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IDENTIFICATION OF PROSTAGLANDINS IN CENTRAL NERVOUS TISSUES OF THE CAT AND CHICKEN

BY

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Brain extracts from several species contain biologically active lipids whose physicochemical properties resemble those of the prostaglandins. Ambache reported the presence in rabbit brain of a smooth muscle-stimulating unsaturated hydroxy fatty acid (Ambache & Reynolds, 1960, 1961; Ambache, Reynolds & Whiting, 1963). Kirschner & Vogt (1961) prepared chloroform–methanol extracts of horse brain and separated three biologically active fractions, one of which consisted of unsaturated fatty acids. Toh (1963) prepared an extract of dog and cat brain, the biological activity of which was due to unsaturated fatty acids. The biological activity of these extracts may have been due, at least in part, to prostaglandins. A prostaglandin has so far been isolated from the brain of only one species, the ox (Samuelsson, 1964), though Coceani & Wolfe (1965) have reported the presence of a prostaglandin-like substance in cat brain using Samuelsson's extraction procedure.

In view of the pharmacological actions of prostaglandins on the central nervous system of cats and chicks (Horton, 1964; Horton & Main, 1965a & b, 1966a, 1967a), we have attempted in this investigation to identify prostaglandins in central nervous tissues of these species. Preliminary reports of this work have been published (Horton & Main, 1966b, 1967b, and c).

METHODS

Chemical Procedures

Collection and ethanol extraction of cat brain

Adult cats of both sexes were anaesthetized with ethyl chloride and ether. All brain tissue above the intercollicular level was removed by classical decerebration technique. The tissue was weighed and either stored at about −10° C for subsequent homogenization or homogenized immediately with a pestle and mortar in 96% aqueous ethanol and sand. The homogenate was stirred for 4 hr at room temperature in 96% aqueous ethanol (4 ml./g of tissue). The mixture was filtered or centrifuged or both, and the residue was re-extracted with 0.5 vol. 96% aqueous ethanol. The combined aqueous ethanol filtrates or supernatants were evaporated to dryness under reduced pressure at 40–45° C using a rotary evaporator. The dried residue was stored at −20° C.

Collection and ethanol extraction of chicken brain and spinal cord

Adult chickens (*Gallus domesticus*) of both sexes were exsanguinated by section of the large blood vessels in the neck. Whole brains and spinal cords were dissected out immediately and

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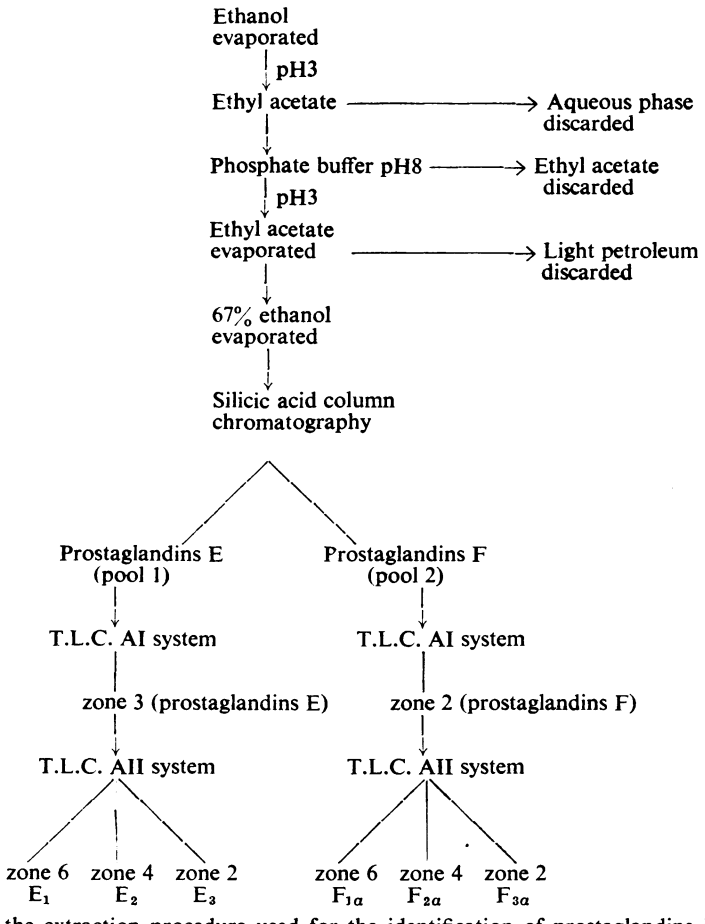

Fig. 1. Outline of the extraction procedure used for the identification of prostaglandins in brain.

weighed. The ethanol extraction procedure was the same as that used for cat brain ; the chicken brains and spinal cords were extracted separately. The dried residues were stored at −20° C.

The subsequent extraction procedure was that described by Samuelsson (1964) and was the same for cat brain, chicken brain and chicken spinal cord (Fig. 1).

Silicic acid chromatography

The dried residue from the ethanol extraction was dissolved in an equal volume of ethyl acetate and 0.1 N hydrochloric acid. After separation the acid aqueous phase was re-extracted with 1 vol. ethyl acetate. The combined ethyl acetate phases were concentrated to a smaller volume on a rotary evaporator. The ethyl acetate was extracted twice with 1 vol. pH 8 phosphate buffer solution. The aqueous phases were pooled and acidified to pH 3 by drop-wise addition of concentrated hydrochloric acid. The acid aqueous solution was then extracted twice with ethyl acetate. The ethyl acetate phases were pooled and evaporated to dryness. The residue was partitioned between equal volumes of 67% aqueous ethanol and light petroleum (B.P. 40–60° C). The aqueous ethanol was evaporated to dryness and subsequently chromatographed on a column of silicic acid.

Silicic acid chromatography

Two grammes silicic acid (Bio-Rad Laboratories, Calif.) was activated by heating for 1 hr at 115° C before use. It was washed twice with heavy petroleum (B.P. 60–80° C) and then suspended

in an ethyl acetate : benzene (30:70) mixture. Columns 80 mm in length and of cross-sectional diameter 8 mm reducing to 4 mm were prepared. The dried residue from the solvent partition procedure or a mixture of purified prostaglandins was dissolved in 1 ml. ethyl acetate:benzene (30:70) and applied to the column drop-wise with a Pasteur pipette. The flask was washed three times with ethyl acetate: benzene and the washings applied to the column.

The column was developed with increasing concentrations of ethyl acetate in benzene. Ten or 20 ml. fractions were collected under reduced pressure with a flow rate of 0.8 to 1.2 ml./min. The fractions were evaporated to dryness immediately after they had been collected and the residues were dissolved in distilled water for assay on the rabbit isolated jejunum. Approximate estimates of the biological activity in each fraction were made as soon as possible after collection, thus monitoring the progress of the chromatography and allowing changes in eluant to be made at appropriate times.

The concentrations of ethyl acetate in benzene used to develop the column were 30, 40, 60, 80 and 100% (v/v), followed finally by 100% methanol to remove the most polar substances from the column. All fractions were assayed more accurately at the end of the experiment and those with biological activity were stored frozen for further investigations.

Thin layer chromatography

Plates of dimensions 200×50 mm coated with Silica Gel G (E. Merck) were used throughout. In some preparative experiments the silica gel was 0.5 mm in thickness but a thickness of 0.25 mm was otherwise routinely used. Silica gel plates impregnated with 4% silver nitrate were prepared by suspending the silica gel (30 g) in a 2% solution of silver nitrate (60 ml.). The plates were activated in an oven at 115–120° C for 1 hr and stored over self-indicating silica gel until used. A strip approximately 3 mm in width was removed from each edge of the silica gel before use. Prostaglandins and brain extracts were dissolved in methanol and applied to the plate with a Agla microsyringe using a length of narrow bore polyethylene tubing (capacity 30 μl.) attached to the syringe needle.

Solvent systems used were the AI and AII systems described by Gréen & Samuelsson (1964). The AI system contains benzene:dioxane:acetic acid 20:20:1, and the AII system contains the less polar (upper) phase of a mixture of ethyl acetate:acetic acid:methanol:2,2,4-trimethylpentane:water 110:30:35:10:100 which had been allowed to equilibrate for 2 hr. All solvents except dioxane were redistilled before use.

Cylindrical glass developing tanks of internal diameter 6 cm and of height 21.5 cm were used. The walls were lined with Whatman No. 1 filter paper impregnated with the solvent mixture. The tanks were sealed with glass lids using silicone grease. Marker plates using pure prostaglandin solutions were prepared by spotting on volumes of not more than 30 μl. of stock (methanol) solutions of the prostaglandins (0.1 or 1.0 mg/ml.). With preparative plates a series of spots (or a continuous band) of the extract dissolved in methanol was applied to the origin. Preparative and marker plates were run simultaneously in the same tank.

Plates were usually developed until the solvent had reached between 11 and 14 cm from the origin. The position of the solvent front was marked on removing the plate from the tank. The plates were dried and marker plates were sprayed with 10% phosphomolybdic acid in ethanol. On heating at 100° C for 10 min grey-blue spots developed on a greenish-yellow background, showing the position of the prostaglandins. About 1 μg of prostaglandin $F_{1\alpha}$ and about 10 μg of prostaglandins E_1 and E_2 could be detected by this method.

Preparative plates were divided into zones corresponding to the Rf values of prostaglandins on the marker plate. With the AI system zones corresponding to prostaglandins E and F were separated; with the AII systems the prostaglandin E fraction was further separated in to E_1, E_2 and E_3, and the prostaglandin F fraction into $F_{1\alpha}$, $F_{2\alpha}$, and $F_{3\alpha}$. Due to shortage of material, marker plates could not be run routinely with $F_{2\alpha}$. The position of $F_{2\alpha}$ on the preparative plate was therefore calculated from the observed Rf value of $F_{1\alpha}$ on the marker plate using the relative Rf values of $F_{1\alpha}$ and $F_{2\alpha}$ reported by Gréen & Samuelsson (1964). In a control experiment $F_{2\alpha}$ was applied to a preparative plate and the predicted $F_{2\alpha}$ zone contained all the biological activity. The

position of the E_3 and $F_{3\alpha}$ zones were also calculated since these prostaglandins were not available for chromatography.

The separated zones were shaken with 3 or 5 ml. methanol and centrifuged. The deposit was re-extracted with methanol and the combined methanol extracts evaporated to dryness. The residue was dissolved in either 0.9% sodium chloride solution or Tyrode solution so that the Ag^+ ions were precipitated as AgCl. This precipitate did not interfere with assays on the rabbit jejunum. In some cases the dried residue from the methanol extraction of these $AgNO_3$ plates was dissolved in 0.1N HCl (which precipitated the Ag^+) and extracted with ethyl acetate. The ethyl acetate was evaporated to dryness, and the residue dissolved in saline or Tyrode solution for assay.

Preparation of $F_{2\alpha}$ from E_2

Reduction of E_2 by sodium borohydride to yield $F_{2\alpha}$ and $F_{2\beta}$ was carried out as described by Bergström, Krabisch, Samuelsson & Sjövall (1962).

Sodium borohydride 1 mg was added to a solution of 400 μg E_2 in 1 ml. methanol at 0° C. The mixture was allowed to stand for 20 min at 0° C and then for 20 min at room temperature. Ten millilitres of 0.1N HCl were then added and the aqueous phase was extracted twice with ether. The combined ether extracts were washed with water and taken to dryness.

The residue was dissolved in methanol and chromatographed on silica gel in the AI solvent system. About 5% of the extract was applied to a marker plate which, after development, was sprayed with phosphomolybdic acid and heated. Two spots appeared, one (Rf 0.32) corresponding to $F_{2\alpha}$ and the other (Rf 0.25) to $F_{2\beta}$. The remainder of the extract was applied to a preparative plate which, after development, was divided into zones corresponding to $F_{2\alpha}$ and $F_{2\beta}$. The zones were eluted with methanol and taken to dryness. The material from the $F_{2\alpha}$ zone was rechromatographed in the AI solvent system to eliminate traces of $F_{2\beta}$ which were present. The biologically active material in the $F_{2\alpha}$ zone from this second chromatogram was standardized on the isolated rabbit jejunum and guinea-pig ileum against pure $F_{2\alpha}$.

Biological preparations

Biological assays

The rabbit isolated jejunum was used routinely for the estimation of biological activity in the various fractions, using prostaglandins E_1 and $F_{1\alpha}$ as the standards for comparison. The concentrations of prostaglandins in brain was calculated from the relative activities previously determined (Table 1).

TABLE 1

BIOLOGICAL ACTIVITY OF PROSTAGLANDINS E_2, E_3, $F_{1\alpha}$ and $F_{2\alpha}$ RELATIVE TO PROSTAGLANDIN E_1 ON RABBIT ISOLATED JEJUNUM

(data from Horton & Main, 1963, 1965, 1966)

E_2	E_3	$F_{1\alpha}$	$F_{2\alpha}$
1·5	1·0	2·2	26

Smooth muscle preparations in vitro

Segments of various organs were suspended in a 4 ml. or 10 ml. organ-bath. Longitudinal contractions were recorded either isotonically with a frontal-writing lever on a smoked drum, or isometrically with a force-displacement transducer (Grass F.T.03) on a Sanborn polygraph. A dose cycle of 4 to 6 min with a contact time of 45–90 sec was used for all preparations except the cat trachea.

Rabbit jejunum

Proximal jejunum, from rabbits weighing 1–3 kg, was suspended in Tyrode solution at 37° C, gassed with air or 5% carbon dioxide in oxygen.

Guinea-pig ileum

Terminal ileum from guinea-pigs weighing 200–400 g was suspended in Tyrode solution at 37° C, gassed with air or 5% carbon dioxide in oxygen.

Rat uterus

A segment of a uterine horn from rats weighing 150–250 g which had been injected subcutaneously, 18 hr previously, with stilboestrol (100 μg), was suspended in de Jalon solution at 30° C, gassed with air.

Jird colon

Segments of ascending colon from Meriones weighing 100–150 g were suspended in Tyrode solution at 37° C, gassed with air.

Rat fundus

Longitudinal strips of the fundus from rats weighing 200–350 g. were prepared as described by Vane (1957) and suspended in Tyrode solution at 37° C, gassed vigorously with oxygen.

Cat trachea

Tracheas were obtained from cats which had been used for other experiments. Two or three rings of trachea were tied together with the muscle in alignment; the cartilage was removed. The preparations were suspended in Krebs-Henseleit solution at 37° C, gassed with 5% carbon dioxide in oxygen. The inhibitory action of a prostaglandin was demonstrated by adding it to the organ bath either 30 sec before a dose of acetylcholine, or after acetylcholine had produced a sustained contraction (Main, 1964).

Spinal chick preparation

The technique is described in the preceding paper (Horton & Main, 1967a).

RESULTS

Identification of prostaglandins in chicken brain

Solvent extraction and partition

Ethanolic extracts of 115 g chicken brain contracted the isolated rabbit jejunum. On partition between ethyl acetate and water at different pHs as described under Methods (Fig. 1) the biologically active material behaved like an acidic lipid. On partition between 67% aqueous ethanol and petroleum ether (B.P. 40–60°) the biological activity was recovered from the aqueous ethanol phase. From the results of previous workers (Samuelsson, 1963; Bergström & Samuelsson, 1963) these results are compatible with the biological activity being due to prostaglandins.

Silicic acid chromatography

The next stage was chromatography on a silicic acid column to purify the extract further and also to separate prostaglandins E from F. According to Samuelsson (1963) Es and Fs are eluted with concentrations of 60% and 80% ethyl acetate in benzene, respectively. Control experiments were first carried out using 10–20 μg of pure E_1 and $F_{1\alpha}$. In these experiments both types of prostaglandins were eluted with 60% ethyl acetate and none with 80% ethyl acetate. However, by using 40% ethyl acetate in benzene E could be eluted first, while F was eluted by 60% ethyl acetate in benzene as shown in Fig. 2.

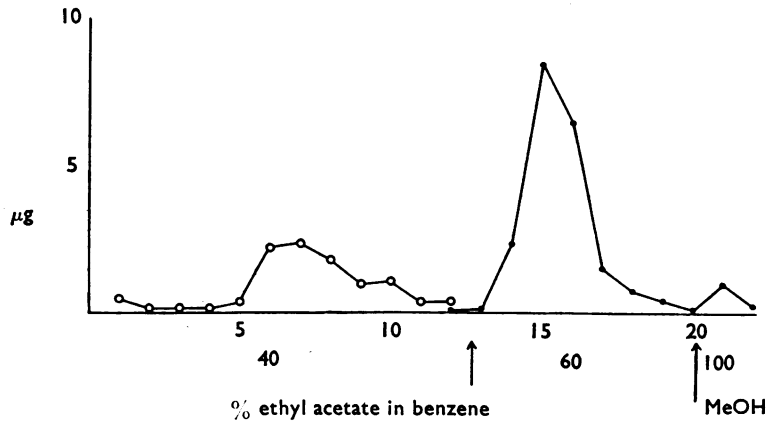

Fig. 2. Chromatography of a mixture of prostaglandins E_1 (10 μg) and $F_{1\alpha}$ (20 μg) on a 2 g column of silicic acid. Elution rate 0.8–1 ml./min. 10 ml. fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of prostaglandin E_1 (open circles) and $F_{1\alpha}$ (closed circles). Abscissa: upper line, fraction number; lower line, nature of eluant.

In this experiment the recoveries of E_1 and $F_{1\alpha}$ applied to the column were 90% and 98% respectively. The identity of the prostaglandins was confirmed by parallel biological assay. Furthermore in experiments in which prostaglandin E_1 was chromatographed alone, all the biological activity was eluted with 40% ethyl acetate.

The chicken brain extract obtained from the solvent partition procedure described above was chromatographed on a silicic acid column. The result is shown in Fig. 3. Apart from a small initial peak of activity (≡2·2 μg $F_{1\alpha}$), there was one peak eluted with 40% ethyl acetate in benzene corresponding to prostaglandins E (≡19 μg E_1) and another peak eluted with 60% ethyl acetate in benzene corresponding to prostaglandins F

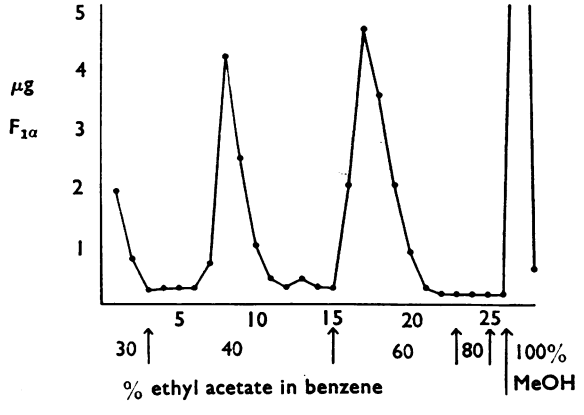

Fig. 3. Chromatography of chicken brain extract on a 2 g column of silicic acid. Elution rate 1–1.2 ml./min. 20 ml. fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of prostaglandin $F_{1\alpha}$. Abscissa: upper line, fraction number; lower line, nature of eluant.

(≡12·1 μg $F_{1\alpha}$). Considerable biological activity (≡16 μg $F_{1\alpha}$) was eluted with methanol, but this did not correspond to either a prostaglandin E or F.

Thin layer chromatography in the AI solvent system

The material in fractions 7–10 (pool 1) and 16–20 (pool 2) was further purified by thin layer chromatography in the AI solvent system. On chromatography of pool 1, 97% of the biological activity was recovered from zone 3 which corresponded to prostaglandins E on the marker plate (Fig. 4). On chromatography of pool 2, 90% of the activity was recovered from zone 2 of the chromatogram corresponding to prostaglandins F. The % biological activity found in the various zones of the chromatogram as assayed on the rabbit isolated jejunum is shown in Table 2.

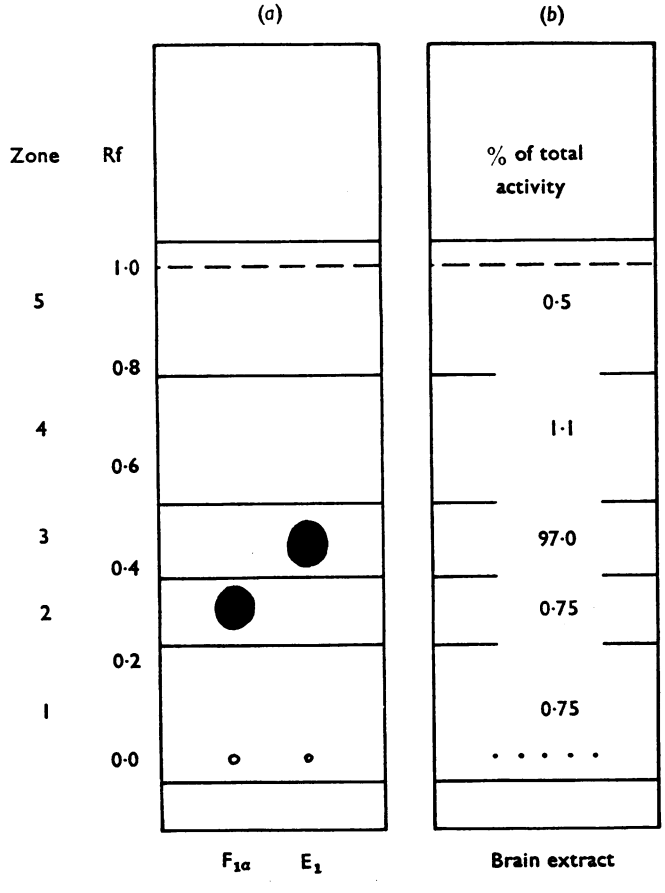

Fig. 4. Thin layer chromatography of chicken brain extract (pool 1, Fig. 3) in the AI solvent system. (a) marker plate, spotted with prostaglandins $F_{1\alpha}$ and E_1 and sprayed with phosphomolybdic acid, (b) preparative plate, spotted with brain extract, divided into zones.

TABLE 2

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF PREPARATIVE THIN LAYER CHROMATOGRAMS OF CHICKEN BRAIN (POOLS 1 AND 2) DEVELOPED IN THE AI SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF E_1 (POOL 1) AND $F_{1\alpha}$ (POOL 2)

Zone No.	Mean Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Pool 1	Pool 2
1		Origin	0·75	7·0
2	0·32	$F\alpha$	0·75	90·0 (≡5 μg $F_{1\alpha}$)
3	0·44	E	97·0 (≡22·5 μg E_1)	<2·0
4			<1·1	<2·0
5		Front	<0·5	<2·0

Thin layer chromatography in the AII solvent system

The material from the active zones corresponding to prostaglandins E and F was further chromatographed on silver nitrate–impregnated plates in the AII solvent system. The results of chromatography of zone 3 (prostaglandins E from the plate run in the AI system) are shown in Table 3. Most of the biological activity as assayed on the

TABLE 3

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CHICKEN BRAIN (POOL 1, ZONE 3, PROSTAGLANDINS E FROM THE PLATE RUN IN AI, TABLE 2) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED IN PARALLEL ON THE RABBIT JEJUNUM AND GUINEA-PIG ILEUM IN TERMS OF PROSTAGLANDIN E_1

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Rabbit jejunum	Guinea-pig ileum
1		Origin	<1	<2
2	*(0·31)	E_3	1	<2
3	—	—	2	<1
4	0·62	E_2	92 (≡25 μg E_1)	89 (≡7 μg E_1)
5	—	—	2	<2
6	0·79	E_1	1	<2
7		Front	<1	<2

* Rf value calculated as described in Methods.

isolated rabbit jejunum and guinea-pig ileum was recovered from zone 4 corresponding to E_2. Traces of activity were detected in the " neutral " zones adjoining E_2 but there was no significant activity in any other zone (Fig. 5). The E_2 zone was assayed on three isolated tissues in terms of E_1 and $F_{1\alpha}$ as shown in Table 4.

The results of the parallel assays confirm that the activity is prostaglandin E-like, it could not be due to a prostaglandin F. The differences between estimates of biological activity (in terms of E_1) on these three tissues may be attributed partly to biological error in the assays and partly to differences in the relative activities of E_2 to E_1 on these preparations (Horton & Main, 1963).

The results of chromatography of the zone 2 (prostaglandins F from the plate run in the AI system) are shown in Table 5.

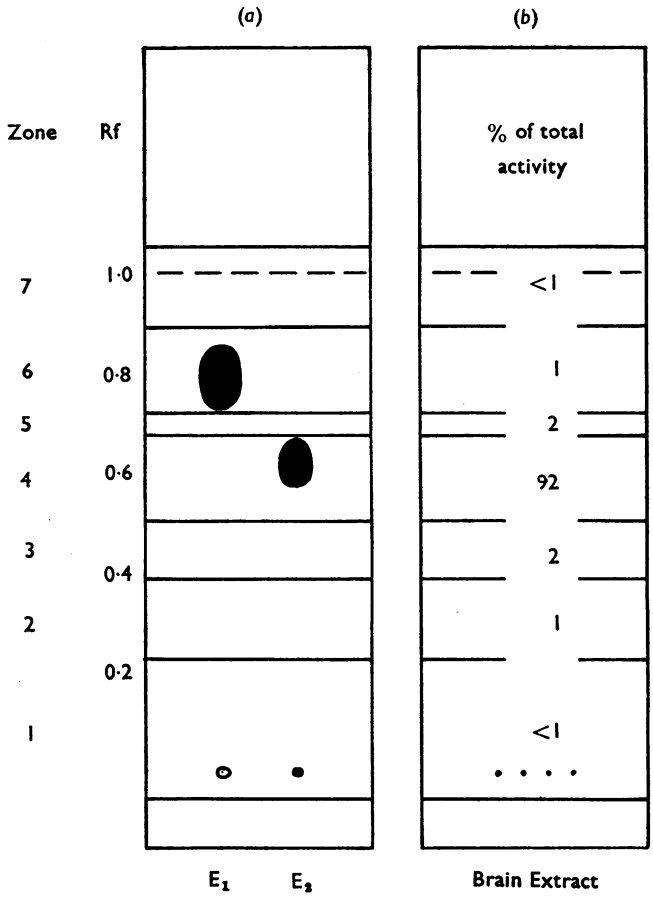

Fig. 5. Thin layer chromatography of chicken brain extract (zone 3, Fig. 4) in the AII solvent system. (a) marker plate, spotted with prostaglandin E_1 and E_2 and sprayed with phosphomolybdic acid, (b) preparative plate, spotted with brain extract divided into zones.

TABLE 4

CHICKEN BRAIN EXTRACT. PARALLEL BIOLOGICAL ASSAYS OF THE MATERIAL ELUTED FROM ZONE 4 OF THE THIN LAYER PLATE DEVELOPED IN THE AII SOLVENT SYSTEM (TABLE 3)

Each figure represents the result of one estimation

	Biological activity	
Preparation	μg E_1 equivalent	μg $F_{1\alpha}$ equivalent
Rabbit jejunum	25, 25	8, 5
Rat fundus	12	96
Guinea-pig ileum	7	87

TABLE 5

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF A THIN LAYER PLATE (PREPARATIVE THIN LAYER CHROMATOGRAM OF CHICKEN BRAIN POOL 2, ZONE 2, FROM THE PLATE RUN IN AI SEE TABLE 2) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN $F_{1\alpha}$

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate
1		Origin	<5
2	(0·22)	$F_{3\alpha}$	<5
3		—	<5
4	(0·47)	$F_{2\alpha}$	67 (≡0·75 μg $F_{1\alpha}$)
5		—	<5
6	0·61	$F_{1\alpha}$	7
7		—	<5
8		Front	<5

Of the total activity 67% were recovered from zone 4, corresponding to $F_{2\alpha}$. A small amount of activity was also present in zone 6, which corresponded to $F_{1\alpha}$.

The $F_{2\alpha}$ zone was assayed on two isolated tissues in terms of $F_{1\alpha}$ as shown in Table 6. The results are compatible with the activity being due to prostaglandin $F_{2\alpha}$. The activity could not be due to any of the prostaglandins E.

TABLE 6

CHICKEN BRAIN EXTRACT. PARALLEL BIOLOGICAL ASSAYS OF THE MATERIAL ELUTED FROM ZONE 4 OF THE THIN LAYER PLATE DEVELOPED IN THE AII SOLVENT SYSTEM (TABLE 5)

Each figure represents the result of one estimation.

Preparation	Biological activity	
	μg $F_{1\alpha}$ equivalent	μg E_1 equivalent
Rabbit jejunum	0·75, 1·0	3·8, 3·0
Rat fundus	0·9	0·11

Concentration of prostaglandins in chicken brain

Although these experiments were primarily designed to identify prostaglandins, some indication of the concentration of prostaglandins E_2 and $F_{2\alpha}$ in chicken brain were obtained from the results of silicic acid chromatography (Fig. 3). Pool 1 (prostaglandins E) contained biological activity on the rabbit jejunum equivalent to 19 μg E_1. The amount of E_2 recovered from the column was therefore about 13 μg (relative activity of E_2 to E_1=1·5, Table 1). The concentration of E_2 in chicken brain was therefore of the order of 100 ng/g wet weight. Pool 2 (prostaglandins F) contained biological activity equivalent to 1 μg $F_{2\alpha}$ (relative activity of $F_{2\alpha}$ to $F_{1\alpha}$=12, Horton & Main, 1965c, Table 1). The concentration of $F_{2\alpha}$ in chicken brain was therefore of the order of 10 ng/g wet weight of tissue.

Identification of prostaglandins in chicken spinal cord

Silicic acid chromatography

Chicken spinal cord (35.7 g) was extracted with ethanol and partitioned as described under Methods. The resultant extract which contained biologically active material as assayed on the rabbit isolated jejunum was chromatographed on a silicic acid column. There were peaks of biological activity corresponding to prostaglandins E (pool 1)

(≡2.2 μg E_1) and prostaglandins F (pool 2) (≡4.5 μg $F_{1\alpha}$). A third peak of activity was eluted with methanol.

Thin layer chromatography in the AI solvent system

Pool 1 and pool 2 were chromatographed separately on thin layer plates in the AI solvent system. The results are shown in Table 7.

TABLE 7

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF PREPARATIVE THIN LAYER CHROMATOGRAMS OF CHICKEN SPINAL CORD DEVELOPED IN THE AI SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN E_1 (POOL 1) AND PROSTAGLANDIN $F_{1\alpha}$ (POOL 2)

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Pool 1	Pool 2
1		Origin	4	11
2	0·3	F_α	4	64 (≡3 μg $F_{1\alpha}$)
3	0·4	E	88 (≡20 μg E_1)	15
4		—	<2	<5
5		Front	<2	<5

Most of the activity in pool 1 was recovered from zone 3 corresponding to prostaglandins E and most of the activity in pool 2 was recovered from zone 2 corresponding to prostaglandins F, though there was a small amount in the adjacent zones.

Thin layer chromatography in the AII solvent system

The material from the active zones corresponding to prostaglandins E and F was further chromatographed on silver nitrate-impregnated plates in the AII solvent system. The results of the two chromatograms are shown in Table 8.

Most of the biological activity of pool 1 zone 3 (prostaglandins E from the plate run in the AI solvent system) was recovered from zone 4 of the silver nitrate-impregnated plate corresponding to prostaglandin E_2. There was no significant activity in the zones corresponding to E_3 (zone 2) or E_1 (zone 6).

TABLE 8

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF PREPARATIVE THIN LAYER CHROMATOGRAMS OF CHICKEN SPINAL CORD (POOL 1, ZONE 3, AND POOL 2, ZONE 2, FROM THE PLATES RUN IN AI, TABLE 7) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN E_1 (POOL 1) AND PROSTAGLANDIN $F_{1\alpha}$ (POOL 2)

Zone No.	% of total biological activity recovered from plate					
	Pool 1 zone 3			Pool 2 zone 2		
	Rf			Rf		
1		Origin	<4		Origin	18
2	(0·34)	E_3	<4	(0·21)	$F_{3\alpha}$	<9
3		—	<8		—	<9
4	(0·67)	E_2	62 (≡4 μg E_1)	(0·45)	$F_{2\alpha}$	46 (≡0·5 μg $F_{1\alpha}$)
5		—	15		—	<9
6	0·77	E_1	<4	0·58	$F_{2\alpha}$	<9
7		Front	<4		Front	

Most of the biological activity of pool 2 zone 2 (prostaglandins F from the plate run in the AI solvent system) was recovered from zone 4 of the silver nitrate-impregnated plate corresponding to prostaglandin $F_{2\alpha}$. Activity was also present at the origin but not in the zones corresponding to $F_{1\alpha}$ or $F_{3\alpha}$.

The activity in the E_2 and $F_{2\alpha}$ zones was assayed in parallel on different isolated tissues and the results are shown in Table 9. The results are compatible with the activity in these zones being due to E_2 and $F_{2\alpha}$ respectively.

TABLE 9

CHICKEN SPINAL CORD EXTRACT. PARALLEL BIOLOGICAL ASSAYS OF THE MATERIAL ELUTED FROM THE E_2 AND $F_{2\alpha}$ ZONES OF THE CHROMATOGRAMS DEVELOPED IN THE AII SOLVENT SYSTEM (TABLE 8)

	Biological activity		
Preparation	E_2 zone (μg E_1 equiv.)	$F_{2\alpha}$ zone (μg $F_{1\alpha}$ equiv.)	Ratio of activity $F_1/E_{1\alpha}$
Rabbit jejunum	4·0	0·5	6·7
Rat fundus	2·0	0·6	0·9
Guinea-pig ileum	1·6	Not tested	<0·1

Concentration of prostaglandins in chicken spinal cord

By assaying the biological activity eluted from the silicic acid column, chicken spinal cord is estimated to contain of the order of 400 ng E_2/g and 10 ng $F_{2\alpha}$/g tissue wet weight.

Identification of prostaglandins in cat brain

Silicic acid chromatography

Cat forebrains obtained by decerebration were extracted with ethanol and partitioned as described under Methods. The resultant extract was chromatographed on a silicic acid column. The result is illustrated in Fig. 6. There were peaks of biological activity corresponding to prostaglandins E and prostaglandins F.

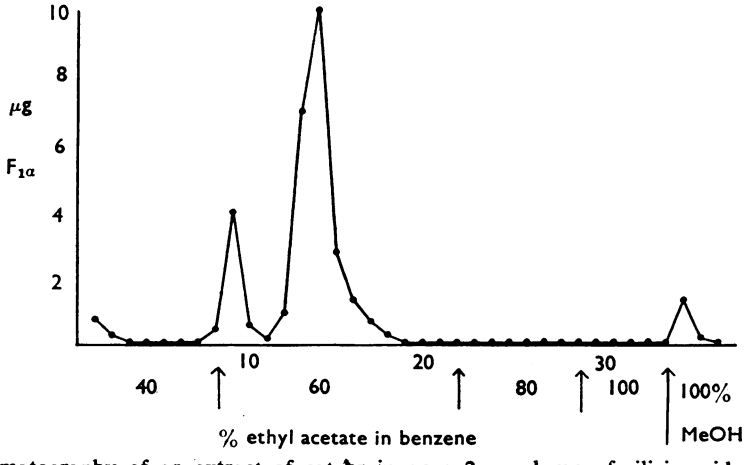

Fig. 6. Chromatography of an extract of cat brain on a 2 g column of silicic acid. Elution rate 0.8–1 ml./min. 10 ml fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of prostaglandin $F_{1\alpha}$. Abscissa: upper line, fraction number; lower line, nature of eluant.

Thin layer chromatography in the AI solvent system

Pool 1 (fraction 9) was chromatographed on a thin layer plate in the AI solvent system. Most of the activity was present in zone 3 corresponding to prostaglandins E. Pool 2 (fractions 13–18) was chromatographed on a thin layer plate in the AI solvent system (Table 10). At least 96% of the activity was present in zone 2 corresponding to prostaglandins F.

TABLE 10

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CAT BRAIN (POOL 2) DEVELOPED IN THE AI SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN $F_{1\alpha}$

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate
1		Origin	3
2	0·31	$F\alpha$	96 ($\equiv$10 μg $F_{1\alpha}$)
3	0·42	E	1
4			<0·2
5		Front	<0·2

TABLE 11

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CAT BRAIN (POOL 1, ZONE 3 FROM THE PLATE RUN IN THE AI SOLVENT SYSTEM) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED IN PARALLEL ON THE RABBIT ISOLATED JEJUNUM AND RAT FUNDUS PREPARATIONS IN TERMS OF PROSTAGLANDIN E_1

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Rabbit jejunum	Rat fundus
1		Origin	6	9
2	(0·3)	E_3	44 ($\equiv$0·35 μg E_1)	<9
3			<6	<9
4	0·61	E_2	<6	9
5			6	<12
6	0·75	E_1	25 ($\equiv$0·2 μg E_1)	44 ($\equiv$0·38 μg E_1)
7		Front	<6	<9

TABLE 12

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CAT BRAIN (POOL 2, ZONE 2 FROM CHROMATOGRAM SHOWN IN TABLE 10) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN $F_{1\alpha}$

Biological activity was found in all zones of the plates even those beyond the solvent front. Figures in the Table are obtained by subtracting the value of this non-specific activity from the total activity in each zone

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate
1			0
2	(0·21)	$F_{3\alpha}$	0
3			17
4	(0·45)	$F_{2\alpha}$	69
5			9
6	0·59	$F_{1\alpha}$	5
7			0
8	Front		0

Thin layer chromatography in the AII solvent system

The material from the active zones corresponding to prostaglandins E and F was chromatographed on silver nitrate impregnated plates in the AII solvent system. The biological activity in each zone was assayed and the results are shown in Tables 11 and 12.

When assayed on the rabbit jejunum, significant activity of the prostaglandins E containing extract was found in zones 2 and 6 corresponding to E_3 and E_1 respectively. No significant activity was present in zone 4 (E_2) or the zones adjacent to it. When assayed on the rat fundus, activity was present only in zone 6, corresponding to E_1 (Table 11).

Most of the activity of the prostaglandins F containing extract corresponded to $F_{2\alpha}$ (zone 4) but a small amount of activity was present in zone 6 corresponding to $F_{1\alpha}$. The " neutral " zones 3 and 5 also contained activity suggesting that, on the preparative plate, the brain $F_{2\alpha}$ spot had extended to the adjacent zones. However, the possibility that a small quantity of $F_{1\alpha}$ was present cannot be excluded.

Parallel biological assays

The material from zone 6 (prostaglandin E_1) of the AII plate of the prostaglandin E extract was assayed on three isolated smooth muscle preparations. The results shown in Table 13, are compatible with the biological activity in zone 6 being due to prostaglandin E_1.

TABLE 13

CAT BRAIN EXTRACT (POOL 1). PARALLEL BIOLOGICAL ASSAYS CF THE MATERIAL ELUTED FROM ZONE 6 OF A THIN LAYER PLATE DEVELOPED IN THE AII SOLVENT SYSTEM (SEE TABLE 11), IN TERMS OF E_1 AND $F_{1\alpha}$

	Biological activity	
Preparation	μg E_1 equivalent	μg $F_{1\alpha}$ equivalent
Rabbit jejunum	0·2	
Rat fundus	0·38	0·38
Cat trachea	0·2	8

The material from zone 4 (prostaglandin $F_{2\alpha}$) of the AII plate of the prostaglandin F containing extract was assayed on the rabbit jejunum and rat fundus in terms of prostaglandins $F_{1\alpha}$ and E_1. The results 0·85 and 1·75 μg $F_{1\alpha}$ and 2.1 and 0.14 μg E_1 respectively were in agreement with its identification as a prostaglandin F.

An aliquot of the active material in zone 2 (prostaglandins F) from the thin layer chromatogram shown in Table 10 was assayed on six isolated smooth muscle preparations using $F_{1\alpha}$ and E_1 as standards. The results are shown in Table 14. The activity in terms of

TABLE 14

CAT BRAIN EXTRACT (POOL 2). PARALLEL BIOLOGICAL ASSAYS OF AN ALIQUOT OF THE MATERIAL ELUTED FROM ZONE 2 OF THE THIN LAYER PLATE DEVELOPED IN THE AI SOLVENT SYSTEM (TABLE 10) EXPRESSED IN TERMS OF $F_{1\alpha}$ AND E_1

	Biological activity		Ratio of activity
Preparation	$F_{1\alpha}$ (μg)	E_1 (μg)	$F_{1\alpha}/E_1$
Rabbit jejunum	3·8	9·4	2·5
Guinea-pig ileum	3·1	0·1	0·04
Rat fundus	3·0	0·3	0·1
Cat trachea	3·0	0·3	0·1
Jird colon	1·7	0·9	0·5
Rat uterus	1·3	7·5	6·0

$F_{1\alpha}$ ranged from 1.3 μg on the rat uterus to 3.8 μg on the rabbit jejunum, thus the greatest index of discrimination between cat brain extract and $F_{1\alpha}$ was about 3. In contrast, the activity in terms of E_1 ranged from 0.1 μg on the guinea-pig ileum to 9.4 μg on the rabbit jejunum, an index of discrimination of 94. These results are compatible with the activity being due to a prostaglandin F, and confirm that it could not be due to a prostaglandin E.

A further aliquot of this material was compared with pure $F_{2\alpha}$ on the arterial blood pressure and crossed extensor reflex of the spinal chick. The intravenous injection of 0.1 μg prostaglandin $F_{2\alpha}$ caused a rise in blood pressure and potentiated the crossed extensor reflex. An amount of brain extract, equivalent to 0.1 μg $F_{2\alpha}$ as assayed on the rabbit jejunum, caused a similar rise in blood pressure and potentiation of the crossed extensor reflex as shown in Fig. 7.

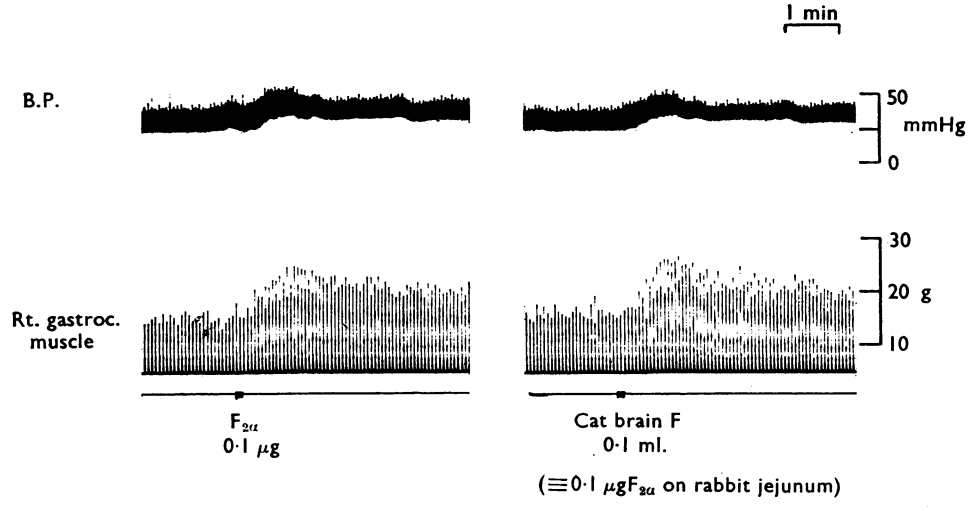

Fig. 7. Spinal chick (decapitated weight 38 g). Blood pressure and crossed extensor reflex. Upper trace: arterial blood pressure. Lower trace: gastrocnemius muscle tension, twitches were elicited by electrical stimulation of the contralateral sciatic nerve. Responses to prostaglandin $F_{2\alpha}$ (0.1 μg) and cat brain extracted (pool 2, zone 2 from AI plate, 0.1 ml.≡0.1 μg $F_{2\alpha}$ on rabbit jejunum) injected intravenously. There was an interval of 25 min between injections.

Concentration of prostaglandins in cat forebrain

Brain tissue, 17 g, removed at decerebration from one cat was extracted and chromatographed, with similar results to those obtained with pooled cat brains. From the silicic acid column peaks of biological activity corresponding to 0.09 μg prostaglandins E_1 and 1.6 μg prostaglandins $F_{1\alpha}$ were eluted.

On the basis of this experiment cat brain was estimated to contain of the order of 10 ng $F_{2\alpha}$/g and 5 ng E_1/g wet weight of tissue.

DISCUSSION

Brain extracts contain acidic lipid-soluble substances which stimulate smooth muscle (Ambache & Reynolds, 1960, 1961; Kirschner & Vogt, 1961; Ambache *et al.*, 1963;

Toh, 1963). One of these substances has been isolated from ox brain and identified as prostaglandin $F_{2\alpha}$ by chromatographic and mass spectrographic analysis (Samuelsson, 1964). Chemical identification, although desirable, is not always possible with the minute quantities of prostaglandins found in small samples of animal tissues. Furthermore, if the compound to be identified is already of known chemical structure and is available in pure form for comparison with the extracted material, such absolute confirmation of its identity may be unnecessary. The question thus arises as to how much and what kind of data are required before a claim to have identified a prostaglandin can be justified. The problem is complicated because there are many different prostaglandins with rather similar biological properties. In the present investigation the following criteria have been used for the identification of a prostaglandin (1) on solvent partition between water (at pH 3 and pH 8) and an organic solvent such as ether or ethylacetate, the unknown should behave like an acidic lipid; (2) on silicic acid chromatography the unknown should have a similar retention volume to one of the known prostaglandins; (3) on thin layer chromatography in the AI and AII (and possibly other) solvent systems of Gréen and Samuelsson (1964), the Rf value should correspond to a known prostaglandin; (4) on parallel biological assay there should be quantitative agreement on two or more tissues. Unequivocal evidence of a negative kind can be obtained from such an approach. Thus it may well be possible to conclude with confidence that an isolated substance is not, say, prostaglandin E_1 because of its different chromatographic behaviour and the lack of agreement between the parallel assays. On the other hand, agreement between the behaviour of the isolated substance and a particular prostaglandin in all these respects does not provide conclusive evidence. It may be permissible to conclude that the chemical and biological properties of the substance are compatible with its identification as, say, prostaglandin $F_{2\alpha}$ but there must always be some element of doubt. Nevertheless the more data of this kind that are accumulated the greater the probability that the identification is correct.

In this investigation solvent partition indicated that the substances extracted from brain and spinal cord were polar acidic lipids. On silicic acid chromatography peaks of biological activity were eluted corresponding to the retention volumes of prostaglandins E and F in control experiments. It should be noted that prostaglandins E and F were eluted with 40 and 60% ethylacetate in benzene respectively, whereas others have used 60 and 80% (Samuelsson, 1964; Coceani & Wolfe, 1965). This may have been due to a different source of silicic acid. Other more polar substances which stimulate the rabbit jejunum were eluted with methanol, but no attempt has been made to identify them. They are possibly hydroperoxides of unsaturated fatty acids.

Confirmation that prostaglandins E and F were present in the extract was obtained by thin layer chromatography in the AI solvent system of Gréen and Samuelsson (1964), which separates the E's from the F_α's and $F\beta$'s. Biological activity in the two peaks from the silicic acid column behaved like prostaglandins E and F_α respectively. In most experiments location of the prostaglandins depended upon biological assay of the eluates of appropriate zones of the thin layer plate, but in one experiment, in which cat brain extract was chromatographed in the AI solvent system and the plate was sprayed with phosphomolybdic acid, a spot appeared which corresponded exactly to F_α. Further evidence that the activity was due to prostaglandins E and F was obtained from the results

of parallel biological assay on smooth muscle preparations. Since there is no way of distinguishing between the different prostaglandins E and F by parallel biological assay, final identification was based on chromatographic behaviour on thin layer plates of silica gel impregnated with silver nitrate developed in the AII solvent system, which separates prostaglandins according to their degree of unsaturation.

From the combination of chromatographic and biological evidence it is concluded that cat forebrain contains prostaglandin $F_{2\alpha}$ and a prostaglandin E, tentatively identified as E_1. Evidence for the presence of E_3 was equivocal (Table 10). There may also be some $F_{1\alpha}$ present but no E_2, or $F_{3\alpha}$ could be detected. Both brain and spinal cord of the chicken contained substances which behaved chromatographically and biologically like prostaglandins E_2 and $F_{2\alpha}$, the presence of detectable quantities of E_1, E_3 and $F_{3\alpha}$,was excluded, but there may have been some $F_{1\alpha}$ in the chicken brain.

The primary object of this investigation was to identify prostaglandins in tissues, not to estimate the amounts present. However, the concentration of $F_{2\alpha}$ in cat fore-brain and of $F_{2\alpha}$ and E_2 in chicken brain and spinal cord has been estimated from the biological activity in the E and F peaks of the silicic acid chromatograms. Since $F_{2\alpha}$ and E_2 were not normally used as prostaglandin standards in the bioassay of fractions from the silicic acid columns, the relative biological activities of the mono- and di-unsaturated F's and E's on the rabbit jejunum (Horton & Main, 1963, 1966a) have been used in the calculation. By this means $F_{2\alpha}$ was estimated to be present in cat fore-brain, chicken brain and chicken spinal cord in concentrations of about 10 ng/g wet weight of tissue. The concentration of E_1 in cat fore-brain was about 5 ng/g; in contrast, the concentration of E_2 in chicken brain and chicken spinal cord was relatively high, 100 ng/g and 400 ng/g respectively.

In calculating these figures, no account was taken of losses during the extraction procedure before silicic acid chromatography; hence, the concentrations of prostaglandins in these tissues may be higher than estimated. It is unlikely, however, that extraction losses could account for the large difference between the estimated concentration of $F_{2\alpha}$ in cat fore-brain, chicken brain and chicken spinal cord (10 ng/g) and that found by Samuelsson (1964) in whole ox brain (300 ng/g). This discrepancy could be due to species variation in the concentration of prostaglandin in the central nervous system or it could also be due to differences in the concentrations of prostaglandins in different parts of the central nervous system. For example, a high concentration of prostaglandins E in the hind brain could account for the differences between cat fore-brain and chicken whole brain. This receives some support from the finding that chicken spinal cord contains even higher concentrations of prostaglandin E_2. It is not certain whether any or all of the hind-brain was included in Samuelsson's experiments or whether a similar uneven distribution of prostaglandins E could account for the absence of prostaglandins E in his extracts. It is of interest that Ambache, Brummer, Rose & Whiting (1966) reported that rabbit cerebral hemispheres contain mainly prostaglandins $F_{2\alpha}$ with some E_2.

If prostaglandins are unevenly distributed in the central nervous system this would suggest that they have a role in the specialized function of the region where their concentration is highest. Further investigations must be carried out to determine the regional distribution of prostaglandins in the central nervous system. It is also important to discover whether, for example, such prostaglandins represent a pool with a constant rate of turnover, or a store which may be released in response to a specific stimulus.

In preceding papers (Horton, 1964; Horton & Main, 1965a, 1967a) pharmacological actions of prostaglandins on the central nervous system of the cat and the chicken have been demonstrated. The identification of prostaglandins in the central nervous system of these species raises the possibility that these actions may reflect a physiological role, though the precise nature of such a role is unknown.

One possibility is that prostaglandins act as chemical transmitters of nerve impulses and evidence related to this hypothesis will be discussed briefly.

Although prostaglandins are present in brain and spinal cord it has not yet been established whether they are located in neurones or, for example, in glial cells. Investigations into the cellular and subcellular distribution of prostaglandins in the central nervous system are required to solve this problem.

That the pharmacological actions of prostaglandins on the central nervous system may be due to a direct action on neurones is suggested by the results of Avanzino, Bradley & Wolstencroft (1966a, b). Prostaglandins E_1, E_2 and $F_{2\alpha}$ applied ionotophoretically in very small amounts, had an excitatory or inhibitory action on the frequency of firing of reticulo-spinal neurones in the decerebrate cat, though desensitization, specific for the prostaglandin applied, was frequently observed. In contrast, prostaglandin E_1 had no effect when applied iontophoretically to cortical neurones in the chloralosed cat (Krnjević, 1965) suggesting either that there are marked regional differences in sensitivity to prostaglandins, or that anaesthesia blocks the effect.

Release of prostaglandins from the central nervous system has been demonstrated (Ramwell & Shaw, 1963a and b, 1966; Ramwell, Shaw & Jessup, 1966; Ramwell, 1967). On direct electrical stimulation, transcallosal stimulation or stimulation of the contralateral superficial radial nerve, prostaglandins (and acetylcholine) were detected in superfusates of the cat somatosensory cortex. Similarly, electrical stimulation of the frog hind limbs caused increased release of prostaglandins into spinal cord perfusates. Prostaglandins (or prostaglandin-like substances) have been detected in superfusates of cat cerebellum (Coceani & Wolfe, 1965) and in fluid perfused through the cerebroventricular system of cats (Feldberg & Myers, 1965, 1966) though in these situations a direct relationship between output of prostaglandins and neuronal activity was not clearly demonstrated.

These results do not exclude the possibility that prostaglandins may be central transmitters. However, evidence has recently been presented that, on nerve stimulation, prostaglandins are released in association with the classical chemical transmitters from several tissues outside the central nervous system including the rat diaphragm (Ramwell, Shaw & Kucharski, 1965), rat adipose tissue (Shaw, 1966), cat adrenal gland (Ramwell, Shaw, Douglas & Poisner, 1966), and dog spleen (Davies, Horton & Withrington, 1967). These results suggest that prostaglandins are not transmitters but may rather be modulators of transmission. Prostaglandins are not released from the rat diaphragm preparation by direct electrical stimulation of the muscle or from the isolated phrenic nerve, but they are released from the intact preparation on nerve stimulation even after muscle twitches have been blocked by d-tubocurarine (Ramwell *et al.*, 1965). Prostaglandins are released from adipose tissue either by nerve stimulation or by adding catecholamines to the incubation medium. Moreover, the amount of prostaglandins released is sufficient to inhibit the concomitant mobilization of free fatty acids from the tissue (Shaw, 1966). These

results raise the possibility that prostaglandins may modulate transmitter action by a local negative feed-back mechanism—for example, the action of the transmitter on the post-synaptic membrane may release prostaglandins which may inhibit further release or action of the transmitter.

Prostaglandins have potent inhibitory actions on hormone-induced lipolysis in adipose tissue (Steinberg, Vaughan, Nestel & Bergström, 1963) and on the hormone-induced increase in permeability of the isolated toad bladder (Orloff, Handler & Bergström, 1965). The concept that prostaglandins are modulators of chemical transmitters at nerve endings could, therefore, be extended to include an action on true hormones throughout the body.

There is evidence that the common factor in the mechanism of action of many hormones is the activation of the adenyl cyclase system which converts adenosine triphosphate (ATP) to 3,5-adenosine mono-phosphate (3,5-AMP) which in turn activates other enzyme systems to produce, for example, lipolysis or increased permeability (Sutherland, Øye & Butcher, 1965). Since, in these situations, prostaglandins do not prevent the action of 3,5-AMP, the action of prostaglandins may be to prevent the activation of adenyl cyclase. Whether the miscellaneous biological actions of prostaglandins can be rationalized in terms of a fundamental action on the adenyl cyclase system cannot be decided from existing evidence. It is of interest to note, however, that the brain forms 3,5-AMP in response to catecholamines (Sutherland *et al.*, 1965).

It is concluded that prostaglandins may have a role in the central nervous system, possibly as modulators of the actions not only of chemical transmitters released from nerve endings but also of other local or true hormones released in the central nervous system or reaching it from the systemic circulation. That this modulator action is not confined to the central nervous system but may be of importance throughout the body does not diminish its significance in the central nervous system.

Human seminal plasma contains by far the highest concentration of prostaglandins in any tissue yet examined. It is known that these prostaglandins are absorbed from the vagina after coitus and circulate in the bloodstream as hormones acting on female reproductive tract smooth muscle. It is tempting to speculate that the function of prostaglandins in human reproduction may also involve a hormonal action on the brain, possibly on those centres which control sexual behaviour.

SUMMARY

1. The object of the investigation was to identify the prostaglandins present in the central nervous system of the cat and the chicken.

2. On the basis of their behaviour on solvent partition, silicic acid column chromatography, thin layer chromatography and on biological preparations prostaglandins $F_{2\alpha}$ and E_1 have been identified in cat brain (supracollicular) and prostaglandins E_2 and $F_{2\alpha}$ in chicken brain and spinal cord.

3. The concentration of prostaglandin $F_{2\alpha}$ in the three tissues was approximately 10 ng/g whereas the concentrations of prostaglandin E_2 in the chicken brain and spinal cord were about 100 ng/g and 400 ng/g respectively.

4. The physiological significance of the prostaglandins in the central nervous system is discussed in the light of the known pharmacological actions.

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