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# THIN-LAYER CHROMATOGRAPHY AND BIOASSAY OF PROSTAGLANDINS IN EXTRACTS OF SEMEN AND TISSUES OF THE MALE REPRODUCTIVE TRACT

BY

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By acid ether extraction and thin-layer chromatography, prostaglandins have been separated from biologically active compounds of other chemical groups. The technique does not, however, separate different prostaglandins from each other. The biological activity of the eluates was estimated on the rabbit isolated jejunum and the hamster isolated colon preparations in terms of prostaglandin $E_1$; the concentrations (thus expressed) of prostaglandin in human semen ranged from 24 to 783 $\mu$g/ml. with a mean of 226 $\mu$g/ml. No prostaglandins (minimal detectable concentration, 0.5 $\mu$g/g) could be detected in the male reproductive organs of several species of laboratory animal.

Bergström and his co-workers have isolated and determined the structure of six prostaglandins (Bergström, Ryhage, Samuelsson & Sjövall, 1962), all of which occur in human semen (Samuelsson, 1963). One or more prostaglandins have also been isolated from sheep prostate glands (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), calf thymus (Bergström & Samuelsson, 1963) and human menstrual fluid (Eglinton, Raphael, Smith, Hall & Pickles, 1963).

In this paper a method is described for the extraction of prostaglandins from tissues and for their separation from biologically active substances of other groups by thin-layer chromatography. After elution, prostaglandin activity was assayed in parallel on the rabbit isolated jejunum and the hamster isolated colon preparations in terms of pure prostaglandin $E_1$. A preliminary report of this method has been published (Horton & Thompson, 1963).

## METHODS

*Collection of semen.* Human semen was obtained from fertility clinics through the kind co-operation of Dr D. F. Hawkins of University College Hospital, London. Ejaculates were poured into a bottle containing 50 ml. of 0.2 M-phthalate buffer (*p*H 4) and 500 ml. of diethyl ether. The pooled samples so collected were extracted at two-weekly intervals. Ram semen, collected out of season by electro-ejaculation, was kindly provided by Dr H. M. Dott of the Agricultural Research Council's Unit of Reproductive Physiology, Cambridge. Rabbit semen was collected by the method of Macirone & Walton (1938).

*Extraction of semen.* The samples of human semen were extracted three-times with 250 ml. of diethyl ether. The pooled ether phases were evaporated to dryness on a rotary film evaporator. The residue was weighed and then dissolved in a mixture of methanol and chloroform (1:1). Ram and rabbit semen in phthalate buffer were extracted with ether in a similar way.

*Extraction of tissues from the male reproductive tract.* The seminal vesicles and prostate glands from rabbits, guinea-pigs, hamsters, rats and mice, and the bulb of the penis of the cat and the testis of the ferret were removed from freshly killed animals. The tissues were weighed and then ground up in 0.1 M-phthalate buffer (*p*H 4). After three extractions with two volumes of ether, the pooled ether phases were evaporated to dryness and the residue was dissolved in the chloroform:methanol mixture and chromatographed as described for the semen extracts.

*Thin-layer chromatography of extracts.* A Desaga Thin Layer Spreader was used to prepare a layer of Silica Gel G (Merck), 250 mμ thick. The plates were then dried in an oven at 120° C for 30 min. The solutions in methanol:chloroform mixture, volumes of 5, 10 and 20 μl., were spotted on to a 10 × 20 cm marker plate, with the origin 1.5 cm from the bottom edge. The developing solvent was a mixture of diethyl ether, petroleum ether (60 to 80° C boiling range), glacial acetic acid and methanol (50:40:5:5), and it was allowed to run to a line 10 cm from the origin. The spots were located under ultra-violet light after spraying with fluorescein and contrasting with bromine vapour. They were then sprayed with 50% sulphuric acid and heated for 15 to 30 min to provide additional confirmation. Pure prostaglandin $E_1$ was used as a marker. Preparative plates (20 × 20 cm) were run under identical conditions to the marker plates, and the solution in methanol:chloroform mixture was applied at 5 mm intervals. Between 1 and 3 mg of residue were applied to the plate. After development, the

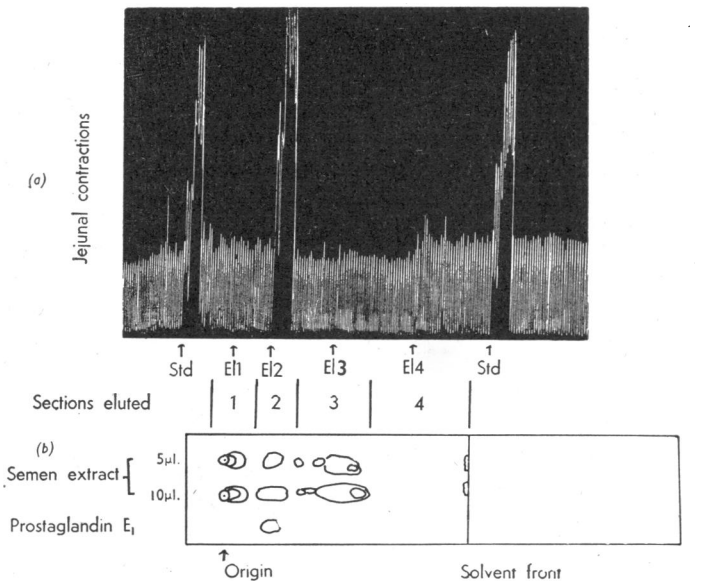


Fig. 1. (*a*) Isotonic contractions of a rabbit's isolated jejunum preparation in a 5 ml. organ-bath containing atropinized (1 μg/ml.) Tyrode solution at 36° C. Responses are shown to the standard prostaglandin $E_1$ (Std, 800 ng) and to the eluates (diluted 1 in 20, 0.2 ml. samples, El1, etc.) from the four zones of a preparative thin-layer chromatogram of an ether-extract of human semen. (*b*) A tracing of the corresponding marker plate on which 5 and 10 μl. of extract of human semen and of pure prostaglandin $E_1$ were chromatographed. The origin is on the left and the solvent front on the right of the diagram. The spots on the marker plate were identified under ultra-violet light after treating the plate as described in Methods.

appropriate zones of silica gel, as indicated by the marker plate and shown in Fig. 1, were scraped off. Elution was carried out by shaking the silica gel with methanol. After filtering off the silica gel with a sintered glass funnel, the methanol was removed on a rotary film evaporator and the dry residue taken up in 1 ml. of water for biological assay.

*Biological assays.* Eluates from the chromatograms of human semen extracts were always assayed in parallel on both rabbit jejunum and hamster colon preparations. Eluates from chromatograms of other tissue extracts were assayed on one preparation only. All estimations are expressed in terms of pure prostaglandin $E_1$ on the basis of bracketing assays.

*Rabbit isolated jejunum.* A 3 to 4 cm segment from the duodeno-jejunal region of the small intestine was removed from rabbits which had been stunned by a blow on the head and killed by exsanguination. The tissue was suspended in a 4 ml. organ-bath containing aerated Tyrode solution at 35 to 36° C. A dose cycle of 3 to 5 min was used with 45 to 90 sec contact. Isotonic contractions were recorded on a smoked drum using a frontal writing point lever, with a tension of 1 to 1.5 g and a five-fold magnification.

*Hamster isolated colon.* A 3 to 5 cm segment of the ascending colon was removed from hamsters, which had been killed by a blow on the head. The procedure was identical to that described for the rabbit jejunum, except that the tension applied was 0.6 to 0.8 g.

## RESULTS

*Thin-layer chromatography of extracts of human semen.* Numerous solvent mixtures were tested as developing solvents in an attempt to separate the prostaglandins from other lipid constituents of human semen by thin-layer chromatography. When the mixture (diethyl ether: petroleum ether: glacial acetic acid: methanol) was used several spots separated, as shown by ultra-violet light. When pure prostaglandin $E_1$ was run in parallel in this solvent system, it had an $R_F$ value of about 0.25, which corresponded to the position of a discrete spot on the chromatogram of the seminal extracts. On the basis of the chromatographic distribution of the components of the seminal extracts, four zones were marked out on the plates (Fig. 1). The first zone, from 0.5 cm below to 1.25 cm above the origin, contained those substances which moved little, if at all, from the origin. The second zone (from 1.25 to 3 cm) contained the prostaglandins. The third zone (from 3 to 6 cm) contained other lipid components of the seminal extracts, and the fourth zone (from 6 to 10 cm) represented the remainder of the plate up to the solvent front which, in the case of the seminal extracts, usually contained no substances. After developing the preparative plates, the four zones were scraped off and eluted separately. Almost all biological activity of the ether extracts of semen was found in zone 2, which corresponded to the position of the prostaglandin $E_1$ spot.

The biological activity of residues which had not been chromatographed varied between 84 and 120% of the activity of the corresponding eluate from zone 2. This variation is within the limits of error of the biological assay.

*Concentration of prostaglandin in human semen.* Two aliquots of each extract were chromatographed on two preparative plates on different days. The eluates from all zones of both plates were assayed on both the rabbit jejunum and the hamster colon preparations. Four separate estimates of the biological activity of the zone of the chromatogram corresponding to the prostaglandin spot were therefore obtained for each sample. In general there was reasonable agreement between the results of the assays (Table 1). The concentration of prostaglandin in fourteen

TABLE 1

CONCENTRATION OF PROSTAGLANDIN IN FOURTEEN POOLED SAMPLES OF HUMAN SEMEN

All samples were run on two thin-layer chromatograms and the zones corresponding to the position of prostaglandin $E_1$ were eluted and assayed using prostaglandin $E_1$ as standard. The four results so obtained and the means are given

| Volume of semen (ml.) | Weight of residue extracted by ether (mg) | Prostaglandin $E_1$-equivalent ($\mu$g/ml. semen) | | |
|---|---|---|---|---|
| | | Rabbit jejunum | Hamster colon | Mean |
| 4·0 | 18 | 320, 212 | 471, 529 | 383 |
| 13·5 | 270 | 796, 844 | 746 | 783 |
| 49·4 | 134 | 111, 109 | 111, 109 | 110 |
| 52 | 98 | 32, 41 | 16, 21 | 27 |
| 3 | 33 | 313, 104 | 835, 42 | 324 |
| 33 | 183 | 11, 21 | 21, 42 | 24 |
| 13 | 240 | 47, 30 | 47, 26 | 37 |
| 10 | 275 | 88, 70 | 88, 70 | 79 |
| 24 | 195 | 466, 391 | 466, 520 | 461 |
| 2·6 | 32 | 133, 190 | 133, 190 | 162 |
| 2·0 | 322 | 164, 261 | 164, 164 | 188 |
| 49 | 847 | 192, 240 | 480, 360 | 318 |
| 29 | 204 | 111, 74 | 37, 37 | 65 |
| 7·0 | 91 | 204, 382 | 115, 130 | 208 |
| | Total means | 213 | 240 | 226 |

pooled samples of human semen, in terms of prostaglandin $E_1$, ranged from 24 to 783 $\mu$g/ml. with a mean of 226 $\mu$g/ml.

*Concentration of prostaglandin in other tissues and tissue fluids.* Sheep semen contained 7.3 $\mu$g/ml. of prostaglandin $E_1$-equivalent and rabbit semen (six samples) less than 0.5 $\mu$g/ml. In none of the following tissues could prostaglandin be detected (minimal detectable concentration, 0.5 $\mu$g/g); the seminal vesicles and prostate glands of rabbits, guinea-pigs, hamsters, rats and mice, the ferret testis and the bulb of the penis of the cat.

## DISCUSSION

Previous estimates of the concentration of prostaglandin in human semen were expressed in terms of arbitrary standards (Asplund, 1947; Eliasson, 1959; Hawkins & Labrum, 1961). Since then prostaglandin $E_1$ has been isolated in pure form (Bergström & Sjövall, 1960b), and it has therefore been possible, in the present investigation, to express the concentrations as prostaglandin $E_1$-equivalents in $\mu$g/ml. The original standard of Euler has been compared directly with pure prostaglandin $E_1$ and, on the rabbit isolated jejunum at least, it contains the prostaglandin $E_1$-equivalent of 4.5 $\mu$g/Euler's unit (Bergström, Eliasson, Euler & Sjövall, 1959). Asplund (1947) used this standard for his assays of prostaglandin in human semen, while Eliasson used a standard which was 1.5-times more active. No direct comparison was made between the standard used by Hawkins & Labrum (1961) and either Euler's standard or pure prostaglandin $E_1$. However, Hawkins & Labrum state that the rabbit isolated jejunum and the guinea-pig isolated ileum preparations respond with small contractions to 0.0005 U/ml. of their standard. Since these

preparations usually respond to a threshold concentration of 8 to 12 ng/ml. (Horton & Main, 1963), the standard of Hawkins & Labrum must have contained the equivalent of about 20 $\mu$g of prostaglandin $E_1$/U. Using these calculated conversion factors, the results of the various workers can be expressed in terms of prostaglandin $E_1$-equivalents in $\mu$g/ml. The results are summarized in Table 2. There is remarkably good agreement between the results of the four investigations.

TABLE 2

COMPARISON OF THE ESTIMATED CONCENTRATIONS OF PROSTAGLANDIN IN HUMAN SEMEN REPORTED BY FOUR GROUPS OF INVESTIGATORS

The results of previous workers have been converted to prostaglandin $E_1$-equivalents ($\mu$g/ml. of semen) using the conversion factor shown in the second column

| Reference | Approximate prostaglandin $E_1$-equivalent of 1 unit ($\mu$g/U) | Prostaglandin $E_1$-equivalent ($\mu$g/ml. semen) Mean | Range | No. of samples | Origin of samples |
|---|---|---|---|---|---|
| Asplund (1947) | 4·5 | 37 | <20–>90 | 155 | Individual ejaculates from fertility clinics |
| Eliasson (1959) | 6·75 | 81 | 10–203 | 16 | Individual ejaculates from sixteen infertile patients |
| | | 187 | 74–405 | 13 | Individual ejaculates from three healthy subjects |
| Hawkins & Labrum (1961) | 20 | 122 | 34–448 | 50 | Individual ejaculates from fertility clinics |
| Present investigation | — | 226 | 24–783 | 14 | Pooled samples from fertility clinics |

Each sample assayed in the present investigation represented a mixture of ejaculates from several individuals all attending fertility clinics. Eliasson (1959) and Hawkins & Labrum (1961) have produced some evidence that subfertile males may have low seminal prostaglandin levels. The contribution from subfertile individuals to the samples collected is unknown, but all the individuals must be regarded as potentially infertile and some probably were so, and the proportion is likely to have varied from one sample to another. This could partly account for the wide range of concentrations.

In agreement with previous workers (Euler, 1936; Bergström *et al.*, 1960), we found that sheep semen contains prostaglandin, though in lower concentrations than does human semen. Our estimate may not present a normal value because the semen was collected by an artificial procedure from rams outside the reproductive season. Rabbit semen and the tissues of the male reproductive tract from the various species investigated contained less than 0.5 $\mu$g/g of prostaglandin. These findings also confirm previous reports that prostaglandins are either absent or present only in low concentrations in these tissues (Euler, 1937; Eliasson, 1959).

The present method has the advantage that the presence of biologically active substances of a non-lipid nature (for example, choline, histamine, adrenaline and bradykinin) in the final eluates can definitely be excluded. Even if these substances passed into the ether phase during extraction, they would not move from the origin when the chromatogram developed with the solvent mixture used in these experiments. It is likely that most of the activity in the final eluates was due to prostaglandins. However, the method does have the disadvantage that a separate estimate

of the concentrations of the different prostaglandins is not possible. When this investigation was begun the chemical identity of the prostaglandins in human semen was unknown. Later prostaglandin $E_1$ was isolated from semen (Bergström & Samuelsson, 1962). More recently the presence in semen of the five other prostaglandins has been reported (Samuelsson, 1963). Clearly the next step must be the development of a method for the separate estimation of these six prostaglandins.

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