symptoms. She is a woman of 50 who a year before had a hysterectomy and has not felt well since. She has backaches, headaches, dizziness, insomnia, and a persistent sense of depression. She sometimes gets up in the night to turn off the gas or to see that the door is locked. She has no serious financial difficulties, but her husband and children take no notice of her and she feels that life in the future stretches out like a desert. She has been seeing her doctor regularly and receiving tranquillizers, but she has not been to him for three months because she feels she is wasting his time.

In many others there are no symptoms but the same life situation. In others there is an acute stress (the woman who shoplifted the day before taking her son, who had a sarcoma of the elbow, to see a surgeon). Rather younger women may have had a recent miscarriage, fear sterility, or mourn the death of children. Current menopausal symptoms are not more common than is to be expected for the age group.

Most women shoplifters of this kind, who represent not

less than 10% and perhaps more than 20% of those arrested, are convicted only once. Those of similarly good character who persist in shoplifting, the rare "compulsive" shoplifters, are often very different. They are of exemplary character, with an attitude of robust mental health, and unwilling to admit the existence of obvious stresses. From our figures, however, it is tempting to suppose that in a fair proportion of cases shoplifting is the first symptom of a frank depressive illness. It is also possible that the conviction has quite a traumatic effect. A number of middle-aged recidivists seem to feel that the first conviction ruined their reputation for honesty and that nothing will efface this memory.

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Prostaglandins and Glaucoma

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Summary

Human aqueous humour was found to contain a substance which contracted the rat stomach strip. The mean activity was significantly higher in specimens from open angle glaucoma cases than in specimens from patients with cataract. The pharmacological and chromatographic properties of the active material were studied in aqueous humour obtained from cadavers; activity seemed to be due to prostaglandin E_1 . These results suggest that prostaglandin E_1 may play some part in the aetiology of open angle glaucoma.

Introduction

Ambache (1959) showed that extracts of iris contained "irin," a hydroxy-unsaturated fatty acid with the ability to cause atropine-resistant meiosis in rabbits. Irin could be released into the aqueous humour (Ambache *et al.*, 1965). This activity of iris extracts was at least partly due to their content of prostaglandins (Änggård and Samuelsson, 1964; Ambache *et al.*, 1966), and it has been shown that several prostaglandins produce a sustained rise in intraocular pressure when injected into the rabbit eye (Waitzman and King, 1967; Beitch and Eakins, 1969). The question therefore arises whether prostaglandins might be implicated in the raised intraocular pressure characteristic of open angle glaucoma. To explore this possibility we studied aqueous from the eyes of 9 cadavers and 17 patients. Eight of the patients had typical open angle glaucoma, the others had cataracts.

Methods

Aqueous was collected 3 to 13 hours after death from both eyes of nine cadavers. Sampling of aqueous from patients was done under cocaine local anaesthesia by needle puncture of the anterior chamber through the limbus. In the cataract cases this was done immediately before cataract extraction; in glaucoma cases samples were taken only from eyes with vision less than 3/60.

The samples were sealed into ampoules under air and frozen at -40°C for up to three weeks. They were refrigerated on CO_2 snow during transfer from Aberdeen or Edinburgh to London, where the assays were performed without knowledge of the clinical condition. The samples were assayed for prostaglandin-like activity on a rat stomach strip (Vane, 1957) suspended in a 3-ml organ bath of Krebs's solution containing hyoscine (10^{-7} g/ml) and methysergide (2×10^{-7} g/ml). To perform an assay the response of the strip was first calibrated with prostaglandin E_2 in the range 0.25-3 ng. Test samples were then assayed, the whole of each sample being injected into the organ bath in 0.4 ml of Krebs's solution. Further calibrating doses of prostaglandin E_2 were then given. The volumes of aqueous samples were estimated by weighing the ampoules before and after removing their contents. Material in cadaver aqueous was tentatively identified by chromatography on a 250- μ layer of silica gel G containing 3% of silver nitrate. The solvent system AII of Grn and Samuelsson (1964) was used.

Results

The Table shows the levels of prostaglandin-like activity in the aqueous from the two groups of patients. For the cataract patients it averaged $1.2 \text{ ng/ml} \pm 0.12 \text{ S.E.}$ of mean and for the glaucoma patients it was significantly higher ($P < 0.025$, 1-tail t test) at $2.3 \text{ ng/ml} \pm 0.46 \text{ S.E.}$ of mean. The variance of the observations was also greater for the glaucoma cases than for the others ($P < 0.01$, F-ratio). The two most clinically severe cases of glaucoma had the highest prostaglandin levels.

The volumes of aqueous available from patients (0.05-0.25 ml)

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Levels of Prostaglandin-like Material in Human Aqueous Humour Assayed as Prostaglandin E₁ on the Rat Stomach Strip

Case No.	Age	Sex	Diagnosis	Prostaglandin Level (ng/ml)
1	65	F.	Cataract	0.9
2	81	F.	"	0.9
3	73	F.	"	1.8
4	74	M.	"	1.5
5	65	F.	"	1.5
6	60	M.	"	0.9
7	83	F.	"	1.2
8	60	F.	"	0.9
9	69	M.	"	0.8
Mean	70.0			1.2
10	70	M.	Glaucoma	4.3
11	75	F.	"	2.5
12	75	M.	"	2.5
13	64	M.	"	2.1
14	80	F.	"	1.3
15	51	M.	"	0.1
16	63	M.	"	3.7
17	55	M.	"	2.2
Mean	65.5			2.3

were insufficient to allow any identification of the spasmogen detected by the rat stomach strip. We therefore studied 5 ml of aqueous from 18 cadaver eyes. Two experiments were performed with this material:

(1) One millilitre was assayed on a rat stomach strip in terms of prostaglandin E₂ before and after adding the following antagonists to the Krebs's solution—hyoscine hydrobromide (10^{-7} g/ml), methysergide maleate (2×10^{-7} g/ml), mepyramine maleate (10^{-7} g/ml), phenoxylbenzamine hydrochloride (10^{-7} g/ml), and propranolol hydrochloride (2×10^{-6} g/ml). (The concentrations refer to the active bases.) The assay value before adding the antagonists was 1.5 ng/ml, afterwards it was 1.7 ng/ml. This showed that histamine, choline compounds, 5-hydroxytryptamine, and catecholamines did not contribute to the activity in the aqueous.

(2) The remaining 4 ml was freeze-dried. The residue was extracted twice with 1 ml of ethanol and the combined ethanol extracts were evaporated to dryness in a centrifugal freeze-drier. The residue was applied to the origin in a thin layer plate in 0.4 ml of methanol and the AII solvent was allowed to climb 13 cm above the origin. After allowing the plate to dry, the silica was scraped into a series of test-tubes from strips 1.5 cm wide starting at the origin (fraction 0) and continuing to the solvent front (fraction 8). Material in the silica was eluted with methanol. After centrifuging, the methanol was transferred to a second series of tubes in which it was evaporated to dryness. The dry material was then taken up in 1 ml of Krebs's solution and assayed on a rat stomach strip in plain Krebs's solution. The Chart shows the result of the assay. Substantial bioactivity occurred only in the position reached by 10 μ g of prostaglandin E₁ run as a marker on another part of the same plate and visualized by spraying with 10% phosphomolybdic acid in ethanol.

Thin layer chromatography of an extract of human aqueous and prostaglandins E₁, E₂, and F_{2α}. Bioactivity in the aqueous was detected by assaying segments of the chromatogram on a rat stomach strip as shown at the top of the figure; the standard prostaglandins were detected by spraying with phosphomolybdic acid as shown at the bottom.

It thus seemed that the traces of biological activity detected in human aqueous were due to prostaglandin E₁. The activity recovered from the plate was equivalent to 7.1 ng of prostaglandin E₂, implying a concentration in the original aqueous equivalent to 1.8 ng/ml. This was in good agreement with the value of 1.5–1.7 ng/ml obtained by direct assay. It was therefore unlikely that any other substance contributed to the activity in the untreated aqueous.

Discussion

The volumes of aqueous available from patients were too small to allow identification of the substance detected by the rat stomach strip. It is, however, probable that the activity was due to prostaglandin E₁ since this substance accounted for all the activity detected in aqueous from cadavers. Also, it seems unlikely that any well-known substance other than a prostaglandin could be present in aqueous in sufficient concentration to affect the strip, which has an exquisite sensitivity to E-type prostaglandins and (when blocked by hyoscine and methysergide) is insensitive to acetylcholine and 5-hydroxytryptamine. Bradykinin contracts the strip, but only in doses of the order of 1 μ g, and bradykinin could not account for the activity in cadaver aqueous since the R_f of small peptides in AII solvent is about 0.1. The strip is also relatively insensitive to histamine. The combined pharmacological and chromatographic evidence therefore make it likely that the activity detected was due to prostaglandin E₁. Since the strip is less sensitive to prostaglandin E₁ than to prostaglandin E₂, the actual concentrations of prostaglandin E₁ would be slightly higher than the values we give in the Table.

The two groups of patients were well matched for age; the mean age of glaucoma cases was 66, range 51–80, while the mean for the cataract cases was 70, range 60–83 years. The sex distribution was not so well matched, however; there were six men and two women in the glaucoma group, and three men and six women in the cataract group. Nevertheless, it seems unlikely that the difference between the groups can be explained purely on the basis of sex since none of the three men with cataract had particularly high values. Obviously, this difference between the groups could be due either to raised prostaglandin levels in glaucoma or to diminished levels in cataract. The former seems the more likely possibility since the two highest values in the glaucoma group were in patients before removal of secondary mature cataracts.

It is therefore likely that the higher levels in the glaucoma group were related to glaucoma and not to some other factor. This conclusion is strengthened by the fact that the two most severe cases of glaucoma gave the highest values. Raised prostaglandin levels in the aqueous in cases of glaucoma might indicate one of two things—the raised levels might be a cause of the chronically raised intraocular pressure or they might represent a reaction to the raised pressure or to drugs used in attempts to control it. One observation suggests that the second alternative can be rejected; the most clinically severe case of glaucoma in the series gave the highest prostaglandin activity (4.3 ng/ml) at a time when a filtration operation had reduced very high pressures to normal and the patient was not using drugs.

Our results therefore suggest that prostaglandin E₁ may be implicated as a causal factor in open angle glaucoma. If this is indeed so, prostaglandin antagonists may be useful in controlling the disease.

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PRELIMINARY COMMUNICATIONS

Polymorphonuclear Leucocyte Chemotaxis in Patients with Bacterial Infections

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Summary

A new in vitro method of measuring the chemotaxis of polymorphonuclear leucocytes from peripheral blood has been used to calculate a chemotactic index. The mean chemotactic index in 15 patients with bacterial infection (434) was significantly less ($P < 0.0005$) than in 15 normal controls (553) matched for age and sex. The reduction in chemotaxis could be correlated with the duration of the infection, with the greatest impairment being found in those patients with the shortest duration of infection. In five patients studied before and after appropriate therapy the chemotactic index returned to normal values with clearing of the infection. It is suggested that the impairment in chemotaxis may be due to prior phagocytosis of antibody-antigen complexes by the polymorphonuclear leucocytes.

Introduction

Chemotaxis is an important function of the polymorphonuclear leucocyte in the protection of the individual against invasive agents. Various methods have been used to study chemotaxis, but the technique devised by Boyden (1962) has proved to be the most used because of its simplicity and to some extent its reproducibility. A number of modifications of this technique have been made (Ward *et al.*, 1965; Ward, 1968), but though these simplify the method they do not alter the requirement for large numbers of cells. Consequently few studies of chemotaxis have been carried out on human cells, and investigations of polymorphonuclear leucocyte chemotaxis in patients with various disease states in which infection plays a part have been almost totally ignored. We have recently developed a simple reproducible method for studying chemotaxis of polymorphonuclear leucocytes permitting the use of small volumes of human peripheral blood (Baum *et al.*, 1971) and have compared the results in patients with bacterial infection and in normal controls.

Material and Methods

The 15 patients with bacterial infection were inpatients in Strong Memorial Hospital who had had a variety of infections lasting from 8 months to 3 days without any other overt disease. Most of the patients were already receiving appropriate antibiotic therapy, but in five (Cases 5, 6, 7, 8, and 13) it was possible to study the polymorphonuclear leucocyte chemotaxis before and after treatment with antibiotics. The age of the patients ranged from 18 to 85 years. The normal subjects, who were matched for age and sex with the patients, were members of the laboratory staff and healthy residents of a home for the aged. The mean age of the patients was 53.2 years and of the normal controls 53.8 years (see Table).

The method for studying the chemotaxis of polymorphonuclear leucocytes, which has been described in greater detail elsewhere (Baum *et al.*, 1971), utilized a Sykes-Moore tissue culture chamber (Belco Glass Company, Vineland, New Jersey) which was modified by the design of a new gasket. The chamber was assembled with a 25 mm round cover slip, the gasket, a 25 mm 3 μ m Millipore filter (Millipore Filter Corporation, Bedford, Massachusetts), another gasket, a 25 mm cover slip again, and then closed with the screw top. Ports in the side wall of the chamber enabled the compartments above and below the Millipore filter to be filled with various solutions.

Polymorphonuclear leucocytes were obtained from peripheral blood. Ten millilitres of blood was drawn into a heparinized tube, 0.75 ml of a 2% solution of methyl cellulose in normal saline was added, and the tube was gently inverted. The methyl cellulose caused the rapid sedimentation of red blood cells and mononuclear leucocytes (Böyum, 1968). After standing for 30-45 minutes the plasma layer, which then contained a concentration of polymorphonuclear leucocytes 20-40% higher than in the peripheral blood, was removed and diluted with approximately one-half its volume of Hank's solution. The cells from four or five drops of this cell suspension were deposited on a circumscribed area of a 3 μ m Millipore filter by means of the Shandon cytocentrifuge (Shandon Scientific Company, Sewickley, Pennsylvania). The filter was placed in the Sykes-Moore chamber, and after filling both compartments of the chamber (approximately 0.5 ml each) this was incubated for three hours at 37°C. After incubation the filters were stained by Boyden's (1962) method, trimmed, and mounted.

Cell viability was studied by using the trypan blue dye exclusion method after centrifugation and after incubation, and better than 99% of the cells from both normal controls and patients with bacterial infection were found to be viable at both times.

Since in other techniques some of the cells entering the filter may have done so owing to inherent motility of the polymorphonuclear leucocytes as well as by chemotactic direction, we eliminated as much of the effects of motility as possible by counting only those cells which had completely penetrated the filter and were present on the bottom or attraction surface of the membrane. This was done by focusing beyond any cells seen and then counting only those cells which appeared first in focus as the membrane was approached. The same technique

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