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Drugs Which Inhibit Prostaglandin Biosynthesis

RODERICK J. FLOWER[1]

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, London, England

I. Introduction

ALMOST 40 years have elapsed since von Euler coined the name "prostaglandin" to describe the pharmacologically active principles of seminal fluid (42), yet it is only within the last decade or so that the chemistry and pharmacology of these compounds has been systematically and extensively investigated, and their potential significance realised. The extraordinary biological activity of the prostaglandins has attracted many speculations concerning the possible function of these compounds, but ironically it has been impossible to establish their precise function in the "physiologically normal" organism. Experimental work along these

[1] Present address: Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, England.

lines has been handicapped by the discovery that even mild physical manipulation of tissue is sufficient to provoke a synthesis of prostaglandins which would not occur under "resting" conditions; furthermore, the ubiquitous nature of the prostaglandin generating enzymes ("prostaglandin synthetase") has rendered classical endocrinological techniques (such as removal or ablation of a particular organ or tissue) less useful. A pharmacologist's solution to such a problem might be the use of specific antagonists of prostaglandins or specific inhibitors of prostaglandin synthesis. Of these two approaches, the latter has proved to be the more promising.

The observation that prostaglandins are synthesised from C-20 "essential" fatty acids led to the discovery of several substrate analogues which were potent inhibitors, and during studies on the enzyme system *in vitro*, it was recognized that several antioxidants were also inhibitory but these, on the whole, have not been used for *in vivo* work. In 1971 a significant development occurred when Vane (151), Ferreira *et al.* (46) and Smith and Willis (136) demonstrated that the so-called "aspirin-like" drugs also inhibited enzymic synthesis of prostaglandins, and suggested that this effect was the basis of their therapeutic action. This discovery has had at least two important consequences; it has led to a most comprehensive hypothesis which explains the apparently unrelated therapeutic actions of the aspirin-like drugs (this aspect of the synthetase inhibition will not be considered here; see section II for references) and also to the recognition that the aspirin-like drugs are potent, cheap and readily available inhibitors of prostaglandin biosynthesis, which could be of great assistance not only to the physiologist concerned with investigating the function of prostaglandins *in vivo*, but also to the biochemist who wishes to study the biosynthetic pathways of these fatty acids and, in view of the growing body of evidence implicating prostaglandins in the pathogenesis of certain disease states, to the clinician also.

In presenting this paper the author has three main points in mind: firstly, to provide for the first time a comprehensive review of all types of prostaglandin synthetase inhibitors (although special emphasis is given to the aspirin-like drugs); secondly, to indicate which sorts of inhibitors are the most suitable for investigating the functions of prostaglandins in biological control systems; and thirdly, to stimulate further interest in this field and to suggest some possible lines of future research. Although a relatively new concept, the literature on this subject is already extensive and I have included only that work in which a decrease in prostaglandin synthesis or release has actually been demonstrated directly, rather than implied, after the administration of a putative inhibitor.

I have also included a small section on inhibition of prostaglandin catabolism, although relevant published data are few at present.

II. Other Reviews

Several other reviews are available which deal, at least in part, with topics covered here. The use of inhibitors of prostaglandin biosynthesis as "tools" is briefly discussed by Hinman (73), Weeks (158) and Samuelsson (129). Lands *et al.* (90) have provided a useful paper in which various aspects of synthetase inhibition (chiefly by fatty acids and antioxidants) are discussed. The experimental work which culminated in the discovery of synthetase inhibition by aspirin-like drugs has been described by Vane and Ferreira in several reviews (43, 48, 49, 152–155) in which they develop the thesis that aspirin-like drugs exert their therapeutic action by inhibition of prostaglandin generation *in vivo*. Also included in these reviews are valuable discussions on the use of synthetase inhibitors as experimental tools, and the clinical aspects of synthetase inhibition.

III. Nomenclature

In accordance with standard practice, the following abbreviations have been adopted:

11α-15-dihydroxy-9-ketoprost-13-enoic acid, PGE_1
11α-15-dihydroxy-9-ketoprosta-5,13-dienoic acid, PGE_2
9α,11α-15-trihydroxyprost-13-enoic acid, $PGF_{1\alpha}$
9α,11α-15-trihydroxyprosta-5,13-dienoic acid, $PGF_{2\alpha}$
15-hydroxy-9-ketoprosta-10,13-dienoic acid, PGA_1
15-hydroxy-9-ketoprosta-5,10,13 trienoic acid, PGA_2
15-hydroxy-9-ketoprosta-8(12),13 dienoic acid, PGB_1
15-hydroxy-9-ketoprosta-5,8(12),13-trienoic acid, PGB_2
9α,15-dihydroxy-11-ketoprosta-13-enoic acid, PGD_1
9α,15-dihydroxy-11-ketoprosta-5,13-dienoic acid, PGD_2

The numbering system for fatty acid carbon skeletons is as follows: each carbon is numbered consecutively along the chain beginning with the carboxyl terminal (hence the carboxylic carbon is C-1), the carbon most remote from C-1 is referred to as the ω-carbon. Prostaglandins which may be considered to be derivatives of prostanoic acid are numbered likewise (see fig. 1). Occasionally it is desirable to indicate a specific position relative to the ω-carbon. In this case the ω-carbon is designated C-1, and the carbon atoms numbered consecutively "backwards" along the chain, the carboxyl carbon being the most distant. Thus, when referring to a 20 carbon fatty acid C-1 (carboxyl carbon) is equivalent to ω-20, C-2 to ω-19, *etc.*

The following abbreviations are sometimes used when describing the nature and geometry of carbon-carbon bonds in fatty acids:

c = cis, *t* = trans } —c=c— (ethylenic bonds)
a = —c≡c— (acetylenic bonds)

IV. Chemistry of Prostaglandin Biosynthesis

It seems appropriate to preface this paper with a brief discussion of prostaglandin biosynthesis itself. Several detailed reviews are available on this subject (13, 37, 73, 88, 128, 129, 130, 133, 143) and the reader is referred to these (particularly 128, 129, 133) for a more complete account, as well as for a comprehensive bibliography.

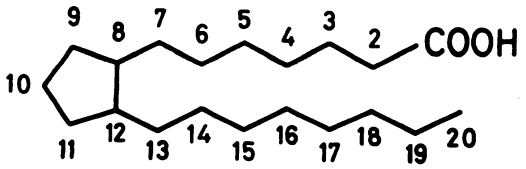


FIG. 1. Prostanoic acid numbering scheme.

The enzymic conversion of certain essential fatty acids into prostaglandins (PGs) was demonstrated in 1964 by two groups of workers led by van Dorp *et al.* (38) in Holland, and Bergström *et al.* (12, 14) in Sweden. Most of the subsequent biochemistry of prostaglandin synthesis has also been pioneered by these two groups. Although much of the original experimental work on the reaction mechanism was conducted with the synthetase from sheep seminal vesicles, there is no reason to suspect that the reaction catalysed by enzymes from other tissues proceeds by a radically different pathway.

The multi-enzyme complex referred to as "prostaglandin synthetase" is located in the high-speed particulate fraction of cells, but may be partially solubilised with non-ionic detergents (133). Although the number of component enzymes in the system is not known, the actual mechanism of the reaction is reasonably well elucidated. The initial step (67, 108; fig. 2) of this dioxygenase reaction is initiated by the stereospecific (L) removal of the (ω-8) hydrogen and the conversion of the substrate to an (ω-10) hydroperoxide. This step is reminiscent of the reaction catalysed by the plant enzyme, soyabean lipoxidase, although in that case an (ω-6) hydroperoxide is formed. The next stage is a concerted reaction; the addition of oxygen at C-15 is followed by isomerisation of the C-13 double bond, ring closure between C-8 and C-12 and attack by the oxygen radical (of the C-11 hydroperoxide) at C-9 thus forming a "cyclic endoperoxide."

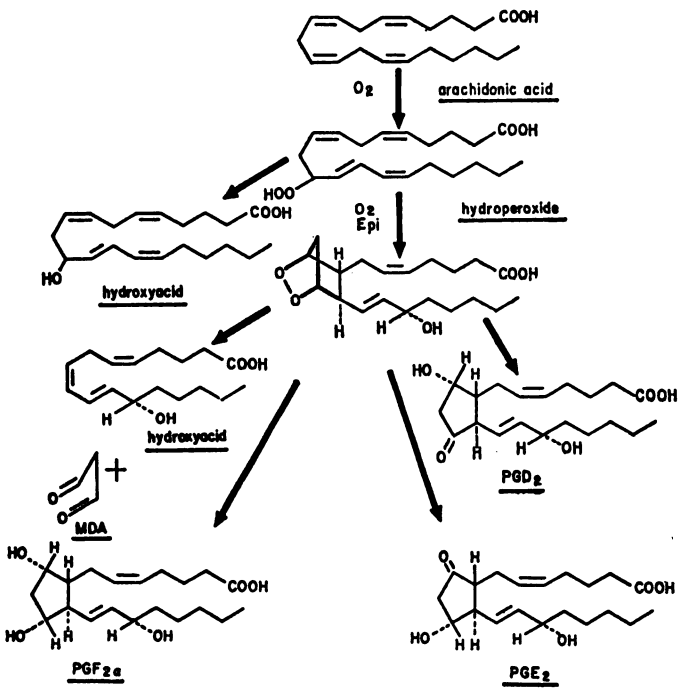


FIG. 2. Proposed reactions during oxygenation of arachidonic acid by the prostaglandin synthetase system of bovine seminal vesicles. This figure taken from Flower *et al.* (51), is based upon the work of Nugteren *et al.* (108) and Hamberg and Samuelsson (67). The abbreviations used are Epi, epinephrine; MDA, malondialdehyde; PGD_2, prostaglandin D_2 or 11-dehydro-$PGF_{2\alpha}$. See text for explanation.

This intermediate (which is most likely somewhat unstable) may be further modified in a number of ways, by "isomerisation" to PGE or PGD, or by reductive cleavage to PGF. Under some conditions the endoperoxide may break down into a 17-carbon hydroxy acid and malondialdehyde (MDA). Altogether 2 moles of oxygen are consumed per mole of product formed, and experiments in which the reaction mixture was incubated in an atmosphere of $^{18}O_2$–$^{16}O_2$ have indicated that the oxygen substituents at C-9 and at C-11 are both derived from the same molecule.

PGA and its isomer PGB (not shown in fig. 2) are dehydration products of PGE and are easily formed by treatment of PGE with acid or base, respectively. It seems likely that much of the PGA which is often detected in tissues and enzyme incubations could have been formed non-enzymically during extraction and isolation procedures (92, 128), which commonly involve acidification, although this prostaglandin does appear to occur naturally in seminal fluid. Despite numerous reports in the literature of the biological activity of PGA (see reviews 72, 75, 158) and its metabolic transformations (77, 82), the existence of an enzyme catalysing the conversion of PGE to PGA—"PGE dehydratase"—has not been unequivocally demonstrated, although there have been some interesting preliminary reports (26, 126). In the light of the foregoing considerations it is difficult to assign a definite role to A (or B) type prostaglandins at the moment. One may conclude, however, that unless some completely novel biosynthetic pathway is reported, PGE is most likely to be the precursor of PGA; hence if the synthesis of PGE is inhibited, one may assume that the synthesis of PGA would also be blocked.

Although prostaglandin synthetase is located in the membraneous fraction of cells, a heat stable cofactor(s) from the soluble fraction is required for appreciable activity

(127, 128). For biosynthetic studies *in vitro*, however, this cofactor is generally replaced by a source of reducing equivalents (often hydroquinone, epinephrine or some other phenolic compound) and reduced glutathione (GSH) (36, 108). Two moles of reducing equivalents are required for the synthesis of PGF but only 1 mole for PGE or PGD formation (133). The requirement for GSH seems to be rather specific (several other thiol compounds are inactive; 36, 133) although the exact role played by this compound in the synthetic reaction is not yet clearly established; possibly it may simply serve to keep the electron donor in a reduced state.

The prostaglandins formed from 8,11,14-eicosatrienoic acid (di-homo-γ-linolenic acid) are denoted by the suffix "1" (*i.e.*, E_1, F_1, A_1, B_1), likewise, if 5,8,11,14-eicosatetraenoic acid (arachidonic acid) is the substrate, they are given the suffix "2," and if they are derived from 5,8,11,14,17-eicosapentaenoic acid, the suffix "3." Only the non-esterified fatty acids are substrates for the enzyme (91) and since the cellular concentration of free fatty acid precursors is generally rather low, it follows that the activity of lipolytic enzymes such as phospholipase may be important in regulating the supply of substrate to the enzyme (87, 127).

V. Distribution of Prostaglandin Synthetase

Enzymes which synthesise prostaglandins appear to be present in every mammalian tissue so far investigated, and several authors (75, 122) have published extensive lists of tissues from which prostaglandin release has been demonstrated after the application of a suitable stimulus.

Although present in many tissue types, the activity in each tissue varies greatly. Christ and van Dorp (28, 29) have systematically investigated the activity of the synthetase from a wide range of tissues, including those from vertebrates, arthropods, molluscs and coelenterates. In mammalian tissues (with the exception of sheep and ox seminal vesicles) the biosynthetic capacity (as measured by the conversion of tritiated di-homo-γ-linolenic acid to PGE_1) fell into three broad catagories; tissues such as kidney (medulla), and lung in which between 10 to 40% conversion occurred, tissues such as gut in which only about 3% occurred, and other tissues such as spleen and aorta in which the conversion was 1% or less.

The synthetase was found in tissues from other vertebrates (carp gills and frog lung) and from members of other phyla (especially in the gills). These authors have pointed out the importance of distinguishing between low enzymatic conversion (less than 2%) and auto-oxidation of the substrate.

The only tissues studied which possess high activity (~75% conversion) are sheep and bovine (ox) seminal vesicles (SSV and BSV enzymes); for this reason the majority of biosynthetic studies have been conducted with these tissues as a source of enzyme.

VI. Inhibition of Prostaglandin Biosynthesis by Substrate Analogues and Other Fatty Acids

One obvious approach in the search for inhibitors of any enzyme is to synthesise analogues of the natural substrate. Various fatty acid derivatives have been prepared and tested against the enzyme system *in vitro* and also in a few whole tissue preparations. The results of this work are summarised in table 1, whilst table 2 contains some structural and kinetic data. (For the sake of convenience, the structures of these fatty acids are drawn in a somewhat artificial manner.)

Ahern and Downing (3) demonstrated a time-dependent inhibition of sheep vesicular gland enzyme by an acetylenic analogue of arachidonic acid, eicosa 5,8,11,14-tetraynoic acid (5*a* 8*a*, 11*a*, 14*a* 20:4, TYA). They reported that inhibition was irreversible, and that the acid was not a substrate for the enzyme system. In addition to a direct action on the enzyme itself, TYA also prevented the hydroxylation (hence inactivation) of linoleic and linolenic acids present in the incubation

TABLE 1

Inhibition of prostaglandin synthetase by some fatty acid derivatives

Prostaglandins Synthesised or Released by:	Fatty Acid Derivatives		Concentration	Inhibition	Reference
			μM	%	
E_2 synthesis by SSV enzymes	5*a* 8*a* 11*a* 14*a*	20:4	4.0	75	3, 40
Release from isolated perfused rabbit heart in response to nerve stimulation	5*a* 8*a* 11*a* 14*a*	20:4	1.0–5.0	100	131
E_2 synthesis by powders of frozen guinea-pig ileum	5*a* 8*a* 11*a* 14*a*	20:4	5.0	50	35
PG production by SSV enzymes	8*c* 12*t* 14*c*	20:3	4.0	≃80	107
	5*c* 8*c* 12*t* 14*c*	20:4	4.0	≃80	
Release of PGs and RCS by isolated perfused guinea-pig lungs	8*c* 12*t* 14*c*	20:3	14.0	100	115
	5*c* 8*c* 12*t* 14*c*	20:4	14.0	100	
E_2 synthesis by SSV enzymes	9*a* 12*a*	18:2	45	96	41
	9*a* 12*c*	18:2	45	85	
	9*a* 12*t*	18:2	45	56	
	9*t* 12*a*	18:2	45	75	
E_2 and $F_{2\alpha}$ synthesis by BSV enzymes	5-Oxaprost-13-*trans*-enoate		150	66–100	103
E_2 and MDA formation by dog spleen particulate fraction	8*c* 12*t* 14*c*	20:3	700.0	≃80	51
	5*c* 8*c* 12*t* 14*c*	20:4	700.0	≃50	
E_1 synthesis by SSV enzymes	Bicyclo (2.2.1) heptene derivative		900.0	35–52	162
E_2 synthesis by SSV enzymes	9*c*	18:1	1800.0	≃40	110
E_2 synthesis by SSV enzymes	9*c* 12*c*	18:2	1800.0	≃40	
E_2 synthesis by SSV or rat stomach enzymes	9*c* 12*c* 15*c*	18:3	1800.0	≃80	
E_1 and E_2 synthesis by SSV enzymes		10:0	5000	100	157
	9*c* 12*c* 15*c*	18:3	5000	12	
	9*c* 12*c*	18:2	5000	5	
	9*c*	18:1	5000	4	

mixture and, since these acids themselves are inhibitors of the enzyme, the authors concluded that this inhibitor has a dual mechanism of action. Interestingly, the fatty acid also inhibited the plant enzyme lipoxidase (40), the mechanism of action of which has certain features in common with prostaglandin synthetase. Eicosatetraynoic acid also blocks prostaglandin output by the isolated perfused heart (131), spleen, vas deferens and seminal vesicle (70), in response to sympathetic nerve stimulation and also prostaglandin synthesis by powdered guinea-pig ileum tissue (35).

In a later paper Downing *et al.* (41) noted that the initial reaction of prostaglandin biosynthesis (removal of the ω-8 hydrogen) is also common to both microsomal hydroxy acid formation (in SSV preparations) and to hydroperoxide formation by soyabean lipoxidase. They speculated that the inhibitory action of the acetylenic analogue arose when enzymic removal of the ω-8 hydrogen led to formation of an allene which then reacted irreversibly with the enzyme. To check this hypothesis and establish further structural requirements for synthetase inhibition, the authors tested the anti-synthetase (and anti-lipoxidase) activity of a wide range of 18-C fatty acids having either two acetylenic, or one acetylenic and one ethylenic bond, at different positions in the hydrocarbon chain. Octadeca-9,12-diynoic acid (9*a*, 12*a* 18:2) was found to be a potent inhibitor of both the SSV synthetase and the soyabean enzyme, but none of the other 10 diynoic acids, nor any of the 15 positional isomers of the active analogue inhibited either enzyme. A group of four compounds containing one acetylenic and one (*cis* or *trans*) ethylenic

bond at C-9 or C-12 failed to inhibit the soyabean enzyme, but only one was without activity against PG synthetase. The authors concluded that the results were consistent with the hypothesis that the ω-8 methylene group was important for inhibition, although they could not account for the fact that one of the compounds, the 9*c*, 12*a* isomer—apparently the naturally occurring form—was inactive.

Further studies of this nature were pursued by Lands *et al.* (89, 90) and by Vanderhoek and Lands (149). This group has reported that TYA exhibits two distinct types of inhibitory activity, an instantaneous concentration dependent effect (confirming the earlier results of Ahern and Downing) and a time-dependent destruction of the enzyme; in the case of the latter action, incubation of the enzyme with TYA resulted in a progressive and irreversible decrease in catalytic activity. The acid was not a substrate for the enzyme since no oxygen uptake was detected during the incubation, although oxygen was apparently essential for inhibitory activity since, under anaerobic conditions, no time dependent loss of activity occurred. When the experiment was repeated under aerobic conditions, but in the presence of the enzyme inhibitor, diethyldithiocarbamic acid, (which also inhibits substrate oxygenation) no destructive activity was observed either. Experiments in which the enzyme and TYA were incubated with glutathione peroxidase, a synthetase inhibitor which apparently acts by reducing the hydroperoxide intermediate (see fig. 2 and section VIII), have established that the lipid peroxide intermediate is also required for inhibition. Three other acetylenic inhibitors were found which also exhibited instantaneous, competitive and reversible behaviour as well as time dependent irreversible destruction; the K_I values for the competitive-reversible phase may be found in table 2 together with similar values for some other acids.

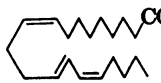
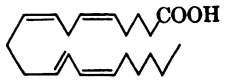
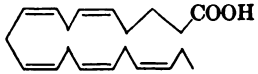
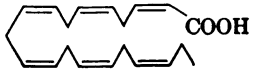
In a summary of their work, Lands' group suggests that acetylenic compounds inhibit prostaglandin synthetase by destruction of the catalytic site and liken this action to "an ultramicrosurgical removal of an enzyme." Presumably by titrating the amount of enzyme against fixed concentrations of TYA it would be possible to determine the number of catalytic centres per mg of microsomal protein.

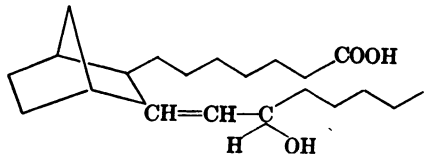
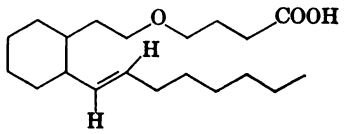
Among a number of fatty acids screened for inhibitory activity against the sheep vesicular gland enzyme, Nugteren (107) discovered two derivatives possessing unusual potency, a 12-*trans* analogue of di-homo-γ-linolenic acid (8*c*, 12*t*, 14*c*, 20:3) and a 12-*trans* analogue of arachidonic acid (5*c*, 8*c*, 12*t*, 14*c* 20:4). These compounds are active in some whole tissue systems, including the conversion of arachidonic acid to prostaglandins and rabbit aorta contracting substance (RCS; 63, 64, 117) by isolated perfused guinea-pig lungs (115), as well as the production of prostaglandins and malondialdehyde by a synthetase from dog spleen *in vitro* (51). In the former case, the block was only maintained during infusion of the inhibitor through the lungs; 20 min after the infusion was discontinued full activity of the enzyme was restored. On the basis of some kinetic studies, Nugteren (107) concluded that these analogues are competitive inhibitors; neither were attacked by the enzyme system, and both could be recovered unchanged at the end of the reaction. The di-homo-γ-linolenic acid analogue was tested against plant lipoxidase and found to be inactive.

Oleic (9*c* 18:1), linoleic (9*c*, 12*c*, 18:2) and linolenic (9*c*, 12*c*, 15*c* 18:3) acids are reported to be inhibitors of the synthetase from sheep seminal vesicles and also of the enzyme in acetone powders of rat stomach, but the concentrations required for inhibition are high (1.8–5.0 mM); Pace-Asciak and Wolfe (110) noted that pre-incubation with the fatty acid results in a greater degree of inhibition, especially at low substrate concentrations; linolenic acid was the most potent although the methyl ester was inactive. Linolenic acid was also amongst several fatty acids which inhibited the conversion of arachidonic acid to PGE_2 by enzymes in

TABLE 2

Structural and kinetic data for some inhibitory fatty acids

Structure	Designation	K_I	Nature of Inhibition	Enzyme Source	Reference
		μM			
	8c 12t 14c 20:3	0.12	Competitive	SSV	107
	also 8c 12c 17c analogue	6.0	Competitive	SSV	90
	5c 8c 12t 14c 20:4		Competitive	SSV	107
	5a 8a 11a 14a 20:4 (TYA)	2.5	Mixed*	SSV	3, 4, 149
	9c 18:1 (oleic acid)	22	Competitive?	SSV	90, 11
	also 10a analogues	10	Mixed*	SSV	149
	13a analogues	25		SSV	
	9c 12c 18:2 (linoleic acid)		Mixed*	SSV	90, 11
	also 9a 12a			SSV	41
	9a 12c analogues		Mixed*	SSV	149
	9a 12t analogues			SSV	
	9t 12a			SSV	
	9c 12c 15c 18:3 (linolenic acid)	15	Mixed*	SSV	90, 110
	5c 8c 11c 14c 17c 20:5	2.5	Competitive	SSV	90
	2c 5c 8c 11c 14c 17c 20:6	1.7	Competitive	SSV	90

	Bicyclo 2.2.1 heptenes		Competitive	SSV	162
	-Oxa-prost-13t-enoate	32.0	Competitive	BSV	103

* These acids have a "competitive irreversible effect." See text for details.

human skin (166). According to these authors as well as Lands *et al.* (90), inhibition by linoleic and linolenic acid is of the same biphasic nature as TYA inhibition.

Wallach and Daniels (157) observed that E_1 and E_2 synthesis by acetone-pentane powders of SSV microsomes was competitively inhibited by decanoate; complete inhibition was seen at a concentration of 5 mM. Caprylic, nonanoic and lauric acids were without effect, indicating some degree of specificity. Decylamine, decyl alcohol, decylaldehyde and decane were also inactive.

An ingenious approach to the problem of synthetase inhibition was made by Wlodawer *et al.* (162); rather than synthesising substrate analogues, they synthesised a series of compounds which were structurally related to the cyclic endoperoxide postulated by Nugteren *et al.* (108) and, Hamberg and Samuelsson (70) to be a common intermediate in the synthesis of E and F type prostaglandins (see fig. 2). When tested against the sheep vesicular gland enzyme, one of these bicyclo (2:2:1) heptene derivatives possessed the interesting property of inhibiting the enzymes controlling the breakdown of the intermediate to PGE_1 but not those responsible for $PGF_{1\alpha}$ formation; in a concentration of 0.9 mM PGE_1 formation was inhibited 35 to 52% but $PGF_{1\alpha}$ formation was unaffected.

Some prostaglandin-like compounds also block synthesis. McDonald-Gibson *et al.* (103) tested a series of racemic analogues of 5- or 7-oxaprostaglandin derivatives for antisynthetase activity. Five of the nine analogues displayed inhibitory activity although the percentage inhibition measured by a radiochemical technique was very different from the corresponding figures obtained by bioassay; the most potent of these, 5-oxaprost-13-*trans*-enoate (90% inhibition of PGE_2 and $PGF_{2\alpha}$ synthesis at 150 μM), was a competitive inhibitor. The remainder of the analogues appeared to increase prostaglandin synthesis. The authors observed that none of the inhibitors had ketonic or hydroxylic substituents at the C-9 or C-11 position of the ring, and also that they lacked the 15-OH group; ring size was unimportant for inhibitory activity.

In summary, several different types of fatty acids inhibit prostaglandin biosynthesis: acetylenic analogues of the substrates, as well as some other naturally occurring fatty acids exhibit a biphasic inhibition which ultimately leads to irreversible destruction of the catalytic site, whereas positional isomers of arachidonic and di-homo-γ-linolenic acid competitively inhibit the enzyme reversibly; also inhibitory are prostaglandin analogues. Analogues of the cyclic endoperoxide intermediate block PGE but not PGF formation. Lands *et al.* have formulated the kinetics of the vesicular gland oxygenase reaction (90) and have noted the existence of a product (as well as a substrate) binding site on the enzyme; this could explain why some prostaglandin analogues are inhibitory.

These fatty acid derivatives are of value in determining the substrate specificity requirements of the synthetase and, especially in the case of the bicyclic acids, in elucidating the reaction mechanism; but even though some of the analogues have been shown to be active *in vivo*, their use as "tools" to investigate specific functions of prostaglandins has not been widespread. This is probably due in part to the uncertain metabolic fate of these compounds and to the lack of availability and difficulty of preparation.

VII. Inhibition of Prostaglandin Biosynthesis by Aspirin-like Drugs and Other Pharmacological Agents

A. General Remarks

The term "aspirin-like" drugs is a pharmacological one; although the drugs which comprise this group are of diverse chemical structures, they all share (to a varying degree) the antipyretic, analgesic and anti-inflammatory actions which are characteristic of aspirin. The steroidal anti-inflammatory drugs and the narcotic analgesics which have some actions in common with the

aspirin-like drugs, are thus specifically excluded by this definition.

In 1971 aspirin-like drugs were simultaneously shown to inhibit prostaglandin release from human platelets by Smith and Willis (136), prostaglandin release from the perfused dog spleen by Ferreira *et al.* (46) and prostaglandin synthesis in cell-free homogenates of guinea-pig lung by Vane (151). In a typical experiment of the last type, guinea-pig lungs were prepared according to the method of Änggård and Samuelsson (7); after homogenisation in a modified Bucher medium and centrifugation at 900 × *g* for 15 min, the supernatant was decanted and mixed with arachidonic acid and various concentrations of aspirin, salicylic acid or indomethacin. After a 30-min incubation, extraction, and in some cases separation by thin-layer chromatography, the $PGF_{2\alpha}$ content was bioassayed with the superfused rat colon preparation. Plotting the log-dose response curves, Vane calculated the following I50 concentrations; indomethacin 0.75 μM, aspirin 35 μM and salicylate 750 μM.

B. Inhibition of Synthesis

Inhibition by these and other aspirin-like drugs has now been demonstrated in some 30 different systems (see table 3) which, for the purposes of this review, have been divided into three categories; inhibition of synthesis in tissue homogenates or subcellular fractions, inhibition of synthesis in isolated "organised" tissues and inhibition of synthesis in whole animals or man.

I shall deal with each category separately.

1. Homogenates and subcellular fractions. Almost all the aspirin-like drugs tested are potent inhibitors of prostaglandin synthetase. Although the original experiments were performed with cell-free homogenates of guinea-pig lungs (151), these drugs are effective against virtually every synthetase preparation so far investigated, even though their relative potencies vary.

"Microsomal" preparations of tissue homogenates have been widely used, since the synthesising enzymes are located in this fraction, whilst the most important inactivating enzymes are located in the soluble fraction; another advantage of such preparations is the relatively high specific activity. In the first experiments prostaglandin synthesis was quantitated biologically, but subsequent workers have employed a variety of techniques including radiometric (51, 65, 147, 165), spectrophotometric (51, 144) and polarographic (138) assays as well as immunochemical techniques (22, 95) and gas-liquid chromatography-mass spectrometry (34, 66, 68). The basic findings are similar regardless of the assay used.

TABLE 3

Summary of systems in which prostaglandin synthesis is inhibited by aspirin-like drugs

Species	Tissue	References
Man	Platelets, semen, skin, urinary metabolite, synovium cells	31, 76, 124, 136, 166
Bull	Thyroid cells, seminal vesicles	22, 51, 144, 147
Sheep	Seminal vesicles	65, 138
Dog	Brain, spleen, kidney, myocardium	5, 32, 46, 52, 53, 71
Cat	Cerebrospinal fluid, spleen, kidney	45, 104, 140
Rabbit	Heart, brain, spleen, retina, gut, polymorphonuclear cells, kidney	15, 17, 34, 44, 53, 63, 78, 102, 105
Guinea-pig	Uterus, lungs, urinary metabolite	71, 111, 117, 120, 142, 151
Rat	Skin, uterus, inflammatory exudate	4, 61, 156, 161,
Mouse	Brain, tumour cells	95, 142, 161
Toad	Bladder	163

In some cases I50 data for these drugs has been published allowing a direct comparison of activity (these values are given in table 4) and it is possible to deduce the following order of (decreasing) potency; meclofenamic acid > niflumic acid or indomethacin > mefenamic acid > flufenamic acid > naproxen > phenylbutazone > aspirin or ibuprofen. This order of potency is, generally speaking, consistent with all the data so far published regardless of the source of the synthetase although some minor variations

TABLE 4

*Inhibition of prostaglandin synthesis in homogenates or subcellular fractions—I50 concentrations of some aspirin-like drugs**

Enzyme Preparation	Prostaglandin	Meclofenamic Acid	Niflumic Acid	Indomethacin	Mefenamic Acid	Flufenamic Acid	Naproxen	Phenylbutazone	Aspirin	Ibuprofen	Reference
Cell-free guinea-pig lung homogenate	$F_{2\alpha}$			0.75					35.0		151
	$F_{2\alpha}$			0.3							142
Dog spleen microsomes	E_2	0.1	0.11	0.17	0.71			7.25	37.0		52
Rabbit brain homogenates	E_2			3.6					61.0		53
Rabbit kidney microsomes	E_2	1.4		3.9				19.5	2500		17
	$F_{2\alpha}$	1.35		3.7				15.0	2800		
BSV enzymes	E_2			7.0			100		15000		147
	Total PGs			2.0	15.0	48.0	220	420	820	1200	144
	E_2	13.0		38.0			370	1400	9000	2000	51
	$F_{2\alpha}$	17.0		30.0			490	1200	10000	2300	51
SSV enzymes	E_2		1.21	0.45	2.1	2.5	6.1	12.6	83.0	1.5	65
	Total PGs								9000		138

* I50 concentrations are expressed as μM.

have been reported (65). That inhibition of prostaglandin formation is a property peculiar to the aspirin-like drugs is suggested by the fact that many other pharmacologically active compounds are inactive (in concentrations of 1.0–5.0 mM) against the enzyme (62). This ineffective group includes morphine, fentanyl, droperidol, chlorpromazine mepyramine, phenergan, mepacrine, primaquine, atropine, methysergide, phenoxybenzamine, propranolol, mersalyl, azathioprine, iproniazid and disodium cromoglycate. Two salicylic acid isomers (which have no therapeutic effect) *m*- and *p*-hydroxybenzoic acid are almost devoid of activity. Various enantiomeric pairs of anti-inflammatory drugs have been tried, in each case the clinically active isomer has been found to be more potent than its partner (see section VII G). Chloroquine is active (61, 62), but only at high doses (50% inhibition at 500 μM). Flower *et al.* (52) reported that aldosterone, triamcinolone, hydrocortisone and fludrocortisone were inactive against dog spleen microsomes in concentrations of up to 280 μM. Greaves and McDonald-Gibson (60), however, reported a 50% inhibition of synthesis in rat skin at the higher concentrations, and Maddox (100) has reported that another steroidal drug, fluocinolone acetonide inhibits E_2 synthesis (SSV enzymes) by 55% at 400 μM, although the extent of inhibition depends on the composition of the reaction mixture.

Paracetamol (4-acetamidophenol) was found to be inactive against the dog spleen or rat skin synthetase (53, 61), but it showed good activity against synthetase preparations from dog, rabbit (53), mouse or gerbil brain tissue (161). The intriguing question of whether the differences in potency of this drug may be attributed to differences in the synthetase enzymes themselves will be discussed in section VII C.

Several authors who used homogenates or subcellular fractions have published kinetic data (see table 5, and section VII E for further comment), the most complete list so far being that of Ku and Wasvary (86).

Several other systems in which aspirin or indomethacin have been found effective, but which are not mentioned in table 4 include

TABLE 5

*Kinetic data for some aspirin-like drugs**

Aspirin-like Drug	K_I μM	Nature of Inhibition†
SU-21524	1.2	C, R
Meclofenamic acid	2.6	C, NR
Mefenamic acid	3.2	C, NR
Indomethacin	6.5	C, NR
Oxyphenbutazone	810	C, R
Phenylbutazone	860	C, NR
Aspirin	8200	C, NR

* Data from Ku and Wasvary (86) reproduced with the permission of the authors.

† C, Competitive; R, reversible; NR, non-reversible.

guinea-pig uterus homogenates [62–77% inhibition of E_2 and $F_{2\alpha}$ production by indomethacin at 14 μM (120)], and mouse tumour cells homogenates [80% inhibition of E_2 synthesis by indomethacin at 15 μM (95, 142)]. Indomethacin was a strong inhibitor of E_2 and $F_{2\alpha}$ production by microsomal preparations or homogenates of rabbit spleen (15), kidney medulla (15, 78) and had variable activity against rabbit ocular tissues (15). Both aspirin and indomethacin inhibited the production of E_2 by human skin (166).

Amongst other miscellaneous compounds which inhibit the enzyme is Δ^1 tetrahydrocannabinol, which gave partial inhibition of E_2 synthesis in a concentration of 10 μM (25). Several other naturally occurring cannabinoids also inhibit the enzyme system (24), the inhibitory activity apparently resides in the aromatic portion of the molecule. Phenelzine and quinidine are reported to be active against the enzyme from rabbit kidney medulla (78). Maddox (100) has reported that fenclozic acid inhibits SSV enzymes (I50 approximately 400 μM) and Ku and Wasvary (86) have reported inhibition of SSV enzymes by a new anti-inflammatory compound designated SU-21524 (2-(3-chloro-4-(3-pyrrolin-1-yl)-phenyl)-propionic acid).

Aspirin, in concentrations which almost completely inhibit the dog spleen synthetase, is reported to be ineffective against the PG synthetase from canine myocardium (98).

Inhibition by aspirin or indomethacin in acetone powders of SSV glands (138) was time dependent as preincubation with the drug for 5 to 10 min was required before maximal inhibition was achieved.

2. Isolated tissues (see table 6). When a certain degree of cellular organisation is present, the problem of quantification of the synthesis of prostaglandins becomes more difficult, and hence the data are less reliable. This is partly due to the presence of prostaglandin metabolising enzymes in the cells, and also because one generally has to rely on prostaglandin release as an indirect measure of cellular synthesis, even though this procedure is generally justifiable [since cells do not store prostaglandins, release is in most cases equivalent to *de novo* synthesis (118)].

The ability of aspirin-like drugs to inhibit release in organised tissues was first shown by Smith and Willis (136), and by Ferreira *et al.* (46). Smith and Willis (136) found that the production of prostaglandins by human platelets *in vitro* was reduced by the addition of aspirin-like drugs to the incubation medium. Indomethacin was about 10 times more potent than aspirin which was in turn about 10 times more potent than salicylate. Ferrerira *et al.* (46) discovered that indomethacin and aspirin reduced the output of PGE_2 from isolated perfused dog spleens in response to adrenaline injections whereas hydrocortisone did not. Indomethacin also abolished the release of PGE_2 from cat spleens in response to electrical stimulation of the splenic nerve (45). Gryglewski and Vane (63) found that prostaglandins are released when slices of rabbit spleen are mechanically stimulated, and used this preparation to test the antienzyme activity of four anti-inflammatory drugs; the order of decreasing potency was found to be meclofenamic acid > indomethacin > oxyphenbutazone > aspirin. The same authors also observed that the production of RCS (thought to be an intermediate in the synthesis of prostaglandins) was also inhibited, though not always to the same extent. Prostaglandins and RCS are also released

TABLE 6

Inhibition of prostaglandin synthesis in isolated tissues by aspirin-like drugs

Prostaglandins Synthesised or Released by:	Inhibitor	Concentration in Bathing Fluid	Inhibition	Reference
		μM	%	
Human platelets, release of E_2	Indomethacin	0.17	≃50	136
	Aspirin	1.7	≃50	
	Salicylate	17.0	≃50	
Perfused dog spleen, release of E_2 and $F_{2\alpha}$ by adrenaline	Indomethacin	1.0	60	46
	Aspirin	7.5	60	
Perfused cat spleen, release of E_2 by nerve stimulation	Indomethacin	0.84–14.0	100	45
Rabbit spleen slices, release of E_2 and RCS by mechanical stimulation	Meclofenamic acid	1.7	≃70	63
	Indomethacin	2.8	≃69	
	Oxyphenbutazone	31.0	≃25	
	Aspirin	220.0	≃56	
Perfused rabbit heart, release of PGE by adenine nucleotides or ischaemia	Indomethacin	1.43	≃100	105
Perfused guinea-pig lungs: prostaglandin and RCS release by various stimuli	Indomethacin	0.28–1.4	100	111, 117
	Mefenamic acid	0.82	100	
	Aspirin	5.5–28.0	100	
Rabbit jejunum, E_2 release into organ bath	Indomethacin	2.8–28.0	100	44
Rat uteri (pregnant), release of E_2 or $F_{2\alpha}$	Indomethacin	0.028–2.8	Variable	4
	Aspirin	560–1680	Variable	
Rat uteri (pregnant), release of E_2 or $F_{2\alpha}$	Indomethacin	2.8–11.2	100	156
Mouse fibrosarcoma cells (in culture), E_2 release	Indomethacin	0.003	50	95
	Aspirin	60.0	50	
Human synovium cells (in culture), PGB and $F_{2\alpha}$ release	Indomethacin	1.4	>97	124
Bovine thyroid cells, stimulation of E_1, $F_{2\alpha}$, A_1 and B_1 content	Indomethacin	2.8	≃100	22
	Aspirin	550.0	≃100	
Rabbit peritoneal polymorphonuclear leucocytes, E_1 release	Indomethacin	84.0	100	102
Toad bladders, E_1 content	Indomethacin	6.0	90	163
	Aspirin	220.0	89	

from intact perfused guinea-pig lungs in response to a variety of stimuli including anaphylactic shock, embolisation, mechanical stimulation and infusions of bradykinin or angiotensin (117, 118) and this release is blocked by several aspirin-like drugs. Nucleotide-induced prostaglandin release from the isolated perfused rabbit heart was blocked by indomethacin (105).

The release of prostaglandins into the fluid bathing isolated organs such as rabbit jejunum (44) and pregnant rat uterus (4, 156) is also inhibited by indomethacin or aspirin; in the spleen experiments mentioned above, a good block by these agents was quickly obtained, but a delay of 45 min to 3 hr occurred before maximal inhibition was seen in the latter experiments. This may simply be a problem of penetration of the drug into a tissue which no longer has an intact vascular supply.

Inhibition of synthesis by aspirin or indomethacin has also been demonstrated in cultured fibrosarcoma (95), human synovium (124) and bovine thyroid cells (22), rabbit polymorphonuclear leucocytes (102) and toad bladders (163), although the concentrations required to inhibit synthesis in these different systems vary greatly.

3. Man and whole animals. Smith and

Willis (136) found that the synthesis of prostaglandins by the platelets of donors who had taken 600 mg of aspirin 1 hr before giving blood was almost abolished, although the platelet release reaction and the release of lysosomal enzymes were unimpaired. Prostaglandin synthesis by platelets from a subject who had previously taken 60 mg codeine was unaffected. Collier and Flower (31) studied the effect of oral aspirin on prostaglandin synthesis in male subjects by measuring the PGE_2 and $PGF_{2\alpha}$ content of semen before and after treatment with aspirin (600 mg ×4/24 hr). After 3 days of treatment, the mean E_2 content was reduced by more than 65% and the mean $PGF_{2\alpha}$ content by more than 75%. At the end of 6 days of treatment, the levels of $PGF_{2\alpha}$ remained low, but the concentration of PGE_2 began to rise, suggesting "escape" from inhibition by the drug.

Horton and his colleagues (76) have confirmed these results with more sophisticated analytical techniques, and by measuring the concentration of 19-hydroxy PGA and 19-hydroxy PGB in addition to PGE and PGF. Two dose schedules were used, 3.6 and 7.2 g aspirin per day. A mean reduction of 56% of PGE content was found in the two subjects after a 3-day treatment with 3.6 g per day. A reduction of 93% of the PGF and 80% PGE content was found during treatment with 7.2 g per day although signs of aspirin toxicity were noticed at this dose level. The content of the hydroxy prostaglandins also decreased during treatment.

Hamberg (66) used the concentration of 7α-hydroxy-5,11-di-keto tetranorprostane-1,16-dioic acid (the major metabolite of E_1 and E_2) in the urine as an index of whole body prostaglandin synthesis. The daily metabolite excretion by subjects receiving

TABLE 7

Inhibition of prostaglandin synthesis in whole animals and man by aspirin-like drugs

Species-Prostaglandins Synthesised or Released by:	Inhibitor*	Dose	Inhibition	Reference
			%	
Man, biosynthesis in platelets	Aspirin	600 mg	80–97	136
Man, E_2 and $F_{2\alpha}$ content of semen	Aspirin	600 mg × 4†	73(E_2) 90($F_{2\alpha}$)	31
Man, PGE, PGF content of semen	Aspirin	3.6 g† 7.2 g†	56(E) 80(E) 93(F)	76
Man, concentration of E_1/E_2 metabolite in urine	Indomethacin Aspirin Salicylate	50 mg × 4† 0.75 g × 4† 0.75 g × 4†	77–98 86 86–98	66
Guinea-pig, concentration of metabolite in urine	Indomethacin	50 mg†	98	68
Dog, release from kidney by angiotensin, by haemorrhage or endotoxin-hypotension, by autoregulation response	Indomethacin Indomethacin Indomethacin	2 mg/kg IV 1 mg/kg IV 105 mg/kg IV	100 100 50–100	5 32 71
Dog, efflux of PGs into the lymph of scalded paws	Indomethacin	10–20 mg/kg IV	100	10
Rat, $F_{2\alpha}$ production by pregnant uteri	Indomethacin	1 mg/kg × 2†	Variable	4
Rat, PG content of carrageenin air bleb exudate	Indomethacin Aspirin Salicylate	12.5 mg/kg 100 mg/kg 100 mg/kg	80–100 75 75	161
Rat (hypertensive), PGE_2 and PGA_2 content of kidney	Indomethacin Aspirin	1 mg/kg 10 mg/kg	80–100 75–100	140
Mouse, production of PGs by BP8/P_1 tumour cells	Indomethacin	5 mg/kg IP	66	142

* Drugs were administered orally unless otherwise stated.

† Dose per day.

therapeutic doses of aspirin, indomethacin or salicylate was greatly reduced. In female subjects (who normally produced 2.5–5.3 μg of metabolite per day) almost maximal inhibition (63–92%) was achieved after only 1 day of treatment. In male subjects (who normally produced 6.5–46.7 μg per day), the excretion continued to decline gradually during the 3-day treatment period. Two days after the drug treatment was discontinued, most metabolite concentrations had returned to near control levels, although there was some variation. Indomethacin was the most potent drug of the three, aspirin and salicylate being roughly 15 times less active on a weight basis. The same author has applied this technique to guinea-pigs. Again, indomethacin abolished the urinary output of the metabolite, although in this species the dose required was higher than that for man; aspirin in the same dose (50 mg/day) was inactive (68).

Inhibition of prostaglandin synthesis in whole animals has been shown in other species; administration of indomethacin to dogs, cats and rabbits abolishes the release of prostaglandins from kidney in response to angiotensin injections (5, 140), haemorrhage or endotoxin-induced hypotension (32) and during autoregulation (71). It also abolishes the release of prostaglandins into the lymph of scalded paws of dogs (10). Intraperitoneal injections of paracetamol prevent the increase in the prostaglandin concentration of the cerebrospinal fluid which is observed during pyrogen-induced fevers in cats (104).

Somova (140) reported that the kidneys of rats with experimental (Goldblatt) hypertension contained some 2 to 3 times the PGE_2 and PGA_2 content of normotensive animals. After treatment with aspirin (10 mg/kg p.o.) or indomethacin (1 mg/kg p.o.) for 6 days, the PGE_2 and PGA_2 content had almost returned to levels found in normotensive animals. The prostaglandin content of normotensive animals which received the same treatment did not significantly change. Treatment of either batch of rats with the prostaglandin antagonists polyphloretin phosphate or 7-oxa-13-prostynoic acid had no effect. Possibly this "drug resistant background" of prostaglandins could be explained by intrarenal synthesis during extraction procedures.

Other workers have also shown blockade of synthesis in rats (4, 161), mice (142) and rabbits (34).

C. Drug Sensitivity of Synthetase Preparations from Different Tissues

Analysis of experimental data such as those presented in the foregoing paragraphs have led to an interesting hypothesis, first proposed by Vane (153), to explain wide variations in drug sensitivity in different tissues; according to this concept the prostaglandin generating system exists in multiple molecular forms within the organism, the synthetase enzymes from each tissue type having a different pharmacological profile to those of any other tissue—a property which has already been noted in connection with some other enzymes, especially phosphodiesterase (74).

Several experimental observations may be cited in support of this idea; the first example to be recorded was that of paracetamol, which was found to be some 10 times more active against synthetase preparations from dog and rabbit brain than against dog spleen (53). Recently, however, some much more striking differences have been reported by Bhattacherjee and Eakins (15) who tested the inhibitory potency of indomethacin against various rabbit tissues. The drug showed good activity against enzyme preparations from spleen ($I50 = 0.14$ μM), but was some 12 times less active against kidney enzyme, 187 times less active against the enzyme from conjunctival tissue and 410 and 1111 times less active against the synthetase prepared from the anterior uvea and retina, respectively.

Table 8 is a list of molar potency ratios of a few standard aspirin-like drugs against enzymes prepared from various tissues; the reader's attention is drawn especially to the differences in activity of paracetamol. Some

TABLE 8

Variation in potency of drugs against different synthetase preparations

Compound	Molar I50 Ratios		
	Dog spleen*	Rabbit kidney†	BSV‡
Meclofenamic acid	370	1801	682
Indomethacin	217	709	236
Niflumic acid	336	NT§	76
Phenylbutazone	5	180	6.4
Aspirin	1	1	1
Paracetamol (4-acetamidophenol)	0.06	4.2	NT§

* Data from Flower *et al.* (52).
† Data from Blackwell *et al.* (17).
‡ Data from Flower *et al.* (51).
§ NT, not tested.

variation of inhibitory potency could obviously be accounted for by differences in experimental techniques, in particular the assay procedure employed [compare for example the inhibitory potency of prostaglandin analogues as assayed by McDonald-Gibson *et al.* (103) by bioassay and by a radiometric technique] and also in the composition of the reaction mixture. Several aspirin-like drugs are competitive inhibitors (see section VII E), and hence the substrate concentration of the reaction mixture has an important influence on the apparent potency of the drug. Flower *et al.* (51) found that the optimal arachidonic acid concentration for their lyophilised BSV enzyme preparation was 1 mM and that at this concentration the I50 (PGE_2) of indomethacin was 40 μM. Decreasing the substrate concentration to 0.02 mM, however, resulted in a 10-fold reduction in I50 value. The substrate concentration is not the only factor which influences inhibitory potency; the optimal substrate concentration for the rabbit kidney enzyme was 2.5 mM and yet the I50 of indomethacin in this preparation was only 3.7 μM. It is worth noting in passing that the biochemical profiles of the BSV and the rabbit kidney enzyme differed in almost every respect—pH optimum, L-epinephrine and glutathione requirements, type and optimum of substrate curve and so on—a finding which lends further credence to the idea that prostaglandin synthetase (or at least one component protein) exists in several forms.

In many cases there is quite a striking correlation between the potency of these drugs against (the same) tissue taken from different species. For example, the I50 concentrations for indomethacin against dog and rabbit spleen are 0.17 μM and 0.14 μM (15, 52); paracetamol concentrations of 92, 83, 130 μM are required to inhibit the synthetase from the brain of rabbit, dog, mouse and gerbil, respectively (53, 161). Unfortunately, perusal of the sheep and bovine seminal vesicle data in table 4 gives no ground for hoping that species differences can be completely ruled out. One point worth noting in passing is that BSV enzymes invariably require much higher doses of drug to produce a given inhibition than do the other synthetase enzymes studied.

It is now possible to argue convincingly that prostaglandins contribute significantly to certain pathological events and that some of the side effects as well as the therapeutic actions of the aspirin-like drugs can be accounted for by interference with endogenous prostaglandin biosynthesis (43, 151–155) and from this standpoint alone, the idea that the synthetase system in different anatomical locations may be genetically different, is a very attractive one. The question of whether these enzymes are immunologically or electrophoretically distinct remains to be settled, but meanwhile it is worth re-iterating that the I50 value varies with the technique of the experimenter and that this should be borne in mind when assessing results of this nature.

D. Specificity of the Aspirin-Like Drugs

In addition to inhibiting prostaglandin synthetase, the aspirin-like drugs exert inhibitory and other effects on a variety of other enzymes and cellular systems (112, 132, 137) and it is especially important to be aware of this when using these drugs to evaluate the participation of prostaglandins in biological events. Fortunately, the concen-

TABLE 9

*Inhibitory and other effects of indomethacin**

Indomethacin Effects	Approximate Concentrations μM	References
Enzyme inhibition		
Prostaglandin synthetase	0.17–38.0	See tables 4 and 6
Prostaglandin 15′ dehydrogenase	15–1000	See section IX, B2
Phosphodiesterase	28	50
DoPa decarboxylase	100	135
Oxidative phosphorylation	250	159
Histidine decarboxylase	400	134
Collagenase	3500	20
Physical effects		
Inhibition of leucocyte motility	0.01	113
Inhibition of urate binding to albumin	200	160
Stabilisation of proteins	400	59
Stabilisation of erythrocyte membranes	500	21
Inhibition of enzyme release from lysosomes	1000	80

* The plasma concentrations of indomethacin are approximately 5 μM total, and 0.5 μM "free."

TABLE 10

Plasma levels of some common aspirin-like drugs

Aspirin-like Drugs	Approximate Peak Plasma Concentration in Man	Plasma Protein Binding	References
	μM	%	
Meclofenamic acid	1.35	99.8	57
Niflumic acid	300	82–98	19
Indomethacin	5.0	90	79
Flufenamic acid	53.0	90?	58
Mefenamic acid	41.0	48*	58
Phenylbutazone	230–500	98	23
Aspirin	280–300	50–80	94, 164
Paracetamol	350	25	121, 164

* Calculated from Glazco (58).

trations of these drugs required to inhibit the synthetase are generally much lower than the concentrations which inhibit other enzymes (although there are one or two important exceptions, see below). Indomethacin is a particularly suitable example, being frequently used both *in vivo* and *in vitro* as a synthetase inhibitor and possessing as it does a number of well documented actions on other enzymes. Several examples are given in table 9 from which it is evident that few other effects could be expected to occur at the low concentrations required for synthetase inhibition.[2]

Similar remarks may be applied to some of the salicylates (and doubtless other aspirin-like drugs also); the concentration of aspirin required for synthetase inhibition in isolated tissues may be as low as 1.5 μM or as high as 1.5 mM and although the inhibition of protein synthesis and the uncoupling effects are sometimes seen in the concentration range of 0.15 to 0.6 mM, many other enzymes require salicylate concentrations of 5 mM or more for inhibition (137).

Flower *et al.* (52) correlating the *in vitro* activity of synthetase inhibitors with their therapeutic potency, pointed out that the concentrations required to inhibit their (dog spleen) enzyme preparation were within the plasma levels achieved by these drugs during normal therapy, even allowing for plasma binding (see table 10) and the work of Hamberg (66), Collier and Flower (31), and Horton *et al.* (76) have conclusively demonstrated that biosynthesis is inhibited after normal therapeutic doses of aspirin, salicylic acid or indomethacin; the same argument cannot be applied to seminal vesicle preparations, however, which, as I have already pointed out, generally require higher concentrations for inhibition. Exceptions may also occur in other species where, either because of accelerated drug metabolism or reduced sensitivity to these compounds, much greater doses are required to produce a given effect. Hamberg (66) found that the dose of indomethacin (per kg) required to inhibit

[2] One enzyme quite sensitive to indomethacin is phosphodiesterase; this should be borne in mind when using indomethacin to investigate prostaglandin-adenosine 3′,5′-cyclic phosphate interaction.

prostaglandin synthesis in the guinea-pig was 10 to 30 times more than the dose required to produce the same inhibition in man. It follows that in guinea-pig large doses of drugs administered to inhibit prostaglandin synthesis may also affect other enzymes.

In summary, there is an old pharmacological adage to the effect that no drug exerts a single action. The aspirin-like drugs are certainly no exception; but, at worst, these drugs may be regarded as "preferential inhibitors" of prostaglandin synthesis, and at best as "selective inhibitors."

E. Mechanism of Inhibitory Action

There are few definite data concerning the mechanism of inhibitory action of these drugs at present and, in view of their wide chemical diversity one might perhaps anticipate more than one mode of action. Amongst the more likely alternatives, however, are competition at the substrate or cofactor site, irreversible inactivation of the enzyme, a chelating action (there is some evidence that PG synthetase contains copper or some other metal; see ref. 133 for a discussion of this), or possibly some free radical destroying mechanism.

A certain similarity in the initial reaction step of PG synthetase and plant lipoxygenase prompted Downing (39) to examine the effects of indomethacin on that enzyme. It was inactive, and on the basis of this and other observations concerning the inhibition of these two enzymes by fatty acids, the author suggested that indomethacin acts against the synthetase by inhibiting the initial attack on the substrate. Tomlinson *et al.* (147), pointing out the apparent absence of intermediates in inhibited preparations of the synthetase, also believed this to be the mode of action of these drugs. At this point it is worth recalling the work of Gryglewski and Vane (64) and Piper and Vane (117), who found that the production of RCS, an unstable biologically active substance thought to be an intermediate in the synthesis of prostaglandins, was also blocked by anti-inflammatory drugs.

The work of Takeguchi and Sih (144) provides some clues to the mechanism of action; these workers assayed prostaglandin synthesis by BSV microsomes indirectly by measuring the oxidation of the cofactor epinephrine. This reducing agent is involved in the transformation of the hydroperoxide to the endoperoxide (see fig. 2). The oxidation was inhibited by several aspirin-like drugs, again suggesting that these compounds act primarily at a very early stage of the reaction.

The majority of the aspirin-like drugs are organic acids (although some non-acidic compounds also inhibit, *i.e.*, the pyrazolone derivatives) and so the simplest hypothesis would envisage a straight competition between drug and substrate at the catalytic site; indeed, some results appear to support this concept. Flower *et al.* (51) found that the degree of indomethacin inhibition was dependent on the substrate concentration, and Ham *et al.* (65), in their kinetic studies, showed that fluoroindomethacin (an anti-inflammatory analogue of indomethacin) also inhibited competitively. If competitive kinetics did obtain, this would provide a convenient explanation for the differences in inhibitory potency of these drugs which are sometimes seen when they are tested under different assay conditions and with different substrate concentrations.

This "simple" hypothesis, however, seems untenable in the light of a more detailed kinetic analysis by Ku and Wasvary (86), Smith and Lands (138) and Lands *et al.* (90). The studies of these workers indicate that the majority of the common aspirin-like drugs are "competitive-irreversible" inhibitors. This term is rather confusing but it seems that two possible kinetic situations could obtain; in the first case the inhibitor combines in an irreversible time-dependent fashion with the catalytic site of the enzyme (for reasons stated above this is assumed to be the dioxygenase component). Presence of the substrate at this site reduces the velocity of the combination but, provided that the inhibitor is in excess of the enzyme, cannot

prevent ultimate (complete) inhibition of the enzyme because there will always be a small fraction of the enzyme (which is in reversible combination with the substrate) which is free to combine in an irreversible fashion with the inhibitor. Because the initial degree of inhibition depends on the substrate concentration, a competitive effect arises.

A similar situation is encountered in the second case, except that the inhibitor does not bind to the catalytic (substrate) site but to another site which is in sufficient proximity to the catalytic site to reduce its affinity for the substrate, perhaps by some "allosteric" effect. It should be possible to distinguish between these two situations by a formal kinetic analysis although this has not apparently been done. Lands *et al.* (90) favour the latter possibility since their data indicate that (in contrast to the destruction of the active site by TYA), inhibition by indomethacin does not require oxygen and is not prevented by diethyldithiocarbamate. Incubation of the enzyme with *o*-phenanthroline, which reversibly inhibits the enzyme, however, does prevent irreversible inhibition by indomethacin. On the basis of this evidence, the authors speculate that a "sensitive hydrophobic site" may be a feature of the synthetase. Smith and Lands (138) were the first to report that treatment of the synthetase with inhibitor prior to addition of substrate led to a progressive irreversible inhibition of the subsequent reaction. By titrating enzyme protein against a fixed concentration of indomethacin (or any other irreversible aspirin-like drug) it should be possible to calculate the number of "inhibitor binding sites" per mg of microsomal protein.

Raz *et al.* (123) also found that indomethacin inhibition was irreversible. When SSV enzyme preparations were preincubated with 4.6 μM indomethacin, almost complete inhibition was observed even if the microsomal fraction was then separated by centrifugation and resuspended in fresh (inhibitor free) buffer. Partial recovery of the enzyme activity took place only after extensive dialysis against large volumes of phosphate buffer.

Of the series of seven aspirin-like drugs tested by Ku and Wasvary (86) only two compounds (SU-21524 and oxyphenbutazone) were found to be truly reversible. Lands *et al.* (89) also found oxyphenbutazone to be reversible. Since phenylbutazone itself is an irreversible inhibitor (86) and the only difference between the two drugs is a *p*-hydroxy substituent on one of the benzene rings, one can conclude that this region of the molecule is somehow important for binding.

E and F type prostaglandins often exert opposite effects within the body and, therefore, from the therapeutic as well as from a purely academic standpoint, it would be of interest to find a drug which interfered with one or another of the pathways of endoperoxide breakdown in a similar fashion to the bicyloheptenes described in section VI. Flower *et al.* (51) made an interesting observation of this type whilst investigating the inhibitory action of the anti-inflammatory drug benzydamine (not a carboxylic acid). This drug appeared to inhibit the formation of $PGF_{2\alpha}$ and PGD_2 by BSV enzymes ($I50 \simeq 1.3$ mM) but to potentiate the synthesis of PGE_2 by approximately 150%. The explanation of this phenomenon appears to lie in the peculiar shape of the velocity-substrate curves for these products (see fig. 3). The optimal substrate concentration for PGE_2 formation was 0.5 mM, but for most experiments a concentration of 1 mM was chosen since this gave almost equal production of PGE, PGF and MDA and smaller amounts of PGD. When tested under the latter conditions, benzydamine potentiated PGE synthesis, but when the reaction was performed under conditions optimal for PGE formation (0.5 mM substrate), no potentiation was observed. The I50 for PGE formation was about 5 mM. The shape of the product-substrate curve for PGE suggests strong substrate or product inhibition and the action of benzydamine could be to prevent this from occurring thus permitting an increase in the amount of PGE formed. Another explanation could be that the drug in some way complexes the arachidonic acid

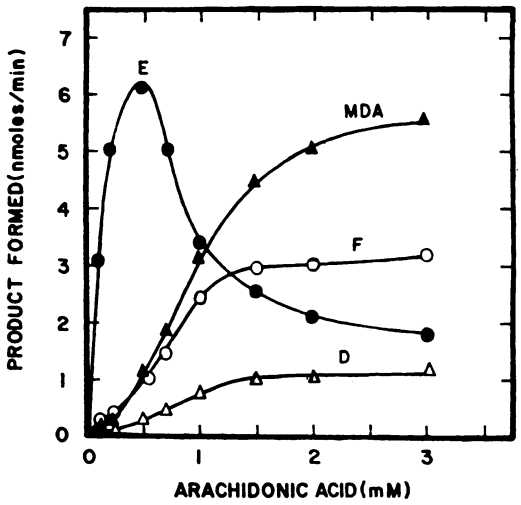


FIG. 3. Effect of the substrate concentration on the rate of product formation by the prostaglandin synthetase system of bovine seminal vesicle; from Flower *et al.* (51). The abbreviations used are MDA, malondialdehyde; E, PGE_2; F, $PGF_{2\alpha}$; D, PGD_2 (11-dehydro-$PGF_{2\alpha}$).

thus reducing the effective substrate concentration; it is, however, difficult to see how this could occur on inspection of the structure (see fig. 4). It is evident that many types of compounds capable of binding fatty acid substrates could give rise to a similar effect in this system and the results underline the importance of basic studies on synthetase enzymes if gross misinterpretations of data are to be avoided.

Another similar observation was also made by Flower *et al.* (51). Most of the aspirin-like drugs tested by these authors (except benzydamine) inhibited the formation of all four products of the synthetase ($PGF_{2\alpha}$, PGE_2, PGD_2, MDA) equally. Phenylbutazone, however, inhibited the formation of PGE_2 and $PGF_{2\alpha}$ at 1.3 mM, but had no effect on the production of PGD or MDA; in some cases a slight potentiation of these products was observed. Some recent unpublished observations suggest that other pyralazone derivatives may have a similar effect. The simplest explanation of this phenomenon is that these drugs interfere with endoperoxide breakdown, but what relevance, if any, this has to the action of phenylbutazone *in vivo* is not clear since the

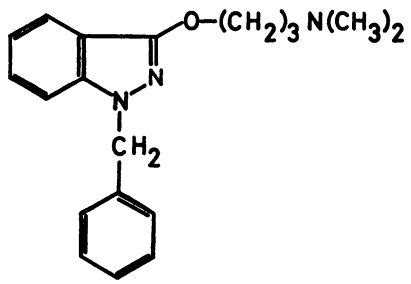


FIG. 4. Structure of benzydamine.

pharmacology of PGD has, for no apparent reason, been neglected.

Maddox (100) has reported some experiments in which selective inactivation of the "isomerase component" of the synthetase apparently occurs. This author incubated homogenates of sheep seminal vesicles in two types of reaction mixture; when incubated in the first type which contained buffer, cofactors and arachidonic acid, only PGE_2 was detected. Aspirin-like drugs when added to this mixture, produced varying degrees of inhibition. The second type of reaction mixture contained in addition to buffer, substrate and cofactors, 0.1 mM $CuCl_2$ also. After incubation in this mixture both PGE_2 and $PGF_{2\alpha}$ were detected; in this case PGE_2 formation was inhibited to approximately the same extent by aspirin-like drugs but $F_{2\alpha}$ synthesis was unimpaired; in fact in the presence of some agents, most notably phenylbutazone, $PGF_{2\alpha}$ production was stimulated by as much as 220%. No biochemical data concerning the enzyme system which might aid interpretation were published, but the author suggested that two separate synthetase complexes exist, one of which produces PGE, and the other, PGF.

Before concluding this section it is worth commenting on the work of Tan *et al.* (145, 146). Certain similarities between steroidogenesis and PG biosynthesis led these authors to speculate that cytochrome P450 might play a part in PG generation in addition to the well established role in steroid biosynthesis. Indeed, precursor acids as well as prostaglandins themselves were shown to bind to microsomal P450 particles from beef adrenals, as evidenced by characteristic

spectral changes. Aspirin, however, had no effect on the binding of arachidonic acid to the preparation. The relevance of this finding is questionable, since the participation of P450 in PG biosynthesis is by no means established; indeed it seems to be absent in preparations of seminal vesicles (119) which are amongst the most active enzyme sources known.

In summary, most of the aspirin-like drugs block the initial stages of the synthetase reaction in a "competitive-irreversible" fashion. They probably exert this effect by combining slowly with a site which, although not the substrate site, is sufficiently close to reduce the catalytic activity of the enzyme in a time-dependent fashion. Some compounds may affect endoperoxide breakdown but this cannot be regarded as definitely proven.

F. Duration of Action of the Aspirin-like Drugs

The action of indomethacin and probably of most other aspirin-like drugs appears to be irreversible in cell free enzyme preparations but obviously many factors including metabolic degradation and *de novo* enzyme synthesis have a profound influence on the duration of action of a drug *in vivo*.

The data of Hamberg (66) suggest that the "whole body" synthesis of prostaglandins in man is depressed for at least 24 hr, and sometimes as much as 48 hr, after treatment with therapeutic doses of aspirin, indomethacin or sodium salicylate. This conclusion receives support from the work of Horton *et al.* (76) who measured the 24-hr seminal prostaglandin content of two subjects before, during and after a 3-day course of aspirin treatment. The semen concentrations of PGE and PGF had returned to control levels within 2 days of discontinuing treatment, but the concentrations of 19-hydroxy PGA and 19-hydroxy PGB remained below control levels for somewhat longer. Collier and Flower (31) measured the seminal prostaglandin concentrations during a weeks course of aspirin treatment. The inhibition of PGE was 57% at day 3 but had declined to 37% by day 7. This suggested that the inhibitory effect of aspirin diminished during prolonged treatment periods although the same phenomenon was not seen for PGF concentrations; a similar trend was seen in the experiments of Horton *et al.* (76).

Kocsis *et al.* (84) measured the time course of platelet prostaglandin biosynthesis inhibition after a single dose of aspirin, salicylate or indomethacin. Inhibition achieved by these three compounds was variable but occurred within 1 hr of oral administration of the drugs. Maximal inhibition by salicylate was maintained for less than 6 hr, and that of indomethacin for less than 24 hr, but the effect of aspirin persisted for 2 to 3 days.

There seems to be a distinct difference between the duration of action of aspirin and sodium salicylate. Aspirin is rapidly hydrolysed to salicylic acid *in vivo* (half-life in human plasma approximately 20 min; see ref. 97) but evidently the acetylated and non-acetylated forms of this acid act as pharmacologically distinct entities. The ability of aspirin to acetylate proteins is well documented (69, 114) and it seems likely that this is the basis for its long lasting action in platelets. It should be remembered, however, that the circulating platelet is only capable of restricted *de novo* enzyme biosynthesis (141) and only has a life of 8 to 11 days in the circulation (1) and hence may be a rather atypical model.

The conclusion to be drawn from these studies outlined above seems to be that there is a considerable variation in the duration of action of the aspirin-like drugs so far tested; aspirin probably has a dual mechanism of action, one consequent upon its possessing an acetylating group and the other subsequent to its rapid metabolism to salicylate. In view of the irreversible nature of inhibition by aspirin-like drugs, replacement of enzyme protein by *de novo* synthesis is a likely method by which action is finally terminated.

G. Structure Activity Relationships

At the time of writing there has been no systematic investigation into the molecular

requirements for synthetase inhibition although Ham *et al.* (65) have investigated representative compounds from several different classes of aspirin-like drugs. Some of their data, together with that of others are collected in tables 11 to 14.

Perhaps the most interesting aspect of this work is the high degree of stereospecificity exhibited by the enzyme towards inhibitors. For example, when the *d*- and *l*-isomers of the *p*-methyl thiobenzyl derivatives of indomethacin (compounds 3 a and b, table 11) were tested against the SSV enzymes by Ham *et al.* (65), the *d*-isomer possessed approximately the same activity as indomethacin, but the *l*-isomer was some 60 times less potent. Takeguchi and Sih (144) tested these two isomers (and found a qualitatively similar effect) and also the *d*- and *l*-isomers of 3-chloro-4-cyclohexyl-α-methylphenylacetic acid (CCMP, see fig. 9). The *d*-isomer was 10 times as potent as indomethacin but the *l*-isomer was found to be inactive.

Another example of this type of stereospecificity was reported by Tomlinson *et al.* (147) who tested the *d*- and *l*-isomers of the aspirin-like drug naproxen (see fig. 10). In this case the naproxen was 70 times more potent against BSV enzymes than its enantiomer.

In all of the *d*- and *l*-enantiomers described above the isomerisation has been identical, *i.e.*, rotation about the optically active group, —CH(CH_3)COOH. It seems safe to assume, therefore, that the correct (*d*) configuration at this centre is essential for binding to the inhibitor site of the enzyme, and one might predict that a similar phenomenon would occur with other drugs possessing this group.

Perhaps the simplest of the synthetase inhibitors are the benzoic acid derivatives. Blackwell *et al.* (18) tested a number of these compounds against a synthetase derived from kidney (see table 14). Benzoic acid itself had little activity, neither did the monohydroxy acids including salicylate itself. Modification of the salicylic acid structure by the addition of another hydroxy group at C-5 or C-6 (gentisic or γ-resorcylic acid) increased the antisynthetase activity by almost 30-fold. It is interesting that gentisic acid (and possibly γ-resorcylic acid) is a metabolite of salicylic acid. Although salicylic acid is as active as aspirin in inhibiting prostaglandin biosynthesis *in vivo* (66, 161), it has always shown little activity against *in vitro* preparations of the synthetase (17, 151, 161). For this reason Willis *et al.* (161) and Vane (153) have suggested that metabolic transformation is required for full activity of this compound. In view of the much greater activity of gentisic acid over salicylic acid, a conversion of less than 10% would be enough to account for the activity of salicylic acid *in vivo*. Estimates of the conversion in man vary between 4 and 8% (9, 11, 83, 125). The other dihydroxy acids tested (protocatechuic and α-resorcylic) had less activity than gentisic acid. Recently another metabolite of salicylic acid, salicyluric acid, has been found to be inactive (16).

Substitution of an amino group for a hydroxy group of salicylic acid (anthranilic acid) gave a much greater antisynthetase activity and *p*-amino benzoic acid was more active than the corresponding hydroxy compound.

It is unfortunately not possible to draw any firm conclusions from the rather diffuse data presented in this section. It would seem, however, that examination of the antisynthetase activity of carefully selected compounds could yield interesting insights into the nature of the binding site and could prove especially fruitful as an approach to the design of new inhibitory drugs.

VIII. Inhibition of Prostaglandin Biosynthesis by Miscellaneous Agents

In addition to the substrate analogues and the pharmacological agents already listed, the literature contains reports of synthetase inhibition by a variety of other compounds including heavy metal ions, nucleotides and anti-oxidants.

As with other enzymes, prostaglandin synthetase is sensitive to fluctuations in sub-

TABLE 11

Structure-activity relationships amongst indomethacin analogues (see fig. 5)

No.	Ring Substituents					Antisynthetase Activity I50	
	X	R_1	R_2	R_3	R_4	BSV*	SSV†
						μM	
1	N	$-N(CH_3)_2$	$-CH_2CO_2H$	$=O$	$-Cl$	NT	0.43
2‡	N	$-CH_3O$	$-CH_2CO_2H$	$=O$	$-Cl$	2.0	0.45
3a (d)	N	$-CH_3O$	$-CH(CH_3)CO_2H$	$-H_2$	$-SCH_3$	30.0	0.46
b (l)	N	$-CH_3O$	$-CH(CH_3)CO_2H$	$-H_2$	$-SCH_3$	55.0	27.4
4	C	$-CH_3O$	$-CH_2CO_2H$	$-H$	$-Cl$	NT	0.49
5§	N	$-CH_3O$	$-CH_2CO_2H$	$=O$	$-Fl$	NT	0.73

* Data compiled from Takeguchi and Sih (144).
† Data compiled from Ham *et al.* (65).
‡ Indomethacin.
§ Fluoroindomethacin.

TABLE 12

Structure-activity relationships amongst fenamates (see fig. 6)

Trivial Name	Substituents				Antisynthetase I50		
	R_1	R_2	R_3	X	BSV*	SSV†	Spleen‡
						μM	
Meclofenamic acid	$-CH_3$	$-Cl$	$-Cl$	$-C-$	NT	NT	0.1
Niflumic acid	$-CF_3$	$-H$	$-H$	$-N-$	NT	1.2	0.11
Mefenamic acid	$-CH_3$	$-CH_3$	$-H$	$-C-$	15.0	2.1	0.71
Flufenamic acid	$-CF_3$	$-H$	$-H$	$-C-$	48.0	2.5	0.64

* Data compiled from Flower *et al.* (51).
† Data compiled from Ham *et al.* (65).
‡ Data compiled from Flower *et al.* (52).

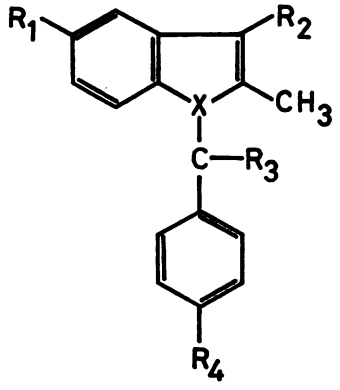


FIG. 5. Skeleton structure of indomethacin derivatives (see table 11).

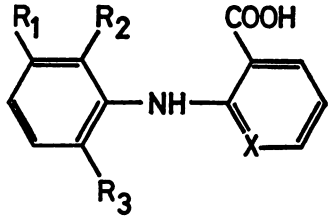


FIG. 6. Skeleton structure of fenamic acid derivatives (see table 12).

strate or cofactor concentrations and to changes in the ionic environment. As far as the concentrations of cofactors and substrate are concerned there are definite optimal concentrations beyond which the addition of further material results in no further conversion, or else becomes inhibitory (51, 108).

Some metal ions are inhibitory; Nugteren *et al.* (108) found that E_1 production by sheep vesicular gland preparations was reduced by Zn^{2+}, Cd^{2+} and Cu^{2+} ions in concentrations of 5×10^{-6} M. The effect was partially reversed by the addition of GSH. Fe^{2+}, Fe^{3+}, Co^{2+}, Sn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+} and AsO_3^{3+} were without effect at these con-

TABLE 13

Structure-activity relationships amongst pyralazone derivatives (see fig. 7)

No.	Trivial Name	Ring Substituents				Antisynthetase I50		
		R_1	R_2	R_3	R_4	R_5	BSV*	SSV†
							μM	
1	Phenylbutazone	—H	$-C_6H_5$	=O	$-(CH_2)_3CH_3$	=O	1.5×10^3	12.3
2	Antipyrine	—H	$-CH_3$	CH_3	—H	=O	4.9×10^3	NT
3	Oxyphenbutazone	—OH	$-C_6H_5$	=O	$-(CH_2)_3CH_3$	=O	NT	49.4
4	Dipyrone	—H	$-CH_3$	$-CH_3$	$-N(CH_3)CH_2SO_3Na$	=O	7.0×10^3	NT
5	Aminopyrine	—H	$-CH_3$	$-CH_3$	$-N(CH_3)_2$	=O	$>10^{-4}$	91.1
6	Phenidone	—H	—H	=O	$-H_2$	$-H_2$	$<10^{-4}$	NT

* Data compiled from Flower *et al.* (51).
† Data compiled from Ham *et al.* (65).

TABLE 14

*Structure-activity relationships amongst benzoic acid derivatives (see fig. 8)**

No.	Derivative	Substituents	Trivial Name	Prostaglandin Synthetase Inhibition
				% at 5 mM
1		None	Benzoic acid	12
2	Monohydroxy acids	2-OH	Salicylic acid	16
3		3-OH	*m*-Hydroxy benzoic acid	5
4		4-OH	*p*-Hydroxy benzoic acid	23
5	Dihydroxy acids	2,5-OH	Gentisic acid	92
6		2,6-OH	γ-Resorcylic acid	89
7		3,4-OH	Protocatachuic acid	38
8		3,5-OH	α-Resorcylic acid	77
9	Amino acids	$2-NH_2$	Anthranilic acid	80
10		$4-NH_2$	*p*-Aminobenzoic acid	41
11		$2-OH,4-NH_2$	*p*-Amino salicylic acid	65
12	Acetoxy acids	$2-OCOCH_3$	Aspirin	66

* Data from Blackwell *et al.* (18).

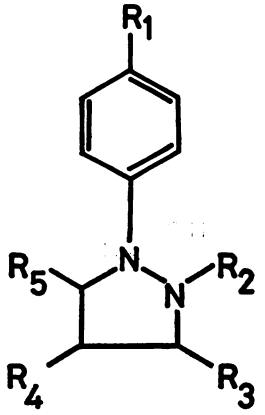


FIG. 7. Skeleton structure of pyralazone derivatives (see table 13).

centrations. Lee and Lands (93) discovered that the inhibition of PGE synthesis by Cu^{2+} was accompanied by a simultaneous increase in the amount of PGF. Copper ions therefore seem to favour an increased synthesis of PGF at the expense of PGE. The effect is enhanced by the addition of a dithiol compound (especially dihydrolipoamide) to the reaction mixture.

Apart from these heavy metals, some other ions also appear to be inhibitory. Wallach and Daniels (157) investigated prostaglandin synthesis by acetone-pentane powders of SSV microsomes at pH 8 in six different buffer systems; maximal conversion of sub-

strate occurred in a sodium ethylenediamine tetraacetate buffer; this was reduced to almost half when tris-HCl of the same molarity was substituted, and to almost a quarter when triethanolamine was used.

The work of Abdulla and McFarlane (2) suggests a possible interaction between adenine nucleotides, sodium ions and prostaglandin biosynthesis, but their experiments were performed with unseparated homogenates of brain tissue and a direct effect by these agents on the synthetic enzymes remains to be established. Dibutyryl adenine 3′,5′-cyclic phosphate (0.1 mM) is reported to inhibit the release of E_2 by colonic carcinoma cells in culture (81).

Cyanide (1 mM) has uncertain activity against the SSV synthetase (90, 108) but inhibits the synthetase from rabbit kidney medulla (78), 8-hydroxyquinolone similarly inhibits the rabbit kidney enzyme but not the SSV synthetase. Other metal complexing agents found to be inactive against the SSV enzymes in concentrations of 1 mM include ethylenediamine tetraacetate, 2, 2¹-dipyridyl and diethyldithiocarbamate (108). *o*-Phenanthroline reversibly inhibited the enzyme at high concentrations (5–10 mM; see ref. 138); carbon monoxide was inactive against the BSV synthetase (67).

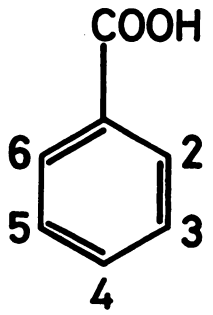


FIG. 8. Skeleton structure of benzoic acid derivatives (see table 14).

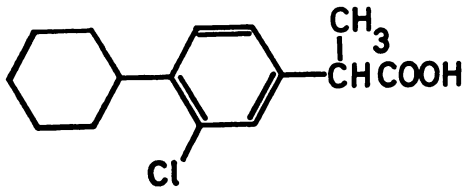


FIG. 9. Structure of CCMP [(+)3-chloro-4-cyclohexyl-α-methylphenylacetic acid].

Lands *et al.* (90) tested a series of Cu^{++} complexing agents against a preparation of SSV enzymes and found several of these to be inhibitory. The most potent, toluene-3,4-dithiol had an I50 of 0.15 mM, the I50 of the other compounds were of the order of 10^{-3} or even 10^{-2} M. This and other data led these workers to speculate that Cu^{++} may play a role in the oxygenation reaction. Maddox (100) suggested that the presence of Cu^{++} in the reaction mixture leads to an activation of the $F_{2\alpha}$ synthesising component and a progressive inactivation of the E_2 synthesising component. This inactivation was accelerated by the Cu^{++} chelating agent DL-penicillamine. Inactivation of the $F_{2\alpha}$ synthesising system also occurred in the presence of excess Cu^{++} (>1 mM) and this process was prevented by Cu^{++} complexing agents. Only one agent, N-phenyl anthranilic acid, was found actually to inhibit the enzyme.

Nugteren *et al.* (108) were the first to observe that anti-oxidants could act as co-factors for the synthetase reaction, although it now seems that this action is not related to their anti-oxidant properties, but rather, to their ability to act as a source of reducing equivalents. Nugteren also observed that high concentrations of anti-oxidants in-

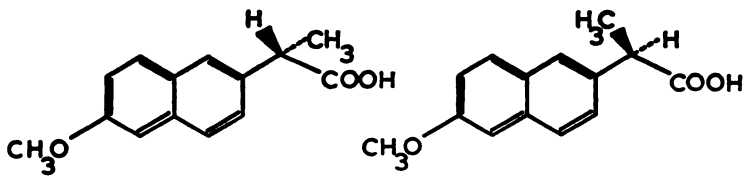


FIG. 10. Enantiomers of naproxen [redrawn from Tomlinson *et al.* (147)].

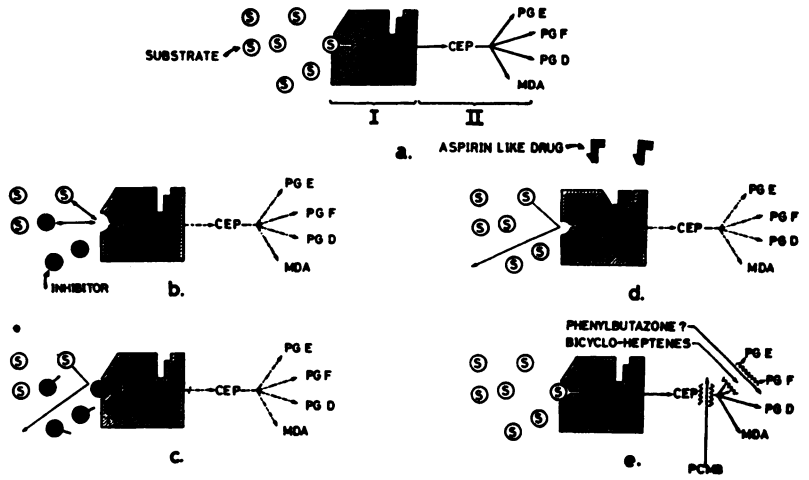


FIG. 11. Alternative modes of synthetase inhibition. A simplified (and inevitably speculative) scheme based on the literature available at the time of writing. a. The uninhibited synthetase. This is depicted as consisting of two components: I, the dioxygenase component responsible for cyclic endoperoxide formation; and II, the enzymatic machinery responsible for endoperoxide breakdown. The dioxygenase component has at least two binding sites, a substrate site (designated S) and an inhibitor binding site (designated I). b. Competitive inhibition by substrate analogues such as the positional isomers described by Nugteren (107). This is characterised by competitive and reversible inhibition at the substrate site. c. Irreversible inhibition by substrate analogues such as the acetylenic analogues described by Ahern and Downing (3) and Lands' group (90, 149). Inhibition by these fatty acids has an initial competitive component, but ultimately, the inhibitor reacts with the active site in some manner such as to irreversibly inactivate the enzyme. This inactivation is prevented by diethyldithiocarbamic acid. d. Inhibition by aspirin-like drugs. The majority of these compounds are irreversible inhibitors although they have an initial competitive component. Evidence suggests that they combine irreversibly with an inhibitor binding site in sufficient proximity to the substrate to reduce the catalytic activity of the enzyme in a time-dependent fashion. This may be an allosteric mechanism, although it is not definitely proven. Combination of the aspirin-like drugs with the active site is prevented by the reversible inhibitor *o*-phenanthroline (90, 138). e. Inhibition by miscellaneous agents. All these compounds act on component II in some way, either by preventing endoperoxide breakdown completely (as in the case of *p*-chloromercuribenzoic acid and other sulphydryl binding drugs), or selectively (as in the case of phenylbutazone and the bicycloheptenes).

hibited the enzyme; for example when propyl gallate was used as a cofactor, a marked stimulation of synthesis was observed at concentrations below 5×10^{-4} M, but a 70% inhibition was seen when the concentration was increased to 9×10^{-4} M. Another anti-oxidant, α-tocopherol caused a 50% inhibition of synthesis at 1 mM. Vanderhoek and Lands (150) and Lands *et al.* (90) investigated the inhibitory activity of a wide range of anti-oxidants. Amongst the most potent were santoquin, α-naphthol and butylated hydroxyanisole, the I50 values for these compounds being of the order of 10^{-6} M. The majority of these agents were non-competitive inhibitors, but none caused any time dependent destruction of the enzyme. Anti-oxidant inhibitors of both the competitive and non-competitive type were also tested against the soyabean lipoxidase enzyme. Some inhibitory activity was found, although this did not necessarily correlate with the antisynthetase potency. An interesting finding was that the antioxidant α-naphthol prevented the time destruction of the enzyme by TYA (see section VI).

Takeguchi and Sih (144) investigated the structural requirements for cofactor activity and reported a series of 14 hydroxy-naphthalene derivatives, some of which were extremely potent inhibitors of the BSV synthetase; the I50 for the most potent deriva-

tive (2,7-dihydroxy naphthalene) was only 2 μM.

Smith and Lands (139) found that PGE_2 synthesis in homogenates of sheep vesicular glands can be suppressed by the addition of rat liver GSH peroxidase and GSH to the reaction mixture. This inhibition may be due to the destruction of the hydroperoxide intermediate (see fig. 2). GSH peroxidase also inhibits the synthesis of prostaglandins by acetone powders of vesicular glands, but only after prior activation of the enzyme by phenol. Plant lipoxygenase is also inhibited.

The inhibitors described in this section have yielded some information about the *in vitro* synthesis of prostaglandins, but none, with the possible exception of the naphthenediols described by Takeguchi and Sih (144), are likely to be of value in studying prostaglandin synthesis *in vivo*.

IX. Inhibition of Prostaglandin Catabolism

A. General Remarks

Most prostaglandins are rapidly inactivated *in vivo*, and by isolated tissues or broken cell homogenates *in vitro*. Four enzymic processes which degrade prostaglandins have been identified; these are the formation of the 15-keto derivative (catalysed by prostaglandin 15-hydroxy dehydrogenase, PGDH), saturation of the Δ^{13} double bond (catalysed by prostaglandin reductase), and oxidation of the hydrocarbon side chains beginning at either the β (carboxylic) or ω terminal (β or ω oxidation) (for references see 116, 128).

The most significant of these appears to be the dehydrogenase; this NAD^+ dependent enzyme, which is found in the high speed supernatant of cells, catalyses the conversion of the prostaglandin substrate to a keto derivative by oxidation of the C-15 alcohol moiety. PGE_1 and PGE_2 are the best substrates; E_3, F and A type prostaglandins are less readily oxidised. B-type prostaglandins and a wide range of other substrates, including steroid alcohols and carbohydrates, are not oxidised at all. The distribution of the enzyme in the tissues of swine has been investigated by Änggård *et al.* (6) who found the highest concentrations in lung, spleen and kidney cortex. Testicle, stomach, small intestine, heart and adipose tissue contained smaller amounts. The enzyme is also present in the tissues of rat, guinea-pig and man; a very high concentration of PGDH is found in human placenta (8). Since the keto derivative possesses little biological activity it is likely that this enzyme is important for inactivation of prostaglandins *in vivo*, indeed, it has been shown to participate in the metabolism of prostaglandins in man and other animals (54).

B. Inhibition of PGDH

1. Substrate or cofactor analogues. As was the case with the synthetase, much attention has been paid to the possibility of finding analogues which interfere with the enzymatic attack on the natural substrate. Such analogues provide not only a means of blocking the metabolism of prostaglandins, but are also of value in investigating the binding of prostaglandin substrates to the active site.

Nakano *et al.* (106) studied the oxidation of PGE_1 by a purified preparation of PGDH

TABLE 15

Compounds which inhibit prostaglandin dehydrogenase

Inhibitors	Reference
rac-prostanoic acid	54
Racemic 7-thia-13-prostynoic acid	54
15-Epimers of PGE_1	101, 106
B-type prostaglandins	106
Stereoisomers of 7-oxa-$PGF_{1\alpha}$	101
Arachidonic, linolenic and oleic acids, and their coenzyme A derivatives	101
Polyphloretin phosphate	55, 101
Substituted pyridines and several nucleosides and nucleotides	101
Methylxanthines	101
Indomethacin	27, 148
Niflumic and meclofenamic acid	27
Aspirin	56
Sulphydryl inhibitors	33, 109
Ca^{++} ions	99

from swine lung. They found that the enzyme was stereospecific with regard to the configuration at C-15 and that a synthetic epimer of E_1, 15-R-PGE_1 was a non-competitive inhibitor of the enzyme. B-type prostaglandins, which are not substrates, were also non-competitive inhibitors but dihydro-PGE_1 and 8-iso-PGE_1 were inactive.

Fried *et al.* (54) studied the inhibition of human placental PGDH by seven prostaglandin analogues. The most active pair, *rac*-prostanoic acid and racemic 7-thia-13-prostynoic acid had I50 values of 15 mM and 13 mM, respectively. All the analogues were "mixed" inhibitors except 7-thia-13-prostynoic acid which was competitive.

In 1972 Marrazzi and Matschinsky (101) published a detailed paper on the structural requirements for substrate and cofactor binding to the purified swine lung PGDH. With $PGF_{1\alpha}$ as a substrate, these authors tested analogues of both substrate and cofactor (NAD^+) as inhibitors. A derivative of $F_{1\alpha}$, 7-oxa-$PGF_{1\alpha}$ (oxygen substituted at C-7) had the same V_{max} as the original substrate (but a much lower affinity) and various stereoisomers of this derivative (the 15-epimer, the optical antipode and an analogue with both these modifications) all showed mixed inhibition of PGDH, as did several fatty acids (arachidonic, linolenic and oleic and their respective coenzyme A derivatives). Polyphloretin phosphate, a high molecular weight compound which antagonises some of the actions of prostaglandins on smooth muscle was also a good competitive inhibitor of the enzyme when E_1 was the substrate, although SC19222, another prostaglandin antagonist was not. In this connection it is interesting to note the observation of Ganesan and Karim (55). These authors reported that polyphloretin phosphate temporarily potentiates the contractile activity of PGE_2 (but not the 15-methyl derivative) on the rat fundus and produced evidence to show that this effect is due to inhibition of PGDH.

Crutchley and Piper (33) have recently reported that both polyphloretin phosphate and diphloretin phosphate inhibit the pulmonary inactivation of PGE_2, $PGF_{2\alpha}$ and $PGF_{2\beta}$ in concentrations of only 10^{-7} M. These compounds also inhibited the metabolism of PGE_2 by a cell free preparation of PGDH.

With regard to the cofactor site, certain NAD^+ analogues or derivatives were inhibitory and a range of substituted pyridines were non-competitive inhibitors. Several nucleosides and nucleotides in concentrations of 3 to 10 mM were also active, as were the methylxanthines caffeine and theophylline as well as aminophylline. Amongst inactive compounds were the barbiturates.

2. Pharmacological agents. Several apparently anomalous observations concerning the inhibition of PG synthetase by aspirin-like drugs prompted a group at the Royal College of Surgeons, London, led by Vane, to suspect that certain of these drugs inhibit prostaglandin inactivation as well as the synthetase (148). It was subsequently found that indomethacin inhibited the NAD^+-dependent destruction of E_1 or E_2 by the high-speed supernatant of dog spleen. At a concentration of 15 μM the destruction of E_1 (as measured by its decrease in biological potency) was inhibited by almost 40%. The destruction of E_2 by a similar preparation was abolished by indomethacin in a concentration of 110 μM. Cheung and Cushman (27), pursuing this line of enquire, used a semipurified preparation of rabbit lung dehydrogenase to study the inhibitory effects of several aspirin-like drugs; indomethacin in concentrations of 1 mM gave 93% inhibition of E_2 metabolism, niflumic acid in a concentration of 5×10^{-4} M inhibited 38% and meclofenamic acid in the same concentration inhibited 14%. Aspirin, naproxen, ibuprofen, phenylbutazone and benzydamine were inactive.

At least one study supports the concept that aspirin-like drugs can inhibit the dehydrogenase *in vivo*; Gibson *et al.* (56) studied the pulmonary inactivation of $F_{2\alpha}$ during one passage through the pulmonary circulation of dogs. The lungs can almost completely in-

activate PGE_1, PGE_2 and $PGF_{2\alpha}$ during one circulation through the pulmonary vascular bed (47). This is thought to be largely due to the activity of PGDH. In Gibson's experiments the mean removal of $F_{2\alpha}$ on passage through the lungs (6 dogs) was 91.9%, after treatment with aspirin (50 mg/kg repeated hourly) there was a small but statistically highly significant decrease (almost 10%) in the destruction. When the same experiment was performed in sheep, however, aspirin was inactive, indicating a species difference. It would be of interest to determine the effects of aspirin-like drugs on other vascular beds, such as those of the liver or the hind quarters, which have also been shown to inactivate prostaglandins. Against Gibson's result one must balance the results of Hamberg and Samuelsson (68) who found that administration of indomethacin to guinea-pigs (50 mg/day) did not change the *in vivo* metabolism of tritiated PGE_2.

It is not known how the aspirin-like drugs inhibit PGDH; however, the salicylates are known to inhibit several dehydrogenases (137), probably by competing for the co-factor site, so possibly a similar action could account for inhibition of PGDH. It seems from what limited data are available that the concentrations required to inhibit the synthetase are considerably less than those which produce a corresponding inhibition of the dehydrogenase; for example, the figure quoted earlier for inhibition of the crude destroying enzyme from particle-free supernatants of spleen was 100 times higher than the I50 concentration for the particulate fraction synthetase from the same tissue.

Marrazzi and Matschinsky (101) pointed out that there are certain similarities between the prostaglandin receptor and PGDH, and proposed that under some circumstances the dehydrogenase may be regarded as a model for the receptor. In this connection it is interesting to recall that certain aspirin-like drugs (especially the fenamates) have been reported to block the actions of prostaglandins on smooth muscle (30, 85, 96).

Sulphydryl inhibitors such as *p*-chloromercuribenzoic acid or N-ethylmaleimide in concentrations of 10^{-3} to 10^{-5} M inhibit PGDH preparations from guinea-pig lung (33), canine myocardium (99) and rat stomach (109).

Limas and Cohn (99) have reported that myocardial PGDH is inhibited by Ca^{++} ions in concentrations of 10^{-7} M and above.

X. Summary

Three classes of compounds inhibit prostaglandin synthetase. The first group consists of the substrate analogues. The specificity of these analogues is unknown but many possess good inhibitory potency which makes them useful tools to demonstrate the involvement of the synthetase enzyme. However, although they are active in some organised tissue preparations, their use *in vivo* has not been widespread. The absorption, excretion and distribution of these fatty acids is not yet fully understood and this tends to detract from their usefulness in whole animal work. Apart from the substrate analogues a number of other fatty acids also inhibit the synthetase but probably in a non-specific manner and only in high concentrations.

The second class of inhibitors consists of the aspirin-like drugs. Although these may not be as specific as the substrate analogues, they have several advantages; they are readily available, easily administered and in many species abolish prostaglandin synthesis almost completely in therapeutic doses. In addition, a considerable amount is known concerning their absorption, distribution and excretion. The final group of inhibitors which inhibit the synthetase includes such diverse agents as metal ions, anti-oxidants and nucleotides. The concentrations necessary to achieve inhibition are often high and no degree of specificity can be claimed. Thus, these agents are not likely to be of value for *in vivo* work, although *in vitro* studies with different cofactors and ions may contribute

to our understanding of how the synthetase system is regulated.

Concerning the inhibition of the dehydrogenase, the situation is far less clear. Certain substrate analogues appear to be good inhibitors of the purified enzyme, although they have not been tested *in vivo*. Some of the aspirin-like drugs are also active in this respect, but the concentrations are again rather high. Polyphloretin phosphate inhibits PGDH and on this basis it may be worth investigating other prostaglandin blocking agents or agonists as inhibitors of PGDH.

In conclusion, the participation of prostaglandins in complex biological events may be investigated with the aid of substrate analogues or with pharmacological agents which block prostaglandin biosynthesis both *in vitro* and *in vivo*. At the time of writing no convenient technique for producing the complementary effect, by inhibition of metabolism, is available.

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REFERENCES

1. Aas, K. A. and Gardner, F. H.: Survival of blood platelets labelled with chromium^{51}. J. Clin. Invest. 37: 1232–1237, 1958.
2. Abdulla, Y. H. and McFarlane, E.: Control of prostaglandin biosynthesis in rat brain homogenates by adenine nucleotides. Biochem. Pharmacol. 21: 2841–2847, 1972.
3. Ahern, D. G. and Downing, D. T.: Inhibition of prostaglandin biosynthesis by eicosa-5,8,11,14-tetraynoic acid. Biochim. Biophys. Acta 210: 456–461, 1970.
4. Aiken, J. W.: Aspirin and indomethacin prolong parturition in rats: evidence that prostaglandins contribute to expulsion of foetus. Nature (London) 240: 21–25, 1972.
5. Aiken, J. W. and Vane, J. R.: Blockade of angiotensin release from dog kidney by indomethacin. Pharmacologist 13: 15, 1971.
6. Änggård, E., Larsson, C. and Samuelsson, B.: The distribution of 15-hydroxy-prostaglandin dehydrogenase and prostaglandin-Δ^{13}-reductase in tissues of the swine. Acta Physiol. Scand. 81: 396–404, 1971.
7. Änggård, E. and Samuelsson, B.: Biosynthesis of prostaglandins from arachidonic acid in guinea-pig lung. J. Biol. Chem. 240: 3518–3521, 1965.
8. Änggård, E. and Samuelsson, B.: Purification and properties of a 15-hydroxy-prostaglandin dehydrogenase from swine lung. Ark. Kemi 25: 293–300, 1966.
9. Arata, L. and Mongola, S.: Richerche sul metabolismo dell'acido salicilico nell'uomo. Progr. Méd. 18: 229–234, 1962.
10. Arturson, G. and Jonsson, C-E.: Effects of indomethacin on the transcapillary leakage of macromolecules and the efflux of prostaglandins in the paw lymph following experimental scalding injury. Uppsala J. Med. Sci., in press, 1974.
11. Batterman, R. C. and Scammer, E. M.: Fate of gentisic acid in man as influenced by alkalinisation and acidification. Proc. Soc. Exp. Biol. 82: 376–379, 1953.
12. Bergström, S., Carlson, L. A. and Orö, L.: Effect of prostaglandins on catecholamine-induced changes in the free fatty acids of plasma and in blood pressure in the dog. Acta Physiol. Scand. 60: 170–180, 1964.
13. Bergström, S., Carlson, L. A. and Weeks, J. R.: The prostaglandins: A family of biologically active lipids. Pharmacol. Rev. 20: 1–48, 1968.
14. Bergström, S., Danielsson, H. and Samuelsson, B.: The enzymatic formation of prostaglandin E_2 from arachidonic acid. Prostaglandins and related factors, 32. Biochim. Biophys. Acta 90: 207–210, 1964.
15. Bhattacherjee, P. and Eakins, K.: Pharmacologist, 15: 209–215, 1973.
16. Blackwell, G. J., Flower, R. J. and Vane, J. R.: Unpublished data.
17. Blackwell, G. J., Flower, R. J. and Vane, J. R.: Studies on prostaglandin metabolism in rabbit kidney. In preparation, 1973.
18. Blackwell, G. J., Flower, R. J. and Vane, J. R.: Anti-synthetase potency of salicylic acid metabolites. In preparation, 1973.
19. Bonsher, J. R., Tillemend, J. G. and Larousse, C.: Metabolisme de l'acide niflumique chez l'homme. Therapie 26: 211, 1971.
20. Brown, J. H. and Pollock, S. H.: Inhibition of elastase and collagenase by anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 135: 793–795, 1970.
21. Brown, J. H., Taylor, J. L. and Watkins, I. W.: Effect of pH on erythrocyte stabilisation by anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 136: 137–140, 1971.
22. Burke, G.: Aspirin and indomethacin abolish thyrotropin-induced increase in thyroid cell prostaglandins. Prostaglandins 2: 413–415, 1972.
23. Burns, J. J., Rose, R. K., Chenkin, T., Goldman, A., Schulert, A. and Brodie, B. B.: The physiological disposition of phenylbutazone (Butazolodin) in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther. 109: 346, 1953.
24. Burstein, S., Levin, E. and Varanelli, C.: Prostaglandins and cannabis. II. Inhibition of biosynthesis by the naturally occurring cannabinoids. Biochem. Pharmacol. 22: 2905–2910, 1973.
25. Burstein, S. and Raz, A.: Inhibition of prostaglandin E_2 biosynthesis by Δ^1-tetrahydrocannabinol. Prostaglandins 2: 369–374, 1972.
26. Cammock, S.: Conversion of PGE_1 to a PGA_1-like compound by rat kidney homogenates. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 10, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg Braunschweig, 1973.
27. Cheung, H. S. and Cushman, D. W.: Unpublished observations, 1972.
28. Christ, E. J. and Dorp, D. A. van: Comparative aspects of prostaglandin biosynthesis in animal tissues. Biochim. Biophys. Acta 270: 537–545, 1972.
29. Christ, E. J. and Dorp, D. A. van: Comparative aspects of prostaglandin biosynthesis in animal tissue. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 35–38, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.
30. Collier, H. O. J. and Swaitman, W. J. F.: Antagonism by fenamates of prostaglandin $F_{2\alpha}$ and of slow reacting substance on human bronchial muscle. Nature (London) 219: 864–865, 1968.
31. Collier, J. G. and Flower, R. J.: Effect of aspirin on human seminal prostaglandins. Lancet ii: 863–863, 1971.

32. Collier, J. G., Herman, A. G. and Vane, J. R.: Appearance of prostaglandins in the renal venous blood of dogs in response to acute systemic hypotension produced by bleeding or endotoxin. J. Physiol. (London) 230: 19–20P, 1973.

33. Crutchley, D. J. and Piper, P. J.: Inhibition of the inactivation of prostaglandins in guinea-pig lungs. Naunyn Schmeideberg's Arch. Pharmacol. Exp. Pathol. Suppl. 279: 27, 1973.

34. Davis, H.: Output of prostaglandins from the rabbit kidney. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 55, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.

35. Davison, P., Ramwell, P. W. and Willis, A. L.: Inhibition of intestinal tone and prostaglandin synthesis by 5,8,11,14-tetraynoic acid. Brit. J. Pharmacol. 46: 547–548, 1973.

36. Dorp, D. A. van: Aspects of the biosynthesis of prostaglandins. Progr. Biochem. Pharmacol. 3: 71–79, 1967.

37. Dorp, D. A. van: Recent developments in the biosynthesis and the analysis of prostaglandins. Ann. N.Y. Acad. Sci. 180: 181–199, 1971.

38. Dorp, D. A. van, Beerthuis, R. K., Nugteren, D. H. and Vonkeman, H.: The biosynthesis of prostaglandins. Biochim. Biophys. Acta 90: 204–207, 1964.

39. Downing, D. T.: Differential inhibition of prostaglandin synthetase and soybean lipoxidase. Prostaglandins 1: 437–441, 1972.

40. Downing, D. T., Ahern, D. G. and Bachta, M.: Enzyme inhibition by acetylenic compounds. Biochem. Biophys. Res. Commun. 46: 218–223, 1970.

41. Downing, D. T., Barve, J. A., Gunstone, F. D., Jacobsberg, M. and Lie, Ken Jie: Structural requirements of acetylenic fatty acids for inhibition of soybean lipoxygenase and prostaglandin synthetase. Biochim. Biophys. Acta 280: 343–347, 1972.

42. Euler, U. S. von: On the specific vaso-dilating and plain muscle stimulating substance from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). J. Physiol. (London) 88: 213–234, 1937.

43. Ferreira, S. H.: Prostaglandins, inflammation and aspirin-like drugs. Med. Today 7: 29–40, 1973.

44. Ferreira, S. H., Herman, A. and Vane, J. R.: Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. Brit. J. Pharmacol. 44: 338P, 1972.

45. Ferreira, S. H. and Moncada, S.: Inhibition of prostaglandin synthesis augments the effects of sympathetic nerve stimulation. Brit. J. Pharmacol. 43: 419–420P, 1971.

46. Ferreira, S. H., Moncada, S. and Vane, J. R.: Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature, New Biol. 231: 237–239, 1971.

47. Ferreira, S. H. and Vane, J. R.: Prostaglandins. Their disappearance from and release into the circulation. Nature (London) 216: 868–873, 1967.

48. Ferreira, S. H. and Vane, J. R.: Inhibition of prostaglandin biosynthesis; an explanation of the therapeutic effects of non-steroid anti-inflammatory agents. *In* Seminaire Inserm Prostaglandines (1973), pp. 345–357, Editions Inserm, Paris, 1973.

49. Ferreira, S. H. and Vane, J. R.: New aspects of the mode of action of non-steroid anti-inflammatory drugs. Annu. Rev. Pharmacol., in press, 1974.

50. Flores, A. G. A. and Sharp, G. W. G.: Exogenous prostaglandins and osmotic water flow in the toad bladder. Amer. J. Physiol. 223: 1302–1307, 1972.

51. Flower, R. J., Cheung, H. S. and Cushman, D. W.: Quantitative determination of prostaglandins and malondialdehyde formed by the arachidonate oxygenase system of bovine seminal vesicles. Prostaglandins 4: 325–341, 1973.

52. Flower, R. J., Gryglewski, R., Herbaczynska-Cedro, K. and Vane, J. R.: The effects of anti-inflammatory drugs on prostaglandin biosynthesis. Nature, New Biol. 238: 104–106, 1972.

53. Flower, R. J. and Vane, J. R.: Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature (London) 240: 410–411, 1972.

54. Fried, J., Mehra, M. M. and Gabbe, B. J.: Novel selective inhibitors of human palcental PG-15-dehydrogenase. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 18, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.

55. Ganesan, P. and Karim, S. M. M.: Polyphloretin phosphate temporarily potentiates PGE_2 on the rat fundus, probably by inhibiting PG-15-hydroxy dehydrogenase. J. Pharm. Pharmacol. 25: 229–233, 1973.

56. Gibson, E. L., Hodge, R. L., Jackson, H. R., Katic, F. P. and Stevens, A. M.: The effect of aspirin on the pulmonary removal of prostaglandin $F_{2\alpha}$ in dogs and sheep. Proceedings of the Australian Physiological Society, Adelaide, May 1972.

57. Glasco, A. J.: Personal communication, 1973.

58. Glasco, A. J.: Fenamates in medicine. *In* Supplementum to Annals of Physical Medicine, A Symposium, ed. by P. Hume Kendall, pp. 23–36, Balliere, Tindall and Cassell, London, 1966.

59. Grant, N. H., Alburn, H. E. and Kryzanavskas, C.: Stabilization of serum albumin by anti-inflammatory drugs. Biochem. Pharmacol. 19: 715–722, 1970.

60. Greaves, M. W. and McDonald-Gibson, W. J.: Inhibition of prostaglandin biosynthesis by cortico-steroids. Brit. Med. J. 2: 83–84, 1972.

61. Greaves, M. W. and McDonald-Gibson, W. J.: Anti-inflammatory agents and prostaglandin biosynthesis. Brit. Med. J. 3: 527, 1972.

62. Gryglewski, R., Flower, R. J., Herbaczynska-Cedro, K. and Vane, J. R.: Inhibition of prostaglandin synthetase by anti-inflammatory drugs. Proceedings of the Fifth International Congress of Pharmacology, San Francisco, p. 90, 1972.

63. Gryglewski, R. and Vane, J. R.: The release of prostaglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs. Brit. J. Pharmacol. 45: 37–47, 1972.

64. Gryglewski, R. and Vane, J. R.: The generation from arachidonic acid of rabbit aorta contracting substance (RCS) by a microsomal enzyme preparation which also generates prostaglandins. Brit. J. Pharmacol. 46: 449–457, 1972.

65. Ham, E. A., Cirillo, K. J., Zanetti, M., Shen, T. Y. and Kuehl, F. A.: Studies on the mode of action of non-steroidal, anti-inflammatory agents. *In* Prostaglandins in Cellular Biology, ed. by P. W. Ramwell and B. B. Pharriss, pp. 345–352, Plenum Press, New York, 1972.

66. Hamberg, M.: Inhibition of prostaglandin synthesis in man. Biochem. Biophys. Res. Commun. 49: 720–726, 1972.

67. Hamberg, M. and Samuelsson, B.: On the mechanism of the biosynthesis of prostaglandins E_1 and $F_{1\alpha}$. J. Biol. Chem. 242: 5336–5343, 1967.

68. Hamberg, M. and Samuelsson, B.: On the metabolism of prostaglandins E_1 and E_2 in the guinea-pig. J. Biol. Chem. 247: 3495–3502, 1972.

69. Hawkins, D., Pinckard, R. N. and Farr, R. S.: Acetylation of human serum albumin by acetyl salicylic acid. Science 160: 780–781, 1968.

70. Hedqvist, P.: Prostaglandin mediated control of sympathetic neuromuscular transmission. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 75, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.

71. Herbaczynska-Cedro, K. and Vane, J. R.: Local prosta-

glandin production contributes to bloodflow autoregulation in the dog kidney. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 45, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.

72. Hinman, J. W.: Prostaglandins: A report on early clinical studies. Postgrad. Med. J. 46: 562–575, 1970.
73. Hinman, J. W.: Prostaglandins. Annu. Rev. Biochem. 41: 161–178, 1972.
74. Horovitz, Z. P., Beer, B., Clody, D. E., Vogel, J. R. and Chasin, M.: Cyclic AMP and anxiety. Psychosomatics 13: 85–92, 1972.
75. Horton, E. W.: Hypothesis on physiological roles of prostaglandins. Physiol. Rev. 49: 122–161, 1969.
76. Horton, E. W., Jones, R. L. and Marr, G. G.: Effects of aspirin on prostaglandin and fructose levels in human semen. J. Reprod. Fert. 33: 385–392, 1973.
77. Horton, E. W., Jones, R. L., Thompson, C. J. and Poyser, N. L.: Release of prostaglandins. Ann. N. Y. Acad. Sci. 180: 351–362, 1971.
78. Hsin-Hsiung Tai and Hollander, C. S.: Regulation of prostaglandin synthetase activity in rabbit kidney medulla: A possible mechanism of hormonal and drug action. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 5, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.
79. Hucker, H. B., Zacchei, A. G., Cox, S. V., Brodie, D. A. and Cantwell, N. H. R.: Studies on the absorption, distribution and excretion of indomethacin in various species. J. Pharmacol. Exp. Ther. 153: 237–249, 1966.
80. Ignarro, L. J.: Effects of anti-inflammatory drugs on the stability of rat liver lysosomes *in vitro*. Biochem. Pharmacol. 20: 2847–2860, 1971.
81. Jaffe, B. M., Philpott, G. W. and Parker, C. W.: Prostaglandin production by cells *in vitro*. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 28. International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.
82. Jones, R. L.: 15-Hydroxy-9-oxaprost-11,13-dienoic acid as the product of a prostaglandin isomerase. J. Lipid Res. 13: 511–518, 1972.
83. Kaff, E. M. and Coburn, A. F.: Urinary metabolites of sodium salicylate. J. Biol. Chem. 145: 549–565, 1942.
84. Kocsis, J. J., Hernandovich, J., Silver, M. J., Smith, J. B. and Ingerman, C.: Duration of inhibition of platelet prostaglandin formation and aggregation by ingested aspirin or indomethacin. Prostaglandins 3: 141–145, 1973.
85. Koss, M. C., Reiger, J. A. and Nakano, J.: Hemodynamic responses to prostaglandin $F_{2\alpha}$ in the cat: Selective blockade by meclofenamic acid. Fed. Proc. 32: 3222A, 1973.
86. Ku, E. C. and Wasvary, J. M.: Inhibition of prostaglandin synthetase by Su-21534. Fed. Proc. 32: 3302, 1973.
87. Kunze, H. and Vogt, W.: Significance of phospholipase A for prostaglandin formation. Ann. N. Y. Acad. Sci. 180: 123–125, 1971.
88. Lands, W., Lee, R. and Smith, W.: Factors regulating the biosynthesis of various prostaglandins. Ann. N. Y. Acad. Sci. 180: 107–122, 1971.
89. Lands, W. E. M., LeTellier, P. R., Rome, L. H. and Vanderhoek, J. Y.: Modes of inhibiting the prostaglandin synthetic capacity of sheep vesicular gland preparations. Fed. Proc. 31: 476A, 1972.
90. Lands, W. E. M., LeTellier, P. R., Rome, L. H., and Vanderhoek, J. Y.: Inhibition of prostaglandin biosynthesis. *In* Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 15–28, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.
91. Lands, W. E. M. and Samuelsson, B.: Phospholipid precursors of prostaglandins. Biochim. Biophys. Acta 164: 426–429, 1968.
92. Lee, J. B., Crowshaw, K., Takman, B. H. and Attrep, K. A.: The identification of prostaglandins E_2, $F_{2\alpha}$ and A from rabbit kidney medulla. Biochem. J. 105: 1251–1260 1967.
93. Lee, R. E. and Lands, W. E. M.: Cofactors in the biosynthesis of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. Biochim. Biophys. Acta 260: 203–211, 1972.
94. Leonards, J. R.: The influence of solubility on the rate of gastrointestinal absorption of aspirin. Clin. Pharmacol. Ther. 4: 476–479, 1963.
95. Levine, L.: Prostaglandin production by mouse fibrosarcoma cells in culture: Inhibition by indomethacin and aspirin. Biochem. Biophys. Res. Commun. 47: 888–896, 1972.
96. Levy, B. and Lindner, H. R.: Selective blockade of the vasodepressor response to prostaglandin $F_{2\alpha}$ in the anesthetized rabbit. Brit. J. Pharmacol. 43: 236–241, 1971.
97. Levy, G. and Leonards, J. R.: Absorption metabolism and excretion of salicylates. *In* The Salicylates, ed. by M. J. H. Smith and P. K. Smith, pp. 1–48, Interscience, New York, 1966.
98. Limas, C. J. and Cohn, J. N.: Isolation and properties of myocardial prostaglandin synthetase. Cardiovasc. Res. 7: 633–638, 1973.
99. Limas, C. J. and Cohn, J. N.: Regulation of myocardial prostaglandin dehydrogenase activity: The role of cyclic 3′-5′-AMP and calcium ions. Cardiovasc. Res. 142: 1230–1234, 1973.
100. Maddox, I. S.: Copper in PG synthesis. Biochim. Biophys. Acta 306: 74–81, 1973.
101. Marrazzi, M. A. and Matschinsky, M.: Properties of 15-hydroxy prostaglandin dehydrogenase: Structural requirements for binding. Prostaglandins 1: 373–383, 1972.
102. McCall, E. and Youlten, L. J. F.: Prostaglandin E_1 synthesis by phagocytosing rabbit polymorphonuclear leucocytes; its inhibition by indomethacin and its role in chemotaxis. J. Physiol.. (London) 234: 98–100P, 1973.
103. McDonald-Gibson, R. G., Flack, J. D. and Ramwell, P. W.: Inhibition of prostaglandin biosynthesis by 7-oxa- and 5-oxa-prostaglandin analogues. Biochem. J. 133: 117–120, 1973.
104. Milton, A. S.: Prostaglandin release in the central nervous system during endotoxin-induced fever. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhart, vol. 9, p. 79, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.
105. Minkes, M. S., Douglas, J. R. and Needleman, P.: Prostaglandin release by the isolated perfused rabbit heart. Prostaglandins 3: 429–445 1973.
106. Nakano, J., Änggård, E. and Samuelsson, B.: 15-Hydroxy-prostanoate dehydrogenase prostaglandins as substrates and inhibitors. Eur. J. Biochem. 11: 386–389, 1969.
107. Nugteren, D. H.: Inhibition of prostaglandin biosynthesis by 8 *cis*, 12 *trans*, 14 *cis*-eicosatetraenoic acid. Biochem. Biophys. Acta 210: 171–176, 1970.
108. Nugteren, D. H., Beerthuis, R. K. and Dorp, D. A. van: The enzymic conversion of all-*cis* 8,11,14-eicosatrienoic acid into prostaglandin E_1. Rec. Trav. Chim. Pays-Bas 85: 405–419, 1966.
109. Pace-Asciak, C., Morawska, K. and Wolfe, L. S.: Metabolism of prostaglandin $F_{2\alpha}$ by the rat stomach. Biochim. Biophys. Acta 218: 288–295, 1970.
110. Pace-Asciak, C. and Wolfe, L. S.: Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids. Biochim. Biophys. Acta 152: 784–787, 1968.
111. Palmer, M. A., Piper, P. J. and Vane, J. R.: Release of rabbit aorta contracting substance (RCS) and prostaglandins induced by chemical or mechanical stimulation of guinea-pig lungs. Brit. J. Pharmacol., 49: 226–242, 1973.
112. Paulus, H. E. and Whitehouse, M. W.: Non-steroid anti-inflammatory agents. Annu. Rev. Pharmacol. 13: 107–125, 1973.

113. PHELPS, P.: PMN leucocyte motility *in vitro*. II. Stimulatory effect of monosodium urate crystals and urate in solution. Partial inhibition by colchicine and indomethacin. Arthritis Rheum. 12: 180–196, 1969.

114. PINCKARD, R. N., HAWKINS, D. AND FARR, R. S.: *In vitro* acetylation of plasma proteins, ensymes and DNA by aspirin. Nature (London) 219: 68–69, 1968.

115. PIPER, P. J.: Personal communication, 1972.

116. PIPER, P. J.: Distribution and metabolism. *In* The Prostaglandins; Pharmacological and Therapeutic Advances, ed. by M. F. Cuthbert, pp. 125–150, Heinemann Medical Books, London, 1972.

117. PIPER, P. J. AND VANE, J. R.: Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature (London) 233: 29–35, 1969.

118. PIPER, P. J. AND VANE, J. R.: The release of prostaglandins from lung and other tissues. Ann. N. Y. Acad. Sci. 180: 363–385, 1971.

119. POLLARD, J. AND FLOWER, R. J.: Unpublished observations, 1973.

120. POYSER, N. L. Production of prostaglandins by guinea-pig uterus. J. Endocrinol. 54: 147–159, 1972.

121. PRESCOTT, L. F., SANDOR, M., LEVIN, W. AND CONNEY, A. H.: The comparative metabolism of phenacetin and N-acetyl-*p*-aminophenol in man, with particular reference to the kidney. Clin. Pharmacol. Ther. 9: 605–614, 1968.

122. RAMWELL, P. W. AND SHAW, J. E.: Biological significance of the prostaglandins. Recent Progr. Hormone Res. 26: 139–178, 1970.

123. RAS, A., STERN, H. AND KENIG-WAKSHAL, R.: Indomethacin and aspirin inhibition of PGE₂ synthesis by sheep seminal vesicles microsome powder and seminal vesicle slices. Prostaglandins 3: 337–352, 1973.

124. ROBINSON, D. R., SMITH, H. AND LEVINE, L.: Prostaglandin (PG) synthesis by human synovial cultures and its stimulation by colchicine. Proceedings of the 16th Interim Scientific Session of the American Rheumatism Association Section of the Arthritis Foundation, p. 21, December, 1972.

125. ROSEMAN, S. AND DORFMAN, A. The determination and metabolism of gentisic acid. J. Biol. Chem. 192: 105–114, 1951.

126. RUSSELL, P. T., ALAM, N. AND CLARY, P.: Impaired placentae conversion of prostaglandin E_1 to A_1 in toxemia of pregnancy. Fed. Proc. 32: 330A, 1973.

127. SAMUELSSON, B.: Biosynthesis of prostaglandins. Progr. Biochem. Pharmacol. 5: 109–128, 1969.

128. SAMUELSSON, B.: Structures, biosynthesis and metabolism of prostaglandins. *In* Lipid Metabolism, ed. by S. Wakil, pp. 107–153, Academic Press, New York, London, 1970.

129. SAMUELSSON, B.: Biosynthesis of prostaglandins. Fed. Proc. 31: 1442–1450, 1972.

130. SAMUELSSON, B., GRANSTRÖM, E. AND HAMBERG, M.: On the mechanism of the biosynthesis of prostaglandins. *In* Nobel Symposium 2 on Prostaglandins, ed. by S. Bergström and B. Samuelsson, pp. 31–44, Almqvist and Wiksell, Stockholm, 1967.

131. SAMUELSSON, B. AND WENNMALM, A.: Increased nerve stimulation induced release of noradrenaline from rabbit heart after inhibition of prostaglandin synthesis. Acta Physiol. Scand. 83: 163–168, 1971.

132. SHEN, T. Y.: Perspective in non-steroidal, anti-inflammatory agents. Angew Chem. Int. Ed. 11: 460–472, 1972.

133. SIH, C. J. AND TAKEGUCHI, C. A.: Biosynthesis. *In* The Prostaglandins, ed. P. W. Ramwell, vol. 1, chap. 3, pp. 83–100, Plenum Press, New York, London, 1973.

134. SKIDMORE, I. F. AND WHITEHOUSE, M. W.: Biochemical properties of anti-inflammatory drugs. VIII. Biochem. Pharmacol. 15: 1965–1983, 1966.

135. SKIDMORE, I. F. AND WHITEHOUSE, M. W.: Concerning the regulation of some diverse biochemical reactions underlying the inflammatory response by salicylic acid, phenylbutazone and other acidic anti-rheumatic drugs. J. Pharm. Pharmacol. 18: 558–560, 1966.

136. SMITH, J. B. AND WILLIS, A. L.: Aspirin selectively inhibits prostaglandin production in human platelets. Nature, New Biol. 231: 235–237, 1971.

137. SMITH, M. J. H. AND DAWKINS, P. D.: Salicylate and ensymes. J. Pharm. Pharmacol. 23: 729–744, 1971.

138. SMITH, W. L. AND LANDS, W. E. M.: Stimulation and blockade of prostaglandin biosynthesis. J. Biol. Chem. 21: 6700–6702, 1971.

139. SMITH, W. L. AND LANDS, W. E. M.: Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland. Biochemistry 11: 3276–3285, 1972.

140. SOMOVA, L.: Inhibition of prostaglandin synthesis in the kidneys by aspirin-like drugs. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 53, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.

141. STEINER, M.: Platelet protein synthesis studied in a cell-free system. Experientia (Basel) 26: 786–789, 1970.

142. STYKS, J. A. C. AND MADDOX, I. S.: Prostaglandin production by experimental tumours and effects of anti-inflammatory compounds. Nature, New Biol. 237: 59–60, 1972.

143. TAKEGUCHI, C., KOHNO, E. AND SIH, C. J.: Mechanism of prostaglandin biosynthesis. I. Characterisation and assay of bovine prostaglandin synthetase. Biochemistry 10: 2372–2376, 1971.

144. TAKEGUCHI, C. AND SIH, C. J.: A rapid spectrophotometric assay for prostaglandin synthetase: Application to the study of non-steroidal, anti-inflammatory agents. Prostaglandins 2: 169–184, 1972.

145. TAN, L., WANG, H. M. AND LE HOUX, J. G.: Binding of prostaglandins and cytochrome P450, I.C.R.S. March 1973 (Prostaglandins (73-3) 15-18-1).

146. TAN, L., WANG, H. M. AND LE HOUX, J. G.: Binding of prostaglandins and cytochrome P450. Prostaglandins 4: 9–16, 1973.

147. TOMLINSON, R. V., RINGOLD, H. J., QURESHI, M. C. AND FORCHIELLI, E.: Relationship between inhibition of prostaglandin synthesis and drug efficacy: support for the current theory on mode of action of aspirin-like drugs. Biochem. Biophys. Res. Commun. 46: 553–559, 1972.

148. Unpublished observations, 1972.

149. VANDERHOEK, J. Y. AND LANDS, W. E. M.: Acetylenic inhibitors of sheep vesicular gland oxygenase. Biochim. Biophys. Acta 296: 374–381, 1973.

150. VANDERHOEK, J. Y. AND LANDS, W. E. M.: The inhibition of the fatty acid oxygenase of sheep vesicular gland by anti-oxidants. Biochim. Biophys. Acta 296: 382–385, 1973.

151. VANE, J. R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature, New Biol. 231: 232–235, 1971.

152. VANE, J. R.: Prostaglandins in inflammation. *In* Inflammation, Mechanisms and Control, ed. by I. H. Lepow and P. A. Ward, pp. 261–279, Academic Press, New York, London, 1972.

153. VANE, J. R.: Prostaglandins and the aspirin-like drugs. Hosp. Pract. 7: 61–71, 1972.

154. VANE, J. R.: Prostaglandins and aspirin-like drugs. *In* Pharmacology and the Future of Man, Proceedings of the Fifth International Congress of Pharmacology, San Francisco (1972), vol. 5, pp. 352–377, Karger, Basel, 1973.

155. VANE, J. R.: Inhibition of prostaglandin biosynthesis as the mechanism of action of aspirin-like drugs. *In* Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, pp. 395–412, Pergamon Press Vieweg, Braunschweig, 1973.

156. VANE, J. R. AND WILLIAMS, K. I.: Prostaglandin production contributes to the contractions of the rat isolated uterus. Brit. J. Pharmacol. 45: 146P, 1972.

157. WALLACH, D. P. AND DANIELS, E. G.: Properties of a novel preparation of prostaglandin synthetase from sheep

seminal vesicles. Biochim. Biophys. Acta 231: 445-457, 1971.

158. WEEKS, J. R.: Prostaglandins. Physiol. Rev. 52: 317-336, 1972.

159. WHITEHOUSE, M. W. AND HASLAM, J. M.: Ability of some antirheumatic drugs to uncouple oxidative phosphorylation. Nature (London) 196: 1323-1324, 1962.

160. WHITEHOUSE, M. W., KIPPEN, I. AND KLINENBERG, J. R.: Biochemical properties of anti-inflammatory drugs. XII. Inhibition of urate binding to human albumin by salicylate and phenylbutazone analogues and some novel anti-inflammatory drugs. Biochem. Pharmacol. 20: 3305-3320, 1971.

161. WILLIS, A. L., DAVISON, P., RAMWELL, P. W., BROCKLEHURST, W. E. AND SMITH, B.: Release and actions of prostaglandins in inflammation and fever: Inhibition by anti-inflammatory and anti-pyretic drugs. *In* Prostaglandins in Cellular Biology, ed. by P. W. Ramwell and B. B. Pharriss, pp. 227-259, Plenum Press, New York, 1972.

162. WLODAWER, P., SAMUELSSON, B., ALBONICO, S. M. AND CORBY, E. J.: Selective inhibition of prostaglandin synthetase by a bicyclo (3.3.1) heptene derivative. J. Amer. Chem. Soc. 93: 2815-2816, 1971.

163. WONG, P. Y. D., BEDWANI, J. R. AND CUTHBERT, A. W.: Hormone action and the levels of cyclic AMP and prostaglandins in the toad bladder. Nature, New Biol. 238: 27-31, 1972.

164. WOODBURY, D. M.: Analgesic-antipyretics, anti-inflammatory agents and inhibitors of uric acid synthesis. *In* The Pharmacological Basis of Therapeutics, 4th edition, ed. by L. S. Goodman and A. Gilman, pp. 314-347, Macmillan, New York, 1970.

165. YOSHIMOTO, A., ITO, H. AND TOMITA, K.: Cofactor requirements of the enzyme synthesising prostaglandins in bovine seminal vesicles. J. Biochem. (Tokyo) 68: 487-490, 1970.

166. ZIBOH, V. A., MCELLIGOT, T. AND HSIA, S. L.: Prostaglandin E_2 biosynthesis in human skin: Subcellular localisation and inhibition by unsaturated fatty acids and anti-inflammatory agents. *In* Supplementum to Advances in the Biosciences, ed. by S. Bergström and S. Bernhard, vol. 9, p. 71, International Conference on Prostaglandins, Vienna, Pergamon Press Vieweg, Braunschweig, 1973.