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A COMPARISON OF THE BIOLOGICAL ACTIVITIES OF FOUR PROSTAGLANDINS

BY

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The biological activities of prostaglandins E_1, E_2, E_3 and $F_{1\alpha}$ have been compared. Prostaglandins E_1, E_2 and E_3 were qualitatively similar; E_1 and E_2 were about equiactive, but E_3 was less active on all preparations. Prostaglandin $F_{1\alpha}$ was a less potent vasodilator than E_1 on the cat gastrocnemius muscle blood flow and skin blood flow and a less potent depressor drug on rabbit blood pressure. On the rabbit isolated jejunum $F_{1\alpha}$ was twice as active as E_1 but on the guinea-pig isolated ileum E_1 was about forty times more active than $F_{1\alpha}$. One qualitative difference between these prostaglandins was observed; on the rabbit fallopian tube *in vivo* prostaglandins of the E series decreased both the tone and the peristalsis of the tube whereas prostaglandin $F_{1\alpha}$ increased tubal tone.

Goldblatt (1933, 1935) and Euler (1934, 1935a) independently described the presence of a substance which contracted smooth muscle and lowered blood pressure and occurred in semen and extracts of prostate glands. The active principle was given the name " prostaglandin " (Euler, 1935b).

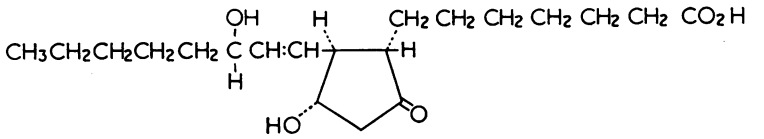
More recently Bergström and his colleagues have isolated several different prostaglandins from sheep prostate gland (Bergström & Sjövall, 1960a, b; Bergström, Dressler, Ryhage, Samuelsson & Sjövall, 1962), sheep semen (Bergström, Krabisch & Sjövall, 1960), sheep and pig lung (Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962) and human semen (Bergström & Samuelsson, 1962).

Six naturally-occurring prostaglandins have been described. Their structures have been elucidated by Bergström and his co-workers (Bergström & Samuelsson, 1962; Bergström *et al.*, 1962a, b). The three prostaglandin E's differ only in the number of double bonds. Reduction of the keto-group in a prostaglandin E can give two isomeric alcohols (Bergström *et al.*, 1962b), those from prostaglandin E_1, for example, being referred to as $F_{1\alpha}$ and $F_{1\beta}$ (Bergström, Ryhage, Samuelsson & Sjövall, 1963).

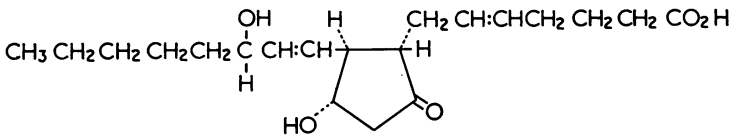
In the present investigation the actions of four prostaglandins (E_1, E_2, E_3 and $F_{1\alpha}$) (Fig. 1) have been compared on various biological preparations. Some of these comparisons have been made previously (Bergström, Eliasson, Euler & Sjövall, 1959; Euler & Bergström, unpublished).

METHODS

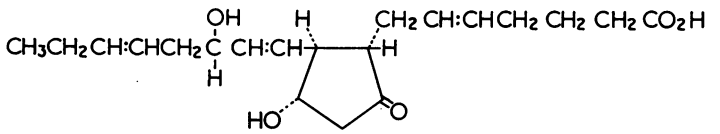
Isolated smooth muscle preparations. Segments of various organs were suspended in a 4 ml. organ-bath. Longitudinal contractions were recorded isotonically with a frontal-writing lever on



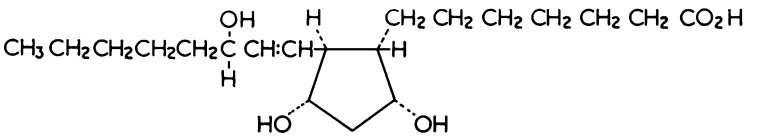
Prostaglandin E_1. 11α,15-dihydroxy-9-keto-prost-13-enoic acid



Prostaglandin E_2. 11α,15-dihydroxy-9-keto-prost-5,13-dienoic acid



Prostaglandin E_3. 11α,15-dihydroxy-9-keto-prost-5,13,17-trienoic acid



Prostaglandin $F_{1\alpha}$. 9α,11α,15-trihydroxy-prost-13-enoic acid

Fig. 1. Formulae of the four naturally-occurring prostaglandins studied in this investigation.

a smoked drum, or isometrically with a force-displacement transducer (Grass FT.03) and an ink-writing polygraph. A dose-cycle of 4 to 6 min was used with 45 to 90 sec contact time.

Guinea-pig ileum. Terminal ileum, from guinea-pigs weighing 200 to 400 g, was suspended in Tyrode solution at 37° C, gassed with air.

Rabbit jejunum. Proximal jejunum, from rabbits weighing 1.5 to 2.5 kg, was suspended in Tyrode solution at 35 to 36° C, gassed with air.

Hamster colon. The ascending colon, from hamsters weighing 100 to 200 g, was suspended in de Jalon solution at 35 to 36° C, gassed with air.

Rat uterus. A segment of uterine horn, from rats weighing 150 to 250 g which had been injected subcutaneously, 18 hr previously, with stilboestrol (100 μg), was suspended in de Jalon solution at 32° C, gassed with air.

Pithed rat blood pressure. Rats, weighing 200 to 350 g, were anaesthetized with ether and pithed by passing a strong wire through the orbit and down the cerebrospinal axis. Artificial ventilation was maintained by a Palmer small-animal respiration pump. Blood pressure was recorded from a carotid artery via a cannula connected to a Statham P23A transducer. Injections were made into a jugular or femoral vein.

Cat gastrocnemius muscle blood flow. Cats, weighing 2.5 to 5 kg, were anaesthetized with sodium pentobarbitone (40 mg/kg) injected intraperitoneally. The trachea, a carotid artery and a jugular vein were cannulated. Blood pressure was recorded by a mercury manometer. The femoral vessels were ligated beyond the branches to the gastrocnemius muscle. All other branches and tributaries proximal to this level were ligated, and the artery supplying the gracilis muscle was

cannulated with fine polyethylene tubing connected to a three-way tap for retrograde intra-arterial injections. Blood flow was maintained by stimulation of the sciatic nerve (3 to 4 V, 0.2 msec duration, 6 shocks/min). Heparin (1,000 U/kg) was injected intravenously and further doses of 500 U/kg were given every 2 hr. Venous outflow was recorded by passing blood from the femoral vein through a Palmer drop-chamber connected to a Gaddum drop-recorder or Thorp impulse-counter, or through a Grass drop-chamber connected to a Grass polygraph, the blood being returned into the jugular vein.

Cat hind-limb skin blood flow. The preparation was identical to that used for recording gastrocnemius muscle blood flow, except that the femoral artery and vein were ligated immediately distal to the saphenous vessels, which were left intact.

Fallopian tubal tone and peristalsis. Rabbits, weighing 1.5 to 3 kg, were anaesthetized with intraperitoneal urethane (175 mg/kg). The trachea was cannulated. A jugular vein was cannulated for intravenous injections. Blood pressure was recorded from a carotid artery with a Statham transducer. The abdomen was opened with a mid-line incision, the alimentary viscera were displaced to one side and a fallopian tube was identified. A polyethylene cannula was inserted into the uterine end of the tube through an incision in the uterine horn and tied in position, taking care not to occlude tubal blood supply. The cannula was attached to a Statham transducer P23AC, the side-limb of which was connected via polyethylene tubing to a 20 ml. syringe placed in a Palmer slow-injection apparatus.

The perfusion system from syringe to cannula contained Tyrode solution. An inflow rate of 27 μl./min was usually adequate to stimulate peristalsis, which was recorded with a suitable pen-writer.

RESULTS

Prostaglandins E_2, E_3 and $F_{1\alpha}$ were assayed by bracketing against prostaglandin E_1 on eight different biological preparations. The activities expressed relative to prostaglandin E_1 (=1) are shown in Table 1. The threshold doses of prostaglandin E_1 are shown in Table 2.

Isolated smooth muscle preparations. Each of the four prostaglandins contracted smooth muscle. In general, prostaglandin E_2 was slightly more active, and prostaglandin E_3 rather less active, than E_1. Prostaglandin $F_{1\alpha}$ was twice as active as E_1 on the rabbit jejunum (Fig. 2) but about forty times less active on the guinea-pig ileum (Fig. 3). The guinea-pig ileum, rabbit jejunum and hamster colon were

TABLE 1

BIOLOGICAL ACTIVITY OF PROSTAGLANDINS E_2, E_3 AND $F_{1\alpha}$ RELATIVE TO PROSTAGLANDIN E_1

Figures represent mean activity (±standard errors) relative to prostaglandin E_1 (=1). Numbers of assays are shown in parentheses. * In four of five preparations prostaglandin $F_{1\alpha}$ increased the tone of the fallopian tubes, in contrast to the prostaglandin E's which caused relaxation

	Biological activity of		
Preparation	E_2	E_3	$F_{1\alpha}$
Guinea-pig ileum	1·56±0·78 (6)	0·23±0·19 (3)	0·023±0·013 (4)
Hamster colon	2·75±1·50 (4)	0·19±0·14 (4)	0·26 ±0·16 (4)
Rabbit jejunum	1·50±0·50 (3)	0·99±0·90 (3)	2·22 ±1·72 (2)
Rat uterus	1·09±0·37 (3)	0·31±0·03 (2)	0·94 ±0·75 (4)
Cat gastrocnemius muscle blood flow	0·76±0·31 (4)	0·53±0·46 (3)	0·22 (1)
Cat hind limb skin blood flow	0·91 (1)	0·23 (1)	0·22 (1)
Rabbit blood pressure	1·00±0·00 (4)	0·34±0·03 (2)	0·075±0·04 (2)
Rabbit fallopian tube	0·93±0·08 (4)	0·43±0·16 (4)	*

TABLE 2

THRESHOLD DOSES OF PROSTAGLANDIN E_1

Figures indicate concentrations (in ng/ml.) for the isolated tissues and doses (in μg/kg) for the *in vivo* experiments

Guinea-pig isolated ileum	Contraction	8
Hamster isolated colon	Contraction	12
Rabbit isolated jejunum	Contraction	12
Rat isolated uterus	Contraction	60
Cat gastrocnemius muscle blood flow	Vasodilatation	0·5
Cat skin blood flow	Vasodilatation	0·1
Rabbit blood pressure	Depression	0·6
Rabbit fallopian tube	Inhibition	0·8

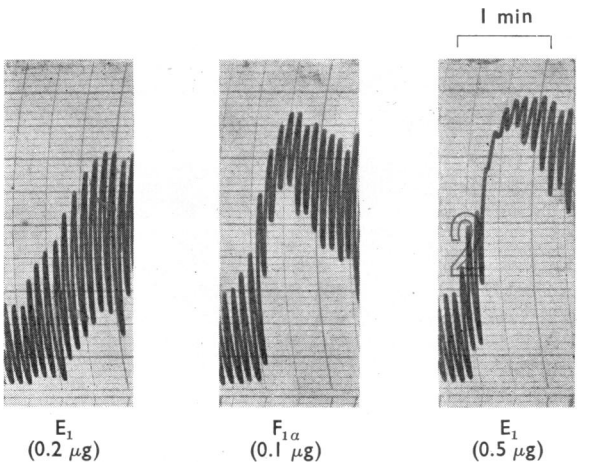


Fig. 2. Contractions of rabbit isolated jejunum, suspended in a 4 ml. organ-bath containing Tyrode solution. Contractions were recorded isometrically with a Grass force-displacement transducer (model FT.03) and recorded on a Grass polygraph. E_1=prostaglandin E_1; $F_{1\alpha}$=prostaglandin $F_{1\alpha}$.

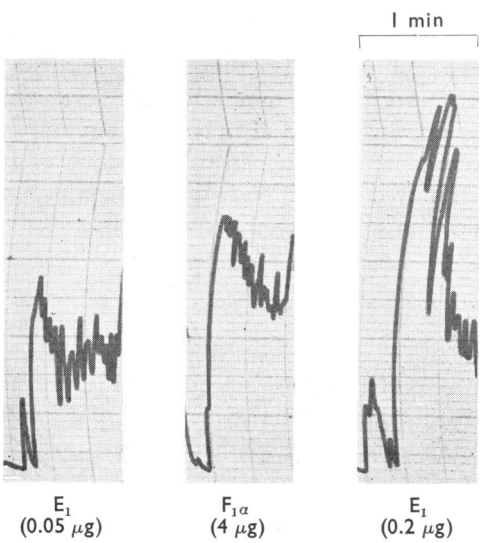


Fig. 3. Contractions of guinea-pig isolated ileum. Records as for Fig. 2.

about equally sensitive to prostaglandin E_1, responding to a concentration of 8 to 12 ng/ml.

Blood pressure and blood flow measurements. Prostaglandins E_1 and E_2 were equiactive in lowering the rabbit blood pressure, and produced a threshold effect in concentrations of 600 ng/kg, but prostaglandin E_3 was less active. Prostaglandin $F_{1\alpha}$ was fifteen to twenty times less potent than E_1 on the blood pressure. Similarly, on the cat gastrocnemius muscle blood flow and the cat skin blood flow prostaglandins E_1 and E_2 were equiactive, but E_3 and $F_{1\alpha}$ were less active (Fig. 4).

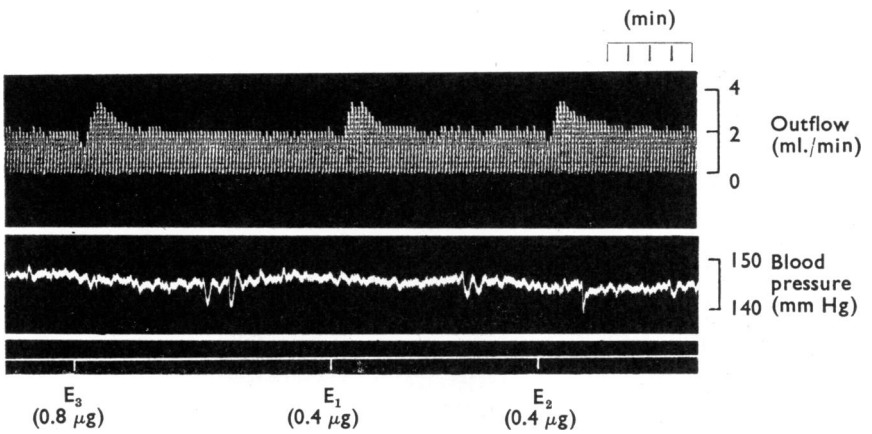


Fig. 4. Cat 3.7 kg, anaesthetized with sodium pentobarbitone (40 mg/kg). Uppermost trace, venous outflow from gastrocnemius muscle recorded with a Thorp impulse counter; middle trace, arterial blood pressure; lowest trace, event marker. E_1, E_2 and E_3=prostaglandins E_1, E_2 and E_3 respectively.

Fallopian tubal tone and peristalsis. Prostaglandin E_1, in doses as low as 800 ng/kg. reduced the tone of the rabbit fallopian tube *in vivo* and diminished its peristalsis (Horton, Main & Thompson, 1963). Similar effects were seen with prostaglandins E_2 and E_3, E_2 being equiactive with E_1 and E_3 being about half as active. In contrast, prostaglandin $F_{1\alpha}$ in doses of 5 μg/kg increased the tone of the fallopian tubes in four of the five rabbits tested (Fig. 5). In the remaining animal 5 μg/kg of $F_{1\alpha}$ caused a transient reduction in tone and in peristalsis.

DISCUSSION

Prostaglandins E_1, E_2 and E_3 differ chemically only in their degree of unsaturation, having one, two and three double bonds respectively (Fig. 1). Biologically, they were qualitatively similar and the ratios of activity were very similar on different preparations. In general prostaglandin E_2 was either equiactive to or slightly more active than E_1, whereas prostaglandin E_3, which has a double bond in the terminal pentyl group (Bergström *et al.*, 1962b), was less active than E_1 and E_2 on all preparations, On the rabbit jejunum and guinea-pig ileum the ratios are in fairly good agreement

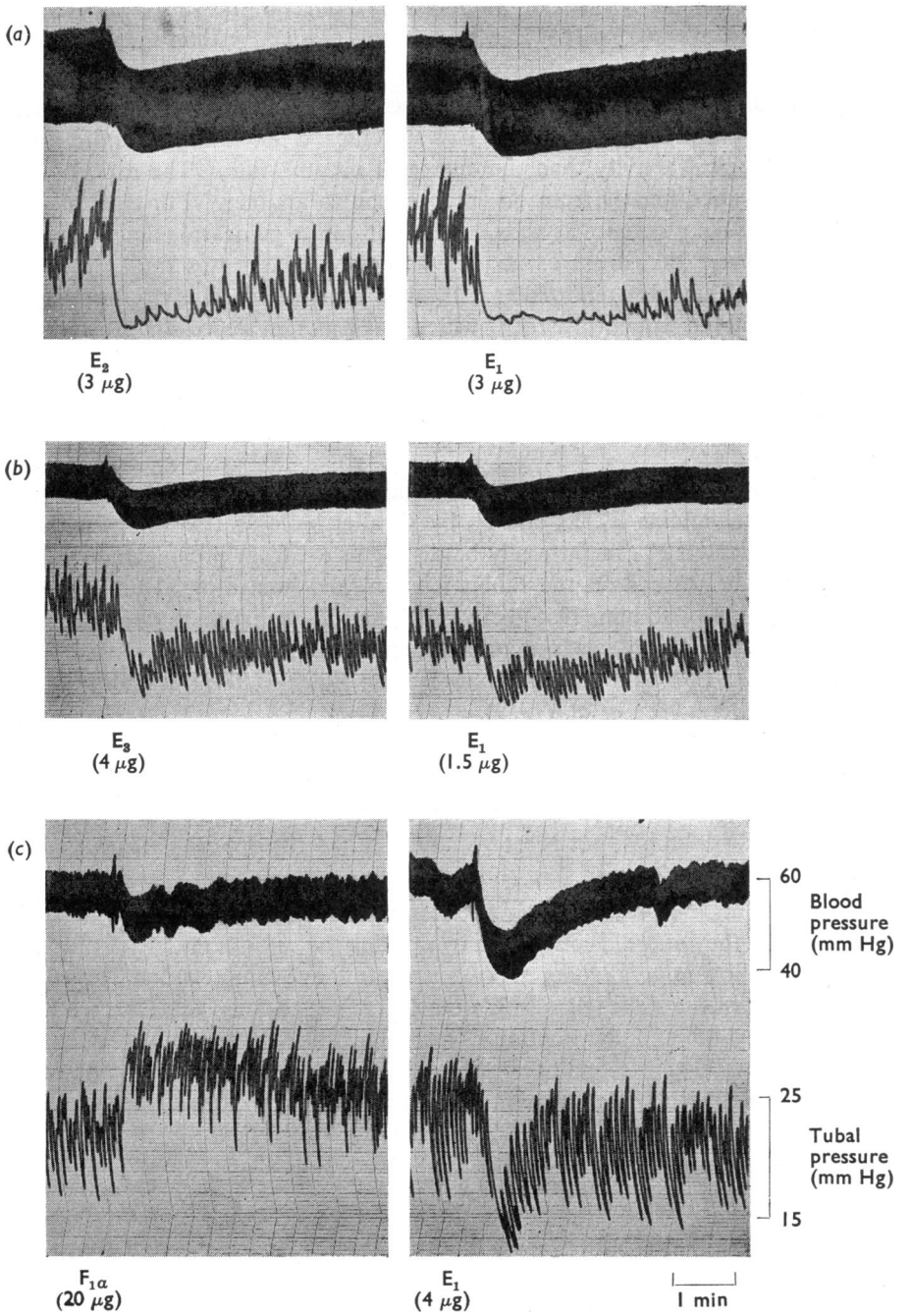


Fig. 5. Records of rabbit blood pressure (upper traces) and intraluminal pressure in a fallopian tube (lower traces). (*a*), (*b*) and (*c*) represent three different experiments. E_1, E_2, E_3 and $F_1\alpha$ = prostaglandins E_1, E_2, E_3 and $F_{1\alpha}$ respectively.

with those of Euler & Bergström (unpublished), but on the rabbit blood pressure Euler & Bergström reported that E_2 was definitely less active than E_1, whereas in our experiments there was no difference.

Prostaglandin $F_{1\alpha}$ has a hydroxyl substituent in the cyclopentane ring instead of the keto-group of prostaglandin E_1. This structural difference has a more profound effect on biological activity than the degree of unsaturation. The ratio of activity of $F_{1\alpha}$ to E_1 varied greatly from one preparation to another. For example, on the rabbit jejunum $F_{1\alpha}$ was twice as active as E_1, but on the guinea-pig ileum it was forty times less active. Bergström *et al.* (1959) found similar ratios for E_1 and $F_{1\alpha}$ on these tissues. They also reported that $F_{1\alpha}$ had no depressor activity in doses up to 10 μg in the rabbit, in contrast to E_1 which was a potent depressor substance. We found that each of these prostaglandins lowered rabbit blood pressure although the threshold dose for $F_{1\alpha}$ (5 μg/kg) was approximately ten times that for E_1. It seems probable that the maximum doses of $F_{1\alpha}$ used by Bergström *et al.* (1959) were just subthreshold.

It is clear that none of the preparations we have tested are suitable for distinguishing between prostaglandins E_1, E_2 and E_3 by parallel biological assay. On the other hand, prostaglandin $F_{1\alpha}$ might easily be distinguished from prostaglandins of the E series by parallel assays on the rabbit jejunum and guinea-pig ileum. The index of discrimination (Gaddum, 1955) between E_1 and $F_{1\alpha}$ using these two tissues would be about 100. Similarly, such a combination of tissues might be used to estimate the amounts of E_1 and $F_{1\alpha}$ in a mixture using Euler's (1948) method, as already suggested by Bergström *et al.* (1959).

Asplund (1947) reported that prostaglandin inhibits tubal peristalsis in the rabbit. This observation has been confirmed using pure prostaglandin E_1 (Horton *et al.*, 1963). In the present investigation prostaglandins E_2 and E_3, like E_1, also inhibited tubal tone and peristalsis, but prostaglandin $F_{1\alpha}$ had the opposite effect, an increase in tubal tone being observed.

Prostaglandins are present in high concentrations in human semen. It has been suggested that they may aid conception by relaxing the smooth muscle of the fallopian tubes, thus allowing easier access of sperm to an ovum (Asplund, 1947). The relaxant effect of the prostaglandin E's on the rabbit fallopian tubes agrees with this hypothesis, but the stimulant action of prostaglandin $F_{1\alpha}$ does not. It is possible that under physiological conditions prostaglandin $F_{1\alpha}$ does not reach the tubes, or at least not in sufficiently high concentration to affect their tone, and that the inhibitory effect of prostaglandins E_1 and E_2, which are more potent, is the predominant physiological response.

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REFERENCES

ASPLUND, J. (1947). Some preliminary experiments in connection with the effect of prostaglandin on the uterus and tubae *in vivo*. *Acta physiol. scand.*, **13**, 109–114.

BERGSTRÖM, S., DRESSLER, F., KRABISCH, L., RYHAGE, R. & SJÖVALL, J. (1962a). The isolation and structure of a smooth muscle stimulating factor in normal sheep and pig lungs. *Ark. Kemi*, **20**, 63–66.

BERGSTRÖM, S., DRESSLER, F., RYHAGE, R., SAMUELSSON, B. & SJÖVALL, J. (1962b). The isolation of two further prostaglandins from sheep prostate glands. *Ark. Kemi*, **19**, 563–567.

BERGSTRÖM, S., ELIASSON, R., EULER, U. S. VON & SJÖVALL, J. (1959). Some biological effects of two crystalline prostaglandin factors. *Acta physiol. scand.*, **45**, 133–144.

BERGSTRÖM, S., KRABISCH, L. & SJÖVALL, J. (1960). Smooth muscle stimulating factors in ram semen. *Acta chem. scand.*, **14**, 1706–1710.

BERGSTRÖM, S., RYHAGE, R., SAMUELSSON, B. & SJÖVALL, J. (1963). *J. biol. Chem.*, in the press.

BERGSTRÖM, S. & SAMUELSSON, B. (1962). Isolation of prostaglandin E_1 from human seminal plasma. *J. biol. Chem.*, **237**, 3005–3006.

BERGSTRÖM, S. & SJÖVALL, J. (1960a). The isolation of prostaglandin E from sheep prostate glands. *Acta chem. scand.*, **14**, 1701–1705.

BERGSTRÖM, S. & SJÖVALL, J. (1960b). The isolation of prostaglandin F from sheep prostate glands. *Acta chem. scand.*, **14**, 1693–1700.

EULER, U. S. VON (1934). Zur Kenntnis der pharmakologischen Wirkungen von Nativsekreten und Extrakten männlicher accessorischer Geschlechtsdrüsen. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **175**, 78–84.

EULER, U. S. VON (1935a). A depressor substance in the vesicular gland. *J. Physiol. (Lond.)*, **84**, 21*P*.

EULER, U. S. VON (1935b). Über die spezifische blutdrucksenkende Substanz des menschlichen Prostata- und Samenblasensekretes. *Klin. Wschr.*, **33**, 1182–1183.

EULER, U. S. VON (1948). Preparation, purification and evaluation of noradrenaline and adrenaline in organ extracts. *Arch. int. Pharmacodyn.*, **77**, 477–485.

EULER, U. S. VON & BERGSTRÖM, S. (1963). *Acta physiol. scand.*, to be published.

GADDUM, J. H. (1955). *Polypeptides which Stimulate Plain Muscle*, pp. 133–135. Edinburgh: Livingstone.

GOLDBLATT, M. W. (1933). A depressor substance in seminal fluid. *Chem. Ind.*, **52**, 1056–1057.

GOLDBLATT, M. W. (1935). Properties of human seminal plasma. *J. Physiol. (Lond.)*, **84**, 208–218.

HORTON, E. W., MAIN, I. H. M. & THOMPSON, C. J. (1963). The action of intra-vaginal prostaglandin E_1 on the female reproductive tract. *J. Physiol. (Lond.)*, in press.