Effects of subarachnoid haemorrhage on intracranial prostaglandins

V WALKER, JD PICKARD, P SMYTHE, S EASTWOOD, S PERRY

From the University Department of Chemical Pathology and Human Metabolism and Wessex Neurological Centre, Southampton General Hospital, Southampton, UK

SUMMARY Prostaglandins E_2 , $F_2\alpha$, 6 OXO $F_1\alpha$ and thromboxane B_2 increased in cisternal CSF following mock subarachnoid haemorrhage in dogs, particularly PGE_2 (X25·5). Concentrations were increased also in lumbar CSF of five patients some 8 days after subarachnoid haemorrhage. Subarachnoid haemorrhage did not alter the production of prostaglandins by dog whole cortex or choroid plexuses in vitro, but production by pooled dissected cerebral arteries of PGE_2 was increased and of 6 OXO $F_1\alpha$ was decreased. Intravenous indomethacin decreased prostaglandin production by cerebral tissues, and caused a marked decrease in the prostacyclin metabolite in CSF. The implications of our findings for the aetiology of cerebral vasospasm are discussed.

How far can excessive production of Thromboxane A_2 , $PGF_2\alpha$ or PGE_2 , or defective synthesis of prostacyclin explain the cerebrovascular changes following subarachnoid haemorrhage? Delayed cerebral ischaemia occurs naturally after subarachnoid haemorrhage but often appears to be aggravated by surgical intervention, particularly when this is performed during the first week after rupture of the aneurysm. This period of greatest risk coincides with the development of cerebral arterial vasospasm which is seldom seen prior to 3 days after the bleed but becomes maximal around the end of the first week and declines thereafter.¹ The capricious relationship between spasm and neurological deficit may reflect the ability of the individual's cerebral circulation to accommodate to such narrowed vessels: tissue ischaemia may be exacerbated when a further stress is applied such as a period of hypotension.^{2,3}

Cerebral vasospasm may be the end result of competition between the changing properties and synthetic capacity of the cerebral endothelium and vascular smooth muscle cell, and the evolving pattern of substances released from clotted blood to which they are exposed.⁴ Human cerebral vasospasm is refractory to any known pharmacological blocker or

synthesis inhibitor.¹ There is a relationship between periarterial haematoma and subsequent arterial narrowing, but the relationship is not a simple one.⁵ Lipid peroxidation may be involved, leading to damage to both endothelium and vascular smooth muscle cell.⁶ Arachidonic acid derivatives such as prostacyclin (Ref 7 for review) may have a physiological role in the control of cerebral blood flow,^{8,9} and part of the sequence of events precipitating cerebral vasospasm may be prostacyclin deficiency.¹⁰ Two recent reports suggest that cerebral arterial generation of prostacyclin in vitro is impaired after subarachnoid haemorrhage.^{11,12} Unfortunately the problems of measuring the concentrations of the different cyclo-oxygenase and lipoxygenase products in the different compartments of the human brain are formidable.¹³ Our present purpose was to obtain quantitative data on changes in CSF prostaglandins/Thromboxane B_2 (TXB_2) following subarachnoid haemorrhage in both man and dog, and to compare these results with the in vitro production of prostaglandins/ TXB_2 by cerebral cortex, choroid plexus and cerebral arteries from the dog. The opportunity was taken to observe the acute effects of indomethacin on intracranial prostaglandin synthesis.

Materials and methods

Materials

Standard prostaglandins (PG) E_2 , 6 OXO $F_1\alpha$, $F_2\alpha$ (tromethamine salt) and TXB_2 were gifts from Dr John Pike, the Upjohn Co, Kalamazoo, Michigan, USA; 3H PG

Address for reprint requests: Mr JD Pickard, Wessex Neurological Centre, Shirley, Southampton SO9 4XY, UK.

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E₂ (specific Activity 160 Ci/mmol), ³H 6 oxo PG F_{1α} (150 Ci/mmol) and ³H PG F_{2α} (180 Ci/mmol) were from the Radiochemical Centre, Amersham. ³H TXB₂ (125 Ci/mmol) was from New England Nuclear, Boston, Mass USA. Antisera to PGE₂, TXB₂ and PG 6 oxo F_{1α} were from Institut Pasteur Production, Marnes-La-Coquette, France. Antiserum to PGF_{2α} was raised in rabbits using PGF_{2α}—bovine serum albumin conjugated with carbodiimide, and was a gift from Dr Keith Hillier, University of Southampton, England. (Per cent cross reactivity at B/BO = 0.5 was PGE₂ < 0.01, PGF_{1α}, 3.8, PGD₂ 6.7, 13.14 dihydro 15 keto PGE₂ < 0.01, 13.14 dihydro 15 keto PGF_{2α} < 0.01.) Water, Analar cyclohexane (BDH) and Analar ethylacetate (BDH) were all twice distilled. Nanograde methanol and silicic acid 100 Mesh (powder), were from Mallinckrodt Inc, St Louis, Missouri. RIA Luma was from LKB Instruments Ltd, Surrey, England. All other laboratory chemicals were of Analar Grade. Siliconised glassware was used throughout. Krebs-Ringer-Bicarbonate buffer containing 1 mmol/l glutathione (KRB) was used for tissue incubations (per litre: NaCl 7.24 g, KCl 0.372 g, KH₂PO₄ 0.163 g, NaHCO₃ 2.184 g, glucose 1.8 g, MgSO₄ 7 H₂O 0.32 g, CaCl₂ 2H₂O 0.184 g, glutathione 0.3072 g; pH adjusted to 7.4 after equilibrating with 95% O₂/5% CO₂ for 15 min). 0.1 mol/l phosphate buffer pH 7.5 was used in the radioimmunoassays.

Methods

Alsatian dogs weighing 10–15 kg were anaesthetised with thiopentone (7.5 mg/kg/iv) and halothane in nitrous oxide 70% and oxygen. Endotracheal intubation was performed and ventilation controlled. A 22 G spinal needle was placed transorbitally into the suprachiasmatic cistern or, if this proved difficult, into the cisterna magna. Five to 7 ml of CSF was withdrawn and fresh autologous blood (0.6–0.75 ml/kg) injected within 30 s, with the dogs in the head-down position. The animals recovered from anaesthesia without neurological deficit and behaved and fed normally. Control animals (3), animals 3–4 days (4), or 6–8 days (3) after subarachnoid haemorrhage were anaesthetised, heparinised and exsanguinated. Three further control animals were given indomethacin (10 mg/kg/iv) under anaesthetic 10–30 min. before exsanguination. CSF was withdrawn from the cisterna magna, and the whole brains removed quickly. The large cerebral arteries were dissected under the operating microscope and used for another related study (to be reported separately). However, the anterior and middle cerebral and basilar arteries and the choroid plexuses from some of the animals, were available for prostaglandin analyses. If immediate incubation (see below) was not feasible, brain cortex, arteries and choroid plexuses were stored at –70°C, and generally incubated within 7 days. CSF was collected on ice into plastic tubes containing 100 μl of Na₂ EDTA (70 g/l) and acetyl salicylic acid (5 g/l) in water. After centrifugation to remove cells, CSF was stored at –20°C until assay.

Human CSF: Lumbar CSF samples were available from five patients following subarachnoid haemorrhage. For comparison lumbar CSF was also obtained from patients undergoing myelography for possible disc lesions. Although not “normal” these patients at least did not have intracranial

pathology. These studies were approved by the Local Ethical Committee.

LABORATORY METHODS

Tissue incubation: Working at 4°C whole cortex was cleared of pial vessels, sliced at 20 μm in two directions, and washed × 1 in KRB. Tissue was resuspended in KRB (approx 3 g tissue in 20 ml buffer). 1 ml of suspension was added to 4 ml of KRB in each of four flasks and incubated at 37°C in a shaking water bath under 95% O₂/5% CO₂. At exactly 60 min, prostaglandin synthesis was terminated by adding 75 μl of indomethacin (1.1 mg/ml), transfer to ice, and centrifugation at 4°C. To determine prostaglandins produced during tissue preparation, suspension was also added to 4 ml of ice cold buffer containing indomethacin in each of two flasks. After mixing, the contents were centrifuged immediately (“zero time incubates”). Supernatants were stored at –20°C for later prostaglandin analysis, and pellets for protein analysis by Lowry’s method. Choroid plexuses and dissected cerebral arteries were incubated using an identical procedure.

Prostaglandin analyses of tissue incubates and CSF: Prostaglandins and TXB₂ were extracted from acidified supernatant or CSF using a method based on that of Mitchell *et al.*¹⁴ After adding approximately 15 000 dpm ³H PGF₂ to monitor recoveries, supernatants and CSF samples (up to 7.5 ml according to availability) were acidified to pH 3.0 with 2.3 mol/l citric acid, extracted twice with 2–3 volumes of diethyl ether, and the extracts dried with Na₂SO₄. Samples were applied to minicolumns containing silicic acid (0.5 g) in cyclohexane (C)–ethyl acetate (E)–methanol (M) (60:40:20 vol/vol). After washing with 5 ml cyclohexane:ethyl acetate (60:40 vol/vol); prostaglandins and TX were eluted under nitrogen pressure with 6 ml of C:E:M. (60:40:20). The eluate was stored in ethanol at –20°C for radioimmunoassay (RIA) of PGE₂, F_{2α} and TXB₂, or in acetonitrile:water (9:1 vol/vol) for 6 oxo F_{1α}. Recovery of labelled F_{2α} usually exceeded 75%.

RIA: Extracts were evaporated to dryness, taken into 0.1 mol/l phosphate buffer, and 100 μl assayed (in duplicate) by RIA. Lower limit of sensitivity in each RIA was 6 pg. Parallelism was demonstrable between serial dilutions of each standard prostanoid and a pooled tissue extract. Within and between batch precision was determined for replicate RIA’s of pooled brain extracts of each prostanoid. Intra-assay coefficient of variation in each case was in the range 3.0 to 7.7% and interassay variation was 7.3% (F_{2α}), 10.0% (E₂), 12.2% (6 oxo F_{1α}) and 13.7% (TXB₂). Intra-assay variation was determined also for the entire extraction

Table 1 Complete extraction and radioimmunoassay procedure for nine aliquots of a CSF sample: intra-assay variation

	Mean conc (ISD) pmol/l of CSF	C of V %
PGF _{2α}	420 (16)	10.7
PGE ₂	227 (13)	16.6
PG 6 oxo F _{1α}	305 (9)	8.1
TXB ₂	46 (2)	13.7

and RIA procedure by simultaneous assay of 9×7.5 ml aliquots of a single CSF sample (human ventricular drainage sample) (table 1). Prostaglandin D_2 was kindly analysed in four CSF samples by Dr S Holgate, Senior Lecturer in Clinical Pharmacology, Southampton University Medical School.

Results

1. Effect of "mock" subarachnoid haemorrhage on prostaglandin concentrations in cisternal CSF

In six samples of cisternal CSF collected from dogs immediately before subarachnoid haemorrhage, the most abundant prostaglandin was PG 6 oxo $F_{1\alpha}$, the prostacyclin metabolite, $PGF_{2\alpha}$, PGE_2 and TXB_2 were all present but in low concentration (table 2). This pattern was unchanged in the three control animals who were anaesthetised and exsanguinated, although in two of the animals increased concentrations of PG 6 oxo $F_{1\alpha}$ were observed (1271 and 1250 pmol/l). Three to four days after subarachnoid haemorrhage (four dogs), all the prostaglandin concentrations were elevated. Comparing the median concentrations with those of the three control animals, the increases were: $PGF_{2\alpha} \times 4.3$; PG 6 oxo $F_{1\alpha} \times 2.3$, $TXB_2 \times 3.1$. The most dramatic increase, however, was in PGE_2 ($\times 25.5$). By 6-8 days after subarachnoid haemorrhage only PGE_2 was elevated above controls in all three animals, $PGF_{2\alpha}$, PG 6 oxo $F_{1\alpha}$, and TXB_2 having fallen to control levels in two of the three. Prostaglandin D_2 , was present at very low concentration (31 pmol/l) in cisternal CSF removed from one dog before subarachnoid haemorrhage, but increased (to a modest level) 7 days after blood injection. Indomethacin injected intravenously 10-30 min before exsanguination, decreased the concentrations of all the prostanoids in cisternal CSF, the most dramatic decrease being in the prostacyclin

metabolite (124 pmol/l compared with 1250 pmol/l in controls).

2. Prostaglandin production by dog cerebral tissues in vitro: effect of mock subarachnoid haemorrhage (table 3)

The procedures used detect the capacity of cerebral tissues to synthesise prostaglandins in vitro under standardised conditions. Whole cortex from the three control animals synthesised PGE_2 in greatest amount, followed by $PGF_{2\alpha}$ and prostacyclin (measured as PG 6 oxo $F_{1\alpha}$). Some TXB_2 was produced. Blood platelets produce TXB_2 during aggregation and platelet contamination from blood in the incubated tissue was unavoidable, despite the wash procedures used. It seems probable that at least some of the TX produced in vitro derived from cellular components of brain tissue other than platelets however, since TXB_2 was always detectable in (clear) CSF samples from the dogs. Results for individual animals showed some scatter, but subarachnoid haemorrhage appears to have had no effect on in vitro PG synthesis.

Because of limited supplies of tissues it was necessary to pool dissected arteries (and similarly choroid plexuses) from individual animals for incubation. The arteries were free of blood contamination and produced PGE_2 and PG 6 oxo $F_{1\alpha}$ in greatest amount. Production of PGE_2 by the subarachnoid haemorrhage "pool" was greater than that of the controls and there was a slight decrease in PG 6 oxo $F_{1\alpha}$. PGE_2 and 6 oxo $F_{1\alpha}$ were also the main products of the choroid plexuses, but for these no difference between the control and subarachnoid haemorrhage pools was apparent. Unavoidably, the choroid plexus incubates were contaminated with blood. Perhaps this accounted for some of the $PGF_{2\alpha}$ produced, although a greater production of TXB_2 might also have been anticipated (see below).

Table 2 Prostaglandins and thromboxane B_2 in dog cisternal CSF

Experiment (no of animals)		CSF prostaglandins (pmol/l)			
		$PGF_{2\alpha}$	E_2	6 oxo $F_{1\alpha}$	TXB_2
CSF collected before SAH (6)	Median	62	57	208	94
	Range	48-73	34-128	181-561	62-162
Controls—terminal (3)	Median	107	60	1250	81
	Range	62-138	51-122	240-1271	62-275
SAH 3-4 days (4)	Median	454	1552	2912	248
	Range	417-643	1506-2701	1673-6505	175-356
SAH 6-8 days (3)	Median	133	377	731	73
	Range	130-158	388-1416	394-2942	62-73
10-30 min after indomethacin (3) (indomethacin-treated controls)	Median	51	34	124	76
	Range	45-59	17-48	100-151	35-140

PG D_2 concentration in cisternal CSF from 1 dog
Before SAH: 31 pmol/l; 7 days after SAH (terminal): 136 pmol/l.

SAH = "mock" subarachnoid haemorrhage.

Table 3 Prostaglandin and thromboxane B_2 synthesised by dog cerebral tissues in vitro

Experiment (no of animals)		* Prostaglandin synthesised (pmol/mg protein)			
		PGF $_2\alpha$	PGE $_2$	PG 6 oxo F $_1\alpha$	TXB $_2$
Whole cortex					
Controls (3)	Median	11.0	20.1	9.6	1.6
	Range	7.9–17.5	18.9–22.8	7.3–10.1	1.1–1.9
SAH 3–4 days (4)	Median	17.2	20.9	6.6	1.4
	Range	11.2–20.4	13.4–24.8	4.9–14.0	0.8–2.1
SAH 6–8 days (3)	Median	15.3	13.3	5.4	1.7
	Range	8.3–19.1	10.4–25.9	3.5–7.2	1.2–1.9
10–30 min after indomethacin ("indomethacin controls") (3)	Median	3.1	2.0	1.8	0.2
	Range	1.6–5.2	0.9–3.3	1.1–1.9	0.1–0.6
† Dissected arteries					
Controls		1.9	7.6	6.7	0.1
Post SAH		2.2	12.6	2.6	0.3
10–30 min after indomethacin		1.3	2.9	2.5	0.1
† Choroid plexuses					
Controls		3.9	25.1	13.3	0.3
Post SAH		6.9	10.6	21.6	0.9
		4.2	22.2	14.9	0.6
10–30 min after indomethacin		1.0	4.2	2.8	0.1

*Prostaglandin synthesised is the mean concentration of the 4 flasks incubated for 60 min, minus the mean concentration of the 2 zero time incubation flasks (see methods section).

†Because of the limited amounts of vascular tissue available, tissue from animals was pooled, and each result is the production for the "pool" (4 × 60 min and 2 zero time incubates per experiment). Two incubation experiments were possible for pooled choroid plexuses from animals after SAH.

Table 4 Prostaglandin production by blood cells incubated in CSF (human) at 37°C

Time of incubation (h)	PGF $_2\alpha$ (pmol/l)		PGE $_2$ (pmol/l)		PG 6 oxo F $_1\alpha$ (pmol/l)		TBX $_2$ (pmol/l)	
	Clear CSF	CSF + blood*	Clear	+ Blood*	Clear	+ Blood*	Clear	+ Blood*
0	59	39	11	34	165	154	51	67
2	45	73	9	85	67	232	51	1412
6	118	161	28	230	108	300	86	2402
24	71	375	34	295	140	327	54	3074
48	lost	618	lost	374	159	502	lost	4146
78	54	485	9	142	103	456	70	2901

*Approx 300 000 RBC per mm 3 CSF.

Table 5 Prostaglandins and thromboxane B_2 in lumbar CSF collected from five patients following SAH

Patient	Day after SAH	Prostaglandin concentration (pmol/l)			
		F $_2\alpha$	E $_2$	6 oxo F $_1\alpha$	TXB $_2$
1	8	852	471	848	81
2 (3rd bleed)	8	1094	740	1552	70
3	6	1661	1719	2570	178
4	6	259	196	540	16
5	9	3869	5126	4113	173
Mean		1548	1651	1924	103
± SEM		± 620	± 905	± 648	± 32
*Controls		87	28	378	103
± SEM		5.67	4.94	47.23	19.76
No.		11	15	19	13
Range		56–107	< 14–74	130–848	13–240
†		p < 0.01	p < 0.01	p < 0.001	NS

*Patients having myelography for possible lumbosacral disc lesions. Samples removed above the site of disc herniation.

†Student's *t* test. NS = not significant.

Indomethacin administration decreased *in vitro* production of all the prostanoids by whole cortex, arteries, and choroid plexuses. The less pronounced effect of indomethacin on the arteries and choroid plexus may reflect washout of the indomethacin from these much thinner tissues—indomethacin does not produce irreversible inhibition of prostaglandin synthesis.¹⁵

3. Prostaglandin production by blood cells incubated in CSF at 37°C (table 4)

Blood platelets release TX during aggregation, and neutrophils and other white blood cells produce a range of prostanoids. To make some assessment of the maximal contribution blood cells themselves might make to the prostanoid content of CSF after subarachnoid haemorrhage, fresh whole blood was mixed quickly with human CSF to give a red cell count of approximately 300 000 per mm³ CSF—that is of the order found in lumbar CSF after a severe subarachnoid haemorrhage. Aliquots were incubated in siliconised tubes at 37°C and prostaglandin production was terminated at timed intervals by addition of acetyl salicylic acid and freezing (−20°C). All prostaglandins were synthesised for up to 48 hours (table 4), the lower concentrations at 78 h probably reflecting loss of immunoreactivity with prolonged incubation. TXB₂ was the main product. Only a small increase in PG 6 oxo F₁α was observed. This experiment of course makes no allowance for removal of prostaglandins from CSF which would occur *in vivo*.

4. Prostaglandins in lumbar CSF collected from five patients following subarachnoid haemorrhage

Six to 9 days following subarachnoid haemorrhage all prostaglandins were significantly increased in the lumbar CSF when compared with “control” CSF collected from orthopaedic patients (table 5) and the changes resembled closely those seen in dogs. TXB₂ concentration was generally quite low after 8 days, but in one sample collected within 24 h after a bleed, high levels were observed (5568 pmol/l). PGD₂ assayed in a single sample, was present in low concentration (91 pmol/l).

Discussion

Our main findings are firstly that the PG spectra of CSF in the dog is not an accurate reflection of *in vitro* PG production by cerebral cortex, cerebral arteries or choroid plexus. Choroid plexus and cerebral arteries have a similar spectrum of PG synthetic activity (see also refs 16, 17, 18). Secondly the proportions of the various PG's/TXB₂ in CSF in man (see also ref 19) and dog are similar. Thirdly, that subarachnoid

haemorrhage results in a great increase in the whole spectrum of PG's/TXB₂ in the CSF in both man and dog, not just PGF₂α²⁰⁻²² and a proportionately greater increase of PGE₂ in both species. Fourthly, the *in vitro* production of PG's/TXB₂ by cerebral cortex and choroid plexus was not affected by subarachnoid haemorrhage, but there is some support for increased PGE₂ synthesis by the larger cerebral arteries, with depression of prostacyclin synthesis *in vitro*.

Are the concentrations of prostaglandins/TXB₂ sufficient to account for cerebral vasoconstriction some days after subarachnoid haemorrhage? Our parallel studies in dogs have shown that cerebral arterial narrowing to less than 67% of control occurs in 20%, and to between 67% and 100% in 30% of dogs 7 days after subarachnoid haemorrhage. (Graham, Matear, Pickard, McPherson, Tamura and Fitch, unpublished observations.) We have no data on cerebral blood flow changes following subarachnoid haemorrhage in the dog; in the baboon, baseline CBF does not change but reactivity is impaired one week after subarachnoid haemorrhage.² Consistent with these findings, the *in vitro* contractile reactivity of cerebral arteries taken from our dogs is altered by subarachnoid haemorrhage—the response to amines is enhanced but the response to pH changes and sodium-loading is depressed (Pickard and Perry, unpublished observations).

Extrapolation of effective vasoconstrictor doses for prostaglandins/TXA₂ from other species or from *in vitro* analysis must be done with care. Brandt *et al*²³ have shown that there is considerable individual variability in the spectrum of reactivity of cerebral arteries from man *in vitro* to different stimuli such as high potassium, prostaglandins and biogenic amines (serotonin, noradrenaline). However, in both dog and man, *in vitro* contractions of cerebral arteries are produced with PGF₂α and PGE₂ of concentrations above 100 nmol/l.^{24 25} Lesser concentrations may produce just detectable responses, and may potentiate the effects of other vasoconstrictor agents at these low levels. The combined concentrations of the two cerebral vasoconstrictors PGF₂α and PGE₂ of around 2 nmol/l in cisternal CSF 3 days and 0.5 nmol/l 7 days after subarachnoid haemorrhage in the dog, and of around 3 nmol/l in lumbar CSF in man 7 days after subarachnoid haemorrhage, seem a little low for potent vasoconstrictor action. The low concentrations of TXB₂ make the more potent TXA₂ an even less likely candidate at 3–7 days after subarachnoid haemorrhage—its concentration is much higher immediately following a bleed but subsides very rapidly. Unfortunately lumbar CSF is an inexact mirror of what is happening around the cerebral arteries, closeted in their subarachnoid haematoma,

and changes in lumbar $\text{PGF}_2\alpha$ levels do not correlate with clinical state or presence of vasospasm.^{20,21}

Although CSF PG 6 oxo $\text{F}_1\alpha$ increases following subarachnoid haemorrhage, we, Maeda *et al*¹² and Sasaki *et al*¹¹ find that production of this metabolite of prostacyclin by isolated arteries is depressed following subarachnoid haemorrhage by around 60%, 34% and 50% respectively. However, some caution is needed before accepting these results. First, following subarachnoid haemorrhage it is technically more difficult to dissect out cerebral arteries, even using microsurgical technique, because of organised clot and fibrous tissue and the endothelium might become injured even though no damage was seen on light microscopic examination of arteries used in these studies. Secondly, different conditions of in vitro incubation and different analytical procedures were used by the three groups, and whereas Maeda *et al*¹² found that $\text{PGF}_2\alpha$ production was 9% and PGE_2 24% of that of PG 6 oxo $\text{F}_1\alpha$, the corresponding figures in our study were 28% and 108%. Thus the choice of conditions may influence the pattern of production in vitro. A third consideration is that prostacyclin synthesis within vessels of differing calibre may differ after subarachnoid haemorrhage—decreased production in larger arteries perhaps being a consequence of spasm, and increased production occurring possibly in smaller vessels. Finally it remains to be shown what level of impairment of prostacyclin synthesis is biologically significant. Clearly more work is needed.

Several factors may have combined to increase CSF prostaglandin concentrations 3–4 days after subarachnoid haemorrhage. Blood cells themselves produce a range of prostanoids, and the increased concentration of $\text{PGF}_2\alpha$ after subarachnoid haemorrhage and the very modest increase in TXB_2 could well be accounted for by production by extravasated blood cells. If blood were the principal source however, higher concentrations of TXB_2 might have been anticipated. The large changes in PGE_2 and PG 6 oxo $\text{F}_1\alpha$ observed moreover require additional explanation. Some of the PGE_2 may have derived from macrophages, since subarachnoid haemorrhage induces a brisk macrophage response, and phagocytosis of extravasated blood cells is maximal 5–7 days after the bleed. PGE_2 is a major prostanoid product of macrophages.²⁶

A further possibility is that increased prostaglandin synthesis by some element of brain tissue may have contributed to the increased levels. Unfortunately arachidonic acid release and metabolism is increased variably by terminal ischaemia, brain dissection, and tissue handling, and measurement of prostaglandin levels in dissected tissues directly, produces uninterpretable results which bear little relationship

to their levels in vivo (see ref 9 for review). Comparison of the capacities of tissues to produce PG's under standardised conditions in vitro (as in the present study), is a more helpful and acceptable alternative, but such studies are quite crude. Any changes in PG production induced in tissues after subarachnoid haemorrhage might be reversed readily under incubation conditions, and masked. Perhaps this explains why no differences were demonstrable in vitro for prostaglandin production by whole cortex or choroid plexuses between controls and subarachnoid haemorrhage animals. Both Maeda *et al*¹² and ourselves find that PGE_2 production by cerebral arteries is enhanced following subarachnoid haemorrhage—if it were, then the raised concentration of PGE_2 in CSF might reflect an abnormal arterial response to subarachnoid haemorrhage, rather than being a primary agent itself in inducing such a response. Finally, impaired clearance of prostanoids from the subarachnoid space probably contributed to raised CSF levels. This would result in part from saturation of the choroidal and extrachoroidal facilitated transport system for the primary prostaglandins and TXB_2 ,^{27,28} and in part because of impaired clearance through the arachnoid granulations and obstruction to bulk flow of CSF into the dural venous sinuses.²⁹

The preliminary observations with indomethacin are interesting. Although intravenous injection decreased the concentrations of all measured prostanoids in cisternal CSF, the decrease in PG 6 oxo $\text{F}_1\alpha$ was very rapid and most dramatic when compared with controls. This supports the notion that the turnover of prostacyclin in brain is normally rather rapid. In vitro production—notably of PGE_2 and of PG 6 oxo $\text{F}_1\alpha$ —was depressed in all the tissues examined. These findings fit well with Pickard's observations and support his proposal that prostacyclin has a role in the control of CBF.^{8,9} However other nonsteroidal anti-inflammatory agents have more complex effects on the cerebral circulation.⁹ Clearly more work with this group of compounds is needed and should be combined with biochemical studies.

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