prostaglandins \(E_2\), \(F_{2a}\), \(D_2\) and 6-oxoprostaglandin \(F_{1a}\) (each at 2 mg/ml in ethanol). The redissolved residue was then quantitatively spotted on to silica-gel plates using alternate prescored lutropin receptors and activation of adenylate cyclase (Marsh, lutropin, which exerts its action by binding to cell-membrane prostaglandins \(E_2\), \(F_{2a}\) and 6-oxoprostaglandin \(F_{1a}\) zones were detected by exposure to \(I_2\) vapour and placed in scintillation vials; 1 ml of ethanol was added to elute the radioactivity followed by the addition of 10 ml of Unisolve I scintillation fluid (Koch-Light). The samples were mixed and radioactivity was counted in an LKB Rackbeta liquid-scintillation counter at a counting efficiency of 40–44%. Counts were corrected for quench and background (56 d.p.m.).

To determine percentage inhibition of biosynthesis of various prostaglandins radioactivity in d.p.m. of prostaglandins \(E_2\), \(F_{2a}\), \(D_2\) and 6-oxoprostaglandin \(F_{1a}\) in zones from test incubations was compared with that of controls. Human serum, haptoglobin, albumin and indomethacin (a known inhibitor of prostaglandin biosynthesis) produced a concentration-related inhibition of the biosynthesis of various prostaglandins under both conditions of the presence and absence of the cofactor reduced glutathione (Table 1). The inhibitory activities of haptoglobin and albumin were enhanced by the presence of reduced glutathione in the assay mixtures. These results demonstrate that human serum, haptoglobin and albumin inhibit the biosynthesis of prostaglandins \(E_2\), \(F_{2a}\), \(D_2\) and 6-oxoprostaglandin \(F_{1a}\) a metabolite of prostacyclin; but formation of this metabolite was less susceptible to inhibition by these proteins than was that of the other prostaglandins. This apparent differential effect on prostaglandin biosynthesis exerted by these proteins may well have physiological significance, since the biological properties of prostaglandins \(E_2\), \(F_{2a}\), \(D_2\) and prostacyclin are different.

**Table 1. Comparative effects of human serum, haptoglobin, albumin and indomethacin on the inhibition of biosynthesis of prostaglandins \(E_2\), \(F_{2a}\) and \(D_2\) and 6-oxoprostaglandin \(F_{1a}\)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Prostaglandin . . .</th>
<th>(E_2)</th>
<th>(E_{2\alpha})</th>
<th>(D_2)</th>
<th>6-Oxo (F_{1a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>285 ± 29</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>NT</td>
<td>341 ± 16</td>
<td>236 ± 19</td>
<td>&gt;600</td>
<td>312 ± 13</td>
</tr>
<tr>
<td>Albumin</td>
<td>650 ± 91</td>
<td>800 ± 122</td>
<td>480 ± 30</td>
<td>1200 ± 109</td>
<td>250 ± 27</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.65 ± 0.05</td>
<td>0.82 ± 0.04</td>
<td>0.72 ± 0.05</td>
<td>1.11 ± 0.34</td>
<td>0.31 ± 0.06</td>
</tr>
</tbody>
</table>

* Results given as \(\mu\)M.

**Reduced glutathione, 1.3 mM, was used as cofactor.

In vitro and in vivo effects of a luteolytic prostaglandin (Estrumate, I.C.I. 80996) on rat ovarian adenylate cyclase activity

ALAN E. WAKELING and LESLIE R. GREEN
Biochemistry Department, Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

Although the role of prostaglandin \(F_{2a}\) as the endogenous mediator of luteolysis in many species is now well established (Horton & Poyser, 1976), the mechanism by which prostaglandin \(F_{2a}\) exerts its luteolytic action is poorly understood. Luteal progesterone synthesis is under the trophic control of lutropin, which exerts its action by binding to cell-membrane lutropin receptors and activation of adenylate cyclase (Marsh, 1976). Progesterone synthesis is also stimulated by catecholamines (Connon & Black, 1976), but the biological role of the luteal \(\beta\)-adrenergic receptors is not known. We have conducted a series of experiments to examine the effects of a potent prostaglandin \(F_{2a}\) analogue, compound I.C.I. 80996 (Estrumate; property of Imperial Chemical Industries Ltd.; Dukes et al., 1974), on rat ovarian adenylate cyclase activity. Pseudopregnancy was induced in immature female rats by injection of 50 units of pregnant-mare serum gonadotropin, followed 64 h later by 50 units of human chorionic gonadotropin. At 6 days after human chorionic gonadotropin treatment rats were treated with a single dose (1–100 \(\mu\)g; subcutaneous injection) of I.C.I. 80996, either alone or in combination with 10 units of ovine prolactin or 25 units of human chorionic gonadotropin, and killed at various times after treatment. The response of adenylate cyclase activity to stimulation by lutropin or isoprenaline in vitro was measured in a crude plasma-membrane fraction, prepared from whole ovaries by the method of Birnbaumer et al. (1976). A single dose of I.C.I. 80996 (1–100 \(\mu\)g) greatly reduced the subsequent membrane adenylate cyclase response to lutropin within 15 min of dosing, an effect that was maintained for at least 24 h. The data in Fig. 1 demonstrate this prostaglandin-
The effects of ionizing radiation and oestrogen treatment on the steroid-receptor concentrations in experimental mammary carcinoma

J. PH. JANSSSENS, C. WITTEVRONGEL, J. VAN DAM,* P. GODDEERIS,† J. M. LAUWERIJNST† and W. DE LOECKER

Departments of Biochemistry,* Radiotherapy and† Histopathology, Faculty of Medicine, University of Louvain, B-3000 Leuven, Belgium

The combined determinations of oestradiol- and progesterone-receptor concentrations in mammary tumours considerably increase the prediction ratio of hormone dependency (Horwitz & McGuire, 1977; Jensen & De Sombre, 1979). Ionizing radiation, however, may affect the steroid-receptor concentrations, which are also affected by the endocrinological status of the animal (Bressot et al., 1979). To evaluate these factors, steroid-receptor concentrations were examined in irradiated and in oestradiol-17β-treated rats bearing mammary adenocarcinoma.

Female Sprague-Dawley rats, locally inbred, received at 50 days of age an intragastric injection of 10 mg of 7,12-dimethylbenz[a]anthracene (Fluka, Buchs, Switzerland) dissolved in 0.5 ml of sesame oil. This treatment was repeated 24 h after the first intubation (Huggins et al., 1961). The tumours appeared after 3 months. Of the six different concentrations of labelled steroids used (0.1–10 nm [2,4,6,7-,3H(N)]-oestradiol-17β (sp. radioactivity 94 Ci/mm, New England Nuclear, Southampton, U.K.) for the oestradiol receptor assay or 0.2 to 12 nm [17α-methyl-3H]-promegestone (sp. radioactivity 87 Ci/mm; New England Nuclear) for the progesterone assay). As well as 5 μl of the labelled steroid solution (which was added to each cuvette) the cuvettes numbered 1 (the blanks) received 50 μl of TED or TEG buffer, pH 7.4. For the determination of progesterone receptors, TEG buffer [TED buffer containing 10% (v/v) glycerol] was used. The receptor assay, in duplicate, was carried out on the supernatant obtained after 70 min centrifugation at 105,000 g (International Ultra-centrifuge B60 swinging bucket SB405; I.E.C., Needham Heights, MA, U.S.A.). Cookes microtiter plates with cuvettes of 0.3 ml volume were used. Four cuvettes were needed for each of the six different concentrations of labelled steroids used. The cuvettes numbered 1 (the blanks) received 50 μl of TED or TEG buffer, pH 7.4. For the determination of progesterone receptors, TEG buffer [TED buffer containing 10% (v/v) glycerol] was used. The receptor assay, in duplicate, was carried out on the supernatant obtained after 70 min centrifugation at 105,000 g (International Ultra-centrifuge B60 swinging bucket SB405; I.E.C., Needham Heights, MA, U.S.A.). Cookes microtiter plates with cuvettes of 0.3 ml volume were used. Four cuvettes were needed for each of the six different concentrations of labelled steroids used

The determination of the steroid receptor concentrations was carried out 65 days after irradiation on tumour tissue, which was also measured for size. The tissue was pulverized at –196°C and homogenized in 3 vol. (v/w) 0.15 M Tris (1.5 mM-EDTA/0.5 mM-dithiothreitol) buffer, pH 7.4. For the determination of progesterone receptors, TEG buffer [TED buffer containing 10% (v/v) glycerol] was used. The receptor assay, in duplicate, was carried out on the supernatant obtained after 70 min centrifugation at 105,000 g (International Ultra-centrifuge B60 swinging bucket SB405; I.E.C., Needham Heights, MA, U.S.A.). Cookes microtiter plates with cuvettes of 0.3 ml volume were used. Four cuvettes were needed for each of the six different concentrations of labelled steroids used.

The effects of ionizing radiation and oestrogen treatment on the steroid-receptor concentrations in experimental mammary carcinoma

J. PH. JANSSSENS, C. WITTEVRONGEL, J. VAN DAM,* P. GODDEERIS,† J. M. LAUWERIJNST† and W. DE LOECKER

Departments of Biochemistry,* Radiotherapy and† Histopathology, Faculty of Medicine, University of Louvain, B-3000 Leuven, Belgium

The combined determinations of oestradiol- and progesterone-receptor concentrations in mammary tumours considerably increase the prediction ratio of hormone dependency (Horwitz & McGuire, 1977; Jensen & De Sombre, 1979). Ionizing radiation, however, may affect the steroid-receptor concentrations, which are also affected by the endocrinological status of the animal (Bressot et al., 1979). To evaluate these factors, steroid-receptor concentrations were examined in irradiated and in oestradiol-17β-treated rats bearing mammary adenocarcinoma.

Female Sprague-Dawley rats, locally inbred, received at 50 days of age an intragastric injection of 10 mg of 7,12-dimethylbenz[a]anthracene (Fluka, Buchs, Switzerland) dissolved in 0.5 ml of sesame oil. This treatment was repeated 24 h after the first intubation (Huggins et al., 1961). The tumours appeared after 3 months. Of the six different concentrations of labelled steroids used (0.1–10 nm [2,4,6,7-,3H(N)]-oestradiol-17β (sp. radioactivity 94 Ci/mm, New England Nuclear, Southampton, U.K.) for the oestradiol receptor assay or 0.2 to 12 nm [17α-methyl-3H]-promegestone (sp. radioactivity 87 Ci/mm; New England Nuclear) for the progesterone assay). As well as 5 μl of the labelled steroid solution (which was added to each cuvette) the cuvettes numbered 1 (the blanks) received 55 μl of TED or TEG buffer, cuvettes 2 (to measure the total added tracer) received 205 μl of TED or TEG buffer, cuvettes 3 (to measure the total bound tracer) received 5 μl of TED or TEG buffer as well as 50 μl of cytosol. Cuvettes 4 (to measure the non-specific binding) received 50 μl of cytosol and 5 μl of a 100-fold excess of unlabelled steroid. The labelled steroids also contained a 100-fold excess of unlabelled dihydrotestosterone (Sigma Chemical Co, St. Louis, MO, U.S.A.) or cortisol (Sigma) to