing cells is due to a loss of a very small fraction (<5%) of receptor sites that are specifically involved in mediating the mitogenic effect of EGF. This receptor loss will be hardly or not detectable since luteinization is associated with an overall 5- to 10-fold increase in the number of EGF receptor sites per cell.

REFERENCES

1. Gospodarowicz, D., III, C. R., and Birdwell, C. R. (1977) Endocrinology 100, 1121-1128
2. Gospodarowicz, D., III, C. R., and Birdwell, C. R. (1977) Endocrinology 100, 1121-1128
3. Kahn, C. R. (1976) J. Cell Biol. 70, 261-286
4. Carpenter, G., and Cohen, S. (1976) J. Cell Biol. 71, 159-171
5. Lees, M. A., and Roth, J. (1975) J. Biol. Chem. 251, 3729-3732
6. Gospodarowicz, D. (1975) J. Biol. Chem. 250, 2515-2520
7. Gospodarowicz, D., Bialecki, H., and Greenburg, G. (1978) J. Biol. Chem. 253, 3736-3743
8. Savage, C. R., Jr., and Cohen, S. (1972) J. Biol. Chem. 247, 7609-7611
9. Cohen, S., Carpenter, G., and Lembach, K. (1975) in Advances in Metabolic Disorders (Luft, R., and Hall, K., eds) Vol. 8, pp. 260-264, Academic Press, New York
10. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
11. Vlodavsky, I., and Sachs, L. (1975) J. Exp. Med. 145, 1345-1353
12. Vlodavsky, I., Fielding, P. E., Fielding, C. J., and Gospodarowicz, D. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 356-360
13. Goldstein, J. L., and Sacks, G. S. (1976) J. Clin. Invest. 58, 562-570
14. Chowning, C. P., and Ledwitz-Rigby, F. (1975) Methods Enzymol. 39, 183-230
15. Gospodarowicz, D., and Gospodarowicz, F. (1975) Endocrinology 97, 114-124
16. Havel, R. J., Eder, H. S., and Bragdon, J. A. (1955) J. Clin. Invest. 34, 1353-1365
17. Havel, R. J., Eder, H. S., and Bragdon, J. A. (1955) J. Clin. Invest. 34, 1353-1365
18. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
19. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
20. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
21. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
22. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
23. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
24. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
25. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
26. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
27. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
28. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
29. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
30. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
31. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
32. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
33. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
34. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
35. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
36. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
37. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
38. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
39. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
40. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
41. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
42. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
43. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
44. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
45. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
46. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
47. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
48. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
49. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
50. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
51. Papaionannou, S., and Gospodarowicz, D. (1975) Endocrinology 97, 144-146
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I Vlodavsky, K D Brown and D Gospodarowicz

J. Biol. Chem. 1978, 253:3744-3750.

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