Although prostaglandin receptors in other tissues may contain thiol groups (Johnson et al., 1974), results obtained in the present study do not support the concept that there are functionally important thiol groups in the prostaglandin E\(_1\) -receptor site on blood platelets.

Prostaglandin was generously donated by the Upjohn Company, Kalamazoo, Mich., U.S.A. This study was supported in part by a grant from Zyma (U.K.) Ltd.

Bousser, M. G. (1973) Biomedicine 18, 95–102

Isoelectric Focusing of \[^3H\]Oestradiol-17\(\beta\) Receptor in Flat Beds of Sephadex

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Isoelectric focusing in sucrose gradients has been used to study \[^3H\]oestradiol-17\(\beta\)-binding proteins (Puca et al., 1972), but this method has disadvantages in respect of the number of samples that can be analysed simultaneously, in the recovery of protein after focusing and in the long focusing times required. Radola (1969) replaced sucrose with a flat bed of Sephadex as the supporting matrix. This overcame the disadvantages mentioned above. This method has been developed for the analysis of \[^3H\]oestradiol-17\(\beta\)-binding proteins.

Uteri from immature (21-day old) or adult intact Wistar rats were homogenized at 4°C in 10mM-Tris-HCl-1 mM-EDTA-0.01% mercaptoethanol (TEM buffer), pH 7.4. After centrifugation of the homogenate for 1h (10000g at 4°C) the cytosols obtained were labelled overnight at 4°C with \[^3H\]oestradiol-17\(\beta\) (10nM) and were analysed directly or after precipitation by 0.25% (w/v) (NH\(_4\))\(_2\)SO\(_4\) followed by chromatography on Sephadex G-25. Unbound \[^3H\]oestradiol-17\(\beta\) was removed by treatment with a pellet obtained from a dextran-coated charcoal suspension (0.25% charcoal and 0.005% Dextran-T suspended in 10mM-Tris-HCl-1 mM-EDTA, pH 7.4).

Sephadex G-75 (40–120 \(\mu\)m) was swelled in TEM buffer containing a 2% concentration of appropriate ampholines (LKB Producter A-B, Stockholm, Sweden) of either pH range 5–8 or pH5–8 ampholines mixed with pH3–10 ampholines in the ratio of 11.5:1. Gel beds (2 or 4 mm thick) were formed in perspex troughs (125mm x 250mm) and their water content decreased by 10% by air evaporation at room temperature. Prefocusing of the gel in the absence of samples (100V for 1h at 4°C) minimized excessive heating in the gel during the electrofocusing of \[^3H\]oestradiol-17\(\beta\)-binding proteins. As a routine a 50 \(\mu\)l sample (250 \(\mu\)g of protein) containing 1% ampholines was applied to the gel 10–15 mm from the cathode and spread over 5 mm, ox haemoglobin was included as a marker. Adsorbent paper electrode strips (6mm wide and 1mm deep soaked in 1% (w/v) NaOH

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(cathode) or 1\% (v/v) H₂SO₄ (anode)] were placed along each edge of the gel, contact was made with platinum wire electrodes and the samples were electrofocused at 250 V (22 V/cm). The gel-cooling plate was supplied with water at 4°C from a pump. Focusing took place in a room at 4°C. Both short (100mm) and long (250mm) gel slabs were used. After focusing, samples (2.5mm x 10mm) removed from the gel matrix by means of a small three-edged stainless-steel knife were placed into scintillation vials together with 6ml of toluene phosphor and counted for radioactivity.

The pH gradient was measured at 5mm intervals by using an antimony contact electrode system (Activion) calibrated at 4°C and inserted into the gel through a perspex template placed above the gel surface (Beeley et al., 1972). At pH 5–6.5 there was good

![Graph](image_url)

**Fig. 1. Effect of focusing time on the resolution of [³H]oestradiol-17β-binding proteins obtained from intact mature rat uteri**

Radioactivity profiles obtained by counting (2.5mm x 10mm) gel segments of samples focused: (a) ●, 4h; (b) ■, 5.5h; (c) ▲, 21h. ○, pH gradient measured with an antimony contact electrode; □, zone of sample application. The arrows denote the position of a haemoglobin marker.
agreement between values obtained with antimony and glass electrodes (in the latter case segments were mixed with 1 ml of water). Below pH 5 the antimony electrode gave lower pH values than the glass electrode, and above pH 6.5 the glass electrode gave lower values than the antimony electrode. The pH gradient was stable for up to 7 h of focusing at 250 V after which time the entire gradient was displaced to somewhat lower values of pH. The resolution of electrofocused zones in the gel remained constant between 5 and 7 h, after which zones became more diffuse until at 20 h the area of focused zones had increased by 50%.

Fig. 2. Electrofocusing of [3H]oestradiol-17β-binding proteins from immature rat uteri
(a), 100 µg of [3H]oestradiol-17β-binding protein electrofocused for 6 h at 250 V; ●, radioactivity profile; □, zone of sample application. The arrow denotes the position of a haemoglobin marker. (b) Sucrose-density-gradient analysis of [3H]oestradiol-17β-binding protein used in electrofocusing: 0.25 ml of the preparation applied to 4.65 ml of 5–20% (w/v) sucrose in 10 mm-Tris-HCl–1 mm-EDTA, pH 7.4, was centrifuged for 16 h at 4°C at 100000 g in the Spinco SW50 1 rotor; ▲, binding of [3H]oestradiol-17β to the binding proteins; the arrow denotes the position of a bovine serum albumin marker.
Contrary to electrofocusing in sucrose gradients free [3H]oestradiol-17β did not electrofocus and did not migrate significantly from the point of application. This could present difficulties in the interpretation and resolution of components if the sample is placed on the gel at a point close to the isoelectric points of the components of interest. By placing the sample 10–15 mm from the cathode, acidic oestrogen receptors migrate towards the anode, whereas unbound [3H]oestradiol-17β does not move.

Fig. 1 shows the effect of focusing time on the resolution of [3H]oestradiol-17β-binding proteins from intact mature rat uteri. The gel contained pH 5–8 and pH 3–10 ampholines. Migration of binding components was incomplete at 4 h (Fig. 1a) with a marked improvement in resolution after 5.5 h (Fig. 1b); at 21 h (Fig. 1c), a general deterioration in resolution was evident.

[3H]Oestradiol-17β-binding proteins from immature (21-day old) rat uteri were prepared and examined both by electrofocusing in a pH 5–8 gradient and by ultracentrifugation through a 5–20% (w/v) sucrose density gradient (Fig. 2); a 50 µl sample (100 µg of protein) was electrofocused for 6 h at 250 V. The sucrose gradient profile (Fig. 2b) indicated that a binding protein with a sedimentation coefficient of about 8S was present together with some intermediate sized aggregates. One major cytosol component having an isoelectric point of 5.8 was detected with a second component at pH 5.4 (Fig. 2a). This agrees with data obtained with the sucrose-column method of focusing (De Sombre et al., 1969).


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Distinction Between Choline and Ethanolamine Phosphorylation in Entodinium caudatum

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The enzyme choline kinase (EC 2.7.1.32) plays an initial role in the synthesis de novo of phosphatidylcholine and phosphatidylethanolamine by catalysing the formation of phosphorylcholine and phosphorylethanolamine from the free bases choline or ethanolamine and ATP. It is widely believed that a single enzyme is responsible for the phosphorylation of both bases. A preparation of the enzyme purified 25-fold from brewer's yeast was found to phosphorylate both choline and ethanolamine, although the reaction occurred at a much slower rate with the latter substrate (Wittenberg & Kornberg, 1953). A preparation purified 200-fold from rabbit brain was also able to phosphorylate both bases (Haubrich, 1973). On the other hand, Ramasarma & Wettner (1957) reported that an enzyme in seeds of Brassica compestris was able to phosphorylate choline but not ethanolamine. Sung & Johnstone (1967) have suggested that two enzymes are present in Ehrlich ascites carcinoma cells on the basis of differential stability of choline and ethanolamine phosphorylation on storage and the variable effects of inhibitors. In the present work we present three different lines of evidence that suggest that in the rumen protozoan, Entodinium caudatum, the two bases are phosphorylated by different enzymes.

E. caudatum cells were grown, recovered and washed as previously described (Coleman, 1962; Broad & Dawson, 1973). The cells were ultrasonicated for 2 min in 0.1 M-potassium phosphate buffer, pH 7.8, at 80 Kc/s in an ultrasonic water bath (Kerry's Ultrasonics Ltd., Basildon, Essex, U.K.). A portion of the sonicated preparation (equivalent to 5 x 10⁹ cells in 0.5 ml) was assayed for choline kinase activity under conditions similar to...