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COMMENTARY

INHIBITION OF PROSTAGLANDIN BIOSYNTHESIS

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ENZYMES in the microsomal fraction of most mammalian cells catalyse a complex series of reactions by which certain C-20 fatty acids are transformed into prostaglandins (PGs). Because prostaglandins possess an unusually wide spectrum of biological activity, considerable interest has been aroused by the discovery of Vane and others[1–3] that the aspirin-like drugs inhibit prostaglandin biosynthesis. Use of the inhibitory activity has led to a better understanding of the function of prostaglandins in the homeostasis of the body and of the metabolic pathways involving prostaglandins. The finding is of clinical interest too, since several disease states have been attributed, at least in part, to aberrant prostaglandin production. Indeed, Vane has suggested that this anti-enzyme action is responsible for the therapeutic actions of the nonsteroid anti-inflammatory agents and although the theory is still too young to feature in most text books, it has gained wide acceptance.

Several other types of inhibitor of prostaglandin biosynthesis have been described in the literature; these include substrate analogues and other fatty acids, metal ions, sulphydryl compounds and anti-oxidants. In this short paper, however, we shall confine our attention to our chief interest: inhibition of prostaglandin synthesis by the aspirin-like drugs. To do this, we shall first review the main aspects of prostaglandin biosynthesis itself.

1. SYNTHESIS OF PROSTAGLANDINS

The 20-carbon unsaturated ("essential") fatty acids having 3, 4 or 5, all-*cis* double bonds (eicosatrienoic, eicosatetraenoic, and eicosapentaenoic acids) are the most usual substrates for the enzyme, the position of the double bonds being C-8, 11, 14; C-5, 8, 11, 14 and C-5, 8, 11, 14, 17 respectively. The major pathways of prostaglandin metabolism are shown in Fig. 1. The substrate acids can be derived from cellular phospholipids, cholesterol esters, plasma triglycerides or non-esterified fatty acids. The actual reaction mechanism of the synthetase has been worked out in some detail by Nugeteren *et al.*[4] and Hamberg and Samuelsson.[5]

The first reaction of the sequence, the incorporation of molecular oxygen at the C-11 position of the fatty acid substrate, has certain features in common with the plant lipoxidase reaction. This is followed by a hydroxylation–cyclisation step during which the linear hydroperoxide is transformed into a cyclic-endoperoxide (a somewhat unstable moiety) and oxygen is inserted at C-15. In the final series of reactions, the endoperoxide is broken down to several products, which include three prostaglandins (distinguished by their C-9 and C-11 substituents). All of these are derived

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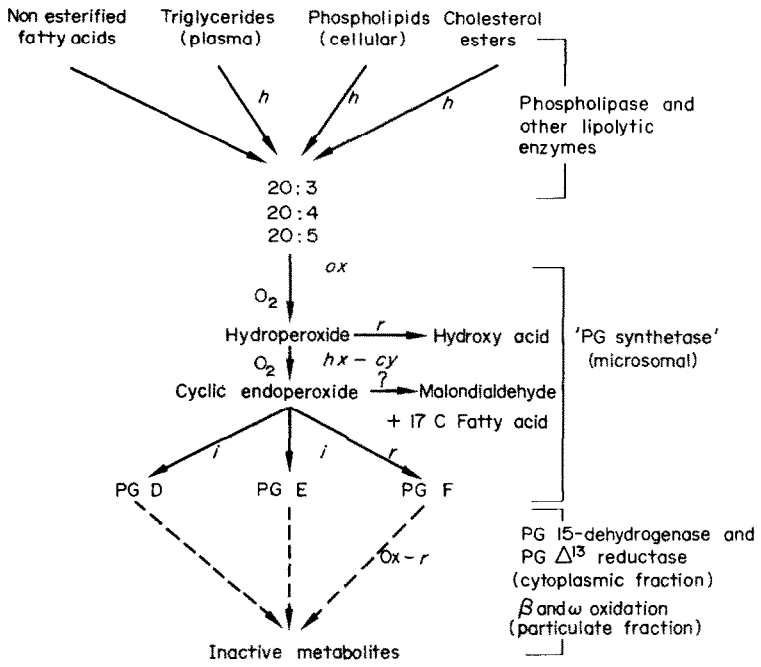

FIG. 1. Key: h = hydrolysis; ox = oxidation; r = reduction; hx = hydroxalation; cy = cyclisation; i = isomerisation.

from the cyclic endoperoxide, for there is no evidence of interconversion by the synthetic enzymes. Further metabolism of prostaglandins to inactive derivatives occurs chiefly in the cytoplasm.

The enzymes and co-enzymes involved in the formation and breakdown of the endoperoxide intermediate constitute 'prostaglandin synthetase'. The enzyme system is membrane bound (though it may be rendered partially soluble with an appropriate non-ionic detergent), but high substrate conversion only takes place in the presence of heat stable cofactor(s) from the cytoplasmic fraction of the cell. For *in vitro* studies, however, this cofactor is generally replaced by a thiol compound, usually reduced glutathione, and a source of reducing equivalents (often a phenolic compound such as (-)adrenaline or hydroquinone). Microsomal fractions of tissue homogenates have been widely used in experimental work, because of their high specific activity and also because they are relatively free of cytoplasmic prostaglandin inactivating enzymes. Two tissues particularly rich in the synthetic enzymes are sheep or bovine seminal vesicles (SSV or BSV enzymes) and the subcellular fractions or homogenates of these tissues have been used extensively as a source of enzyme material.

2. INHIBITION OF SYNTHESIS BY ASPIRIN-LIKE DRUGS

The term "aspirin-like" drugs is a pharmacological rather than a chemical definition. The compounds are of a diverse nature (although the majority are organic acids), yet all share to some extent the antipyretic, anti-inflammatory and analgesic actions of aspirin. The main usefulness of the term is to exclude the narcotic analgesics and the anti-inflammatory steroids, which act by different mechanisms.

In 1971 the inhibition of prostaglandin biosynthesis by aspirin and indomethacin was demonstrated simultaneously in three different systems: cell-free homogenates of guinea-pig lungs,[1] human platelets[2] and dog perfused spleens.[3] In these pioneering experiments prostaglandin synthesis was quantitated biologically, but the basic findings have since been repeated using a wide range of analytical techniques including thin layer and gas chromatography, radiometric and polarographic assays as well as immunological techniques.

At present there are almost thirty different systems in which inhibition of prostaglandin biosynthesis by aspirin or indomethacin has been demonstrated (see Table 1). Inhibition of biosynthesis, therefore, is not restricted to any one species or tissue, and can be demonstrated in *in vitro* preparations of subcellular fractions, homogenates, or isolated organs and tissue slices, as well as *in vivo*. Thus, all the evidence points to the effect being a general one depending, *in vivo*, only on the drug reaching the enzyme.

TABLE 1. SYSTEMS IN WHICH ASPIRIN-LIKE DRUGS ARE KNOWN TO INHIBIT PROSTAGLANDIN BIOSYNTHESIS

Species	Tissue
Man	Platelets, semen, skin, "whole body" (estimated by urinary metabolites of PGs)
Bull	Thyroid cells, seminal vesicles
Sheep	Seminal vesicles
Dog	Brain, spleen, kidney
Cat	Cerebral ventricles, spleen, kidney
Rabbit	Brain, spleen, retina, iris, ciliary body, gut, polymorphonuclear cells
Guinea-pig	Uterus, lungs, "whole body"
Rat	Skin, uterus, inflammatory exudate
Mouse	Brain, tumour cells
Toad	Bladder

Inhibition of prostaglandin biosynthesis also appears to be a property peculiar to drugs of the aspirin type since many otherwise pharmacologically active agents are not active against the enzyme system. This group of inactive compounds includes centrally acting analgesics (such as the opiates), antihistamines, *alpha* and *beta* adrenoceptor blocking agents and antagonists of acetylcholine and 5-hydroxytryptamine. The anti-inflammatory steroids are generally inactive, or show poor activity, against the enzyme. Several therapeutically inactive analogues, or enantiomers of aspirin-like drugs are also inactive against the enzyme (see Section 6).

3. POTENCY OF INHIBITORS

Most of the data on the inhibitory potency of the aspirin-like drugs has been obtained on microsomal preparations. Apart from the convenience and high specific activity of such preparations, their use also avoids formation of the two major metabolites of prostaglandins (13-dihydro. and 15-keto derivatives), which is catalysed by enzymes present in 100,000 *g* supernatant. When measuring prostaglandin biosynthesis by intact cells, unseparated homogenates, or *in vivo*, it is important to realise that it is only the prostaglandins which escape destruction that are in fact assayed. Confusing results may sometimes be obtained with unseparated broken cell sytems,

TABLE 2. I_{50} CONCENTRATIONS (IN μM) OF SOME COMMON ASPIRIN-LIKE DRUGS AGAINST PG SYNTHETASE

Compound	Source of synthetase		
	Dog spleen[6]	BSV[7]	SSV[8]
Melcofenamic acid	0·1	15	NT
Niflumic acid	0·11	125	1·2
Indomethacin	0·17	34	0·5
Mefenamic acid	0·71	NT	2·1
Flufenamic acid	0·64	NT	2·5
Naproxen	NT	425	6·1
Phenylbulazone	7·25	1300	12·6
Ibuprofen	NT	2150	1·5
Aspirin	37·0	9500	83·0

NT = not tested.

for some aspirin-like drugs also inhibit prostaglandin inactivating enzymes. This double action may result in an apparent potentiation of prostaglandin formation.

The most usual method of expressing anti-enzyme potency is to calculate the concentration of a compound required to produce a 50 per cent inhibition of a given reaction (I_{50}). Table 2 lists some of the more comprehensive I_{50} values for aspirin-like drugs against prostaglandin synthetase. It is evident that the *relative potencies* of the drugs vary considerably from one enzyme to another (and from one laboratory to another!). This is at least partly due to the differences in assay conditions, but the much more important question of whether enzymes from different sources are differentially sensitive to the inhibitory action of these drugs constitutes a fascinating problem in itself. There is already some evidence for this, for instance, with paracetamol (see Section 7).

4. MECHANISM OF INHIBITORY ACTION

Not much is known about the mechanism of enzyme inhibition by these drugs; since many are organic acids, competition at the substrate site of the synthetase is a likely possibility, although there are several basic compounds which are also effective inhibitors. If competitive kinetics do obtain (and thus the degree of inhibition is dependent on the substrate concentration) this would help to explain the variations in published I_{50} values.

Several workers have reported that the aspirin-like drugs are competitive inhibitors,[7,8] but the work of Ku and Wasvary[9] and particularly that of Lands[10] and his co-workers, shows that inhibition by these drugs is of a dual nature, termed 'competitive-irreversible.' According to this concept, the drug interacts with a binding site sufficiently close to the active centre to reduce the catalytic activity of the enzyme in a time-dependent fashion. Whilst in combination with the substrate the enzyme cannot bind the inhibitor and this gives rise to a competitive effect, but since there is always a fraction of the enzyme which is not in combination with substrate and which is therefore free to combine (irreversibly) with the inhibitor, the substrate concentration cannot influence the ultimate inhibition of the synthetase by these drugs.

In a multi-enzyme system such as prostaglandin synthetase, inhibition could be exerted at a number of sites but most the available evidence suggests that aspirin-like

drugs inhibit the initial stages of the reaction, probably the attack on the substrate. For example, a source of reducing equivalents is required for the transformation of the linear hydroperoxide to the cyclic endoperoxide (see Fig. 1) and Takeguchi and Sih[11] have demonstrated that the oxidation of this co-factor (in this case adrenaline) was inhibited by most of the standard aspirin-like drugs, suggesting that it was the initial phase of the reaction mechanism which was inhibited. Tomlinson *et al.*[12] came to a similar conclusion because of the apparent absence of intermediates in inhibited synthetase preparations.

Flower *et al.*[7] measured the production of E, F and D type prostaglandins by the BSV synthetase during partial inhibition of the enzyme system. With most of the standard drugs, there was equal inhibition of all products, but with phenylbutazone in concentrations which inhibited by 50 per cent the production of E and F type prostaglandins the production of prostaglandin D and malondialdehyde was not inhibited at all (see Fig. 1). Thus, phenylbutazone (and perhaps some other pyrazalone derivatives as well) interferes with breakdown of the endoperoxide rather than with its formation. This result may lead to a sub-division of aspirin-like drugs into groups acting at different sites on the multi-enzyme system. Clearly, structure/activity relationships and theories on mechanism of action will have to take this into account.

5. SPECIFICITY OF THE ASPIRIN-LIKE DRUGS

The aspirin-like drugs inhibit or interfere with a variety of other enzymes and cellular systems; indeed there have been several attempts to explain their clinical effects by these actions. A telling objection to many of the proposals has been that the concentrations of drug required were unrealistically high. It is, however, important to be aware of these other actions, especially if the drugs are used as a test for the involvement of endogenous prostaglandin formation in biological control systems. It is our contention that few of the other enzymes known to be susceptible to aspirin-like drugs are inhibited at concentrations which inhibit the synthetase although there are one or two interesting exceptions (see below). An example will make this clearer; indomethacin is a potent inhibitor of the synthetase and is known to possess many anti-enzyme effects. It is evident, however, from Table 3 that the concentrations required to bring about many of these actions are so high that they are unlikely to occur *in vivo* after therapeutic dosage, although they could contribute to the toxic effects after an overdosage.

Exceptions to this general rule may occur when, either because of an accelerated drug metabolism or a reduced sensitivity, much greater doses of aspirin-like drugs are required to produce inhibition of prostaglandin biosynthesis. For example, the dose of indomethacin (per kg) required to inhibit prostaglandin synthesis in the guinea pig was 10–30 times higher than the dose needed to produce the same effect in man.[13,14] It follows that in the guinea-pig the large doses required to inhibit prostaglandin biosynthesis may also affect other enzymes (unless they, too, are less sensitive to indomethacin). Phosphodiesterase appears to be more sensitive than most enzymes to indomethacin;[15] this should be borne in mind when investigating prostaglandin–cyclic AMP interactions. Use of other potent inhibitors of prostaglandin biosynthesis which lack the activity on phosphodiesterase should eliminate complications of this sort. Indeed, when investigating the role of prostaglandins by the use

TABLE 3. ACTIVITY OF INDOMETHACIN* *in vitro*

Enzyme inhibition	Approx. I_{50} concn (μM)
Prostaglandin synthetase	0·17 38·0
Prostaglandin 15' dehydrogenase	15–1000
Phosphodiesterase	28
DOPA decarboxylase	100
Oxidative phosphorylation	250
Histidine decarboxylase	400
Collagenase	3500
Physical effects	**Approx. I_{50} concn (μM)**
Inhibition of leucocyte motility	0·01
Inhibition of urate binding to albumin	200
Stabilisation of proteins	400
Stabilisation of erythrocyte membranes	500
Enzyme release from lysosomes	1000

* Peak plasma concentrations of indomethacin after therapeutic dosage in man are approximately 5 μM total, and 0·5 μM "free".

of synthetase inhibitors, consistent results with two or three different anti-inflammatory drugs add tremendously to the value of the observations.

6. THERAPEUTIC ACTIONS

So far we have considered results which are mainly of interest to the experimental scientist. What influence has this work had on our understanding of the therapeutic effects of these compounds?

When his findings were first published, Vane argued that the well known antipyretic and anti-inflammatory actions of aspirin-like drugs could easily be attributed to their anti-synthetase actions but was more cautious about the analgesic action. Since his original proposals, a great deal of corroborative evidence has been published and, furthermore, important steps have been taken towards an understanding of the analgesic actions of the drugs in terms of inhibition of prostaglandin biosynthesis.

Implicit in Vane's original proposals was the notion that prostaglandins play a pivotal role in the pathogenesis of fever, inflammation and pain. The reader is referred to other reviews[34–38] for an extended account of this evidence and for a comprehensive bibliography. For the present commentary, it may be summarised as follows.

(a) Increased concentrations of prostaglandins (especially of the E-type) are found in inflammatory exudates and damaged tissues. When injected into man or animals, prostaglandins cause erythema and oedema, and in lower concentrations greatly potentiate the pro-inflammatory effects of other mediators such as histamine and bradykinin. Inflammation caused by prostaglandins is unaffected by anti-inflammatory drugs.[16]

(b) Prostaglandins are found in the cerebrospinal fluid during fever,[17] and are themselves amongst the most pyrogenic substances known.[18] When aspirin-like drugs are given to animals during a fever, prostaglandins disappear from the cerebrospinal fluid and the temperature returns to normal. Fever caused

by a central injection of prostaglandins, however, is not reduced by aspirin-like drugs.

(c) Although injected prostaglandins are themselves painful it is unlikely that this is an important facet of their action, for the amounts required to elicit overt pain are high. However, in low concentrations, similar to those found in inflammatory exudates, prostaglandins sensitize afferent nerve endings to the algesic action of other endogenous pain-producing substances such as histamine or bradykinin and to mechanical stimuli such as touch. Hyperalgesia produced by exogenous prostaglandins is unaffected by analgesic doses of aspirin-like drugs.[16]

In the opinion of the authors, any hypothesis which purports to explain the action of a drug in terms of an anti-enzyme action must satisfy at least two basic criteria. First, the free concentrations achieved in plasma during therapy must be sufficient to inhibit the enzyme in question. Secondly, there must be a correlation between the anti-enzyme activity and the therapeutic potency. Table 4 provides good evidence to satisfy these criteria for the aspirin-like drugs and prostaglandin synthetase inhibition, which we shall now discuss in more detail.

TABLE 4. ANTI-INFLAMMATORY AND ANTISYNTHETASE ACTIVITY COMPARED

Compound	Synthetase I_{50} (μM)	Rat paw oedema (I_{50} moles/kg)	Peak plasma concn (μM)	Plasma protein binding (%)
Meclofenamic acid	0·1	0·05	≃6·84	99·8
Niflumic acid	0·11	0·145	300	82–98
Indomethacin	0·17	0·017	5·0	90
Flufenamic acid	0·64	0·142	53·0	90
Mefenamic acid	0·71	0·282	41	48
Phenylbutazone	7·25	0·325	230–500	98
Aclofenac	14·5	0·442	120	10
Bufexamac	≃18·0	0·561	23·0	5
Aspirin	37·0	0·833	280–300	50–80
Paracetamol	≃660·0	Inactive	350	25

Table adapted from Flower *et al.*[6]

In an earlier paper[6] we pointed out that the concentrations of drug required to inhibit prostaglandin synthetase were well within the plasma levels found after therapeutic dosage in man, even taking binding to plasma proteins into consideration. Further evidence has come from results in man. Therapeutic doses of aspirin or indomethacin inhibit prostaglandin production by human platelets[2] and reduce the prostaglandin content of human semen.[19] The most persuasive evidence, however, arises from the work of Hamberg[13] who monitored the concentrations of the major metabolite of PGE_1 and PGE_2 in the urine of males and females before and after treatment with therapeutic doses of indomethacin, aspirin and salicylate. In females, almost maximal inhibition (63–92 per cent) of prostaglandin turnover was obtained after 1 day's treatment; in males, however, (who generally excreted more metabolite than females) the initial reduction was less, but the output continued to decline throughout the 3 day treatment period. Two days after treatment was discontinued

the metabolite excretion had mostly returned to control levels. Indomethacin was the most potent, aspirin and salicylate each being some 15 times less active on a weight basis.

Another important point emerges from consideration of Table 4. There is a good rank-order correlation between the anti-enzyme activity (on dog spleen synthetase) and the anti-inflammatory activity (estimated by a popular laboratory model of inflammation, the carrageenin oedema test in rat hind paw). The only outstanding exception is indomethacin, which is apparently more potent in the paw oedema test than in the anti-enzyme assay; this may reflect differences between the pharmacodynamic properties of indomethacin and the fenamates.

Other results also point to a close correlation between anti-inflammatory and anti-enzyme activity. Ham *et al.*[8] found a good correlation for individual members of a structurally-related group of aspirin-like drugs (except for the fenamates) and a reasonably good overall correlation. More interesting still was that the high degree of stereospecificity exhibited for the anti-inflammatory activity of several pairs of enantiomers of *alpha* methyl aryl-acetic acids, was also shown for their anti-synthetase activity; in each case the dextrorotatory isomer was more potent that the laevorotatory partner. Tomlinson and his colleagues[12] obtained similar results with an enantiomer of naproxen. Naproxen itself was 150 times as potent as aspirin against the synthetase from bovine seminal vesicles, and some 200 times more potent against adjuvant-induced arthritis in rats. The enantiomer of naproxen was much less potent against the synthetase (only twice as potent as aspirin) and had negligible activity in the arthritis test. Indomethacin in the arthritis test was 2000 times more potent than aspirin, and 2140 times more potent against the synthetase. Takeguchi and Sih[11] also investigated the anti-enzyme activity of several enantiomeric pairs of anti-inflammatory drugs and have reported similar findings. The ability of the synthetase test to distiguish between dextro- and laevo-rotatory isomers is lacking in other *in vitro* tests for anti-inflammatory drugs, and reinforces the idea that the synthetase test could be used as a basis for an effective method of screening for anti-inflammatory substances. As far as we are aware, however, there is only one published instance in which this test has been used predictively: during the course of their studies on co-factor requirements for the bovine seminal vesicle synthetase, Takeguchi and Sih[11] discovered that 2,7-naphthalenediol was a potent inhibitor of the enzyme. When it was subsequently tested in the rat paw oedema test, it was also found to possess powerful anti-inflammatory activity.

7. DIFFERENCES IN SYNTHETASE ACTIVITY AND DRUG SPECIFICITY

Evidence is accumulating that synthetase preparations from different tissues may have quite different characteristics. In a careful study of the synthetase systems from BSV[7] and rabbit kidney,[20] for example, we have found clear differences in such fundamental properties as pH optimum, substrate and co-factor requirements. It is clear from the work of Christ and Van Dorp[21] that although the capacity for generating prostaglandins is shared by most tissues, the level of activity of the enzyme in different tissues varies enormously.

The degree to which microsomal synthetase preparations from different tissues are inhibited by the aspirin-like drugs also varies considerably. Table 5 shows the molar

TABLE 5. VARIATION IN POTENCY OF DRUGS AGAINST DIFFERENT SYNTHETASE PREPARATIONS

Compound	Molar I_{50} ratios			
	Dog spleen[6]	Rabbit kidney[20]	BSV[7]	Rabbit brain[24]
Meclofenamic acid	370	1801	682	NT
Indomethacin	217	709	236	17
Niflumic acid	336	NT	76	NT
Phenylbutazone	5	180	6·4	NT
Aspirin	1	1	1	1
Paracetamol (4-acetamidophenol)	0·06	4·2	NT	0·7

NT = not tested.

potency ratios (aspirin = 1) of several standard compounds against synthetase systems from dog spleen, rabbit brain and kidney and bovine seminal vesicles. Bhattacherjee and Eakins[22] also found that the inhibitory potency of indomethacin varied greatly when tested against enzyme preparations of different rabbit tissues. The I_{50} for the spleen enzyme was 0·125 μM, but the drug was 12 times less active against the kidney enzyme and 187, 410 and 1111 times less active against enzymes prepared from conjunctiva, anterior uvea and retina respectively. These results could explain why indomethacin is relatively ineffective against ocular inflammation.

Minor variations in I_{50} are explicable by differences in incubation conditions or assay procedures, but variations of an order or of several orders of magnitude cannot be explained on this basis. We have, therefore, come to the conclusion that the synthetase system (or at least one component protein) exists in multiple forms within the organism and that each has its own drug specificity. Such a variation in drug specificity is not without precedent; for instance, phosphodiesterase preparations from different tissues vary in their response to inhibitors.[23] Nevertheless, we shall only be certain that synthetase complexes from different tissues exist as a series of isoenzymes when purification of individual components of the system allows distinction by immunological or electrophoretic methods.

The possibility of separate genetic control over prostaglandin synthetase in each organ is of considerable practical interest; for example, would it be possible to find a drug which is only active against the enzyme from a particular tissue? Certainly, our results with the drug paracetamol (4-acetamidophenol, acetaminophen) encourage this idea. Paracetamol has analgesic and anti-pyretic effects, but no anti-inflammatory activity. The latter property would certainly fit with its lack of action against the dog spleen synthetase (see Table 5), and yet according to the original hypothesis, paracetamol should exert its anti-pyretic action by inhibiting prostaglandin synthetase too. It is well established that paracetamol is a centrally-acting anti-pyretic, and this prompted us to examine its effects on a prostaglandin synthetase system derived from brain.[24] When these results are compared with the original findings on the dog spleen enzymes[6] (Table 5), it is clear that the activity of indomethacin and paracetamol are strikingly different against the two enzyme preparations. Paracetamol is considerably more active against the brain enzyme than against the spleen enzyme. Expressed as ratios of potency, paracetamol is less than one tenth as active as aspirin against the spleen enzyme, but is almost equipotent to aspirin against the brain enzyme, as it is in the anti-pyresis test. The results with indomethacin are just as

striking; it has a much greater potency against the spleen synthetase than aspirin, and this is also reflected in the rat paw oedema test (see Table 4). However, against the brain enzyme, indomethacin is only seventeen times as potent as aspirin, which fits well with the anti-pyresis test where it is about twelve times as potent (see Ref. 24). Several other results also reinforce the concept of synthetases from different tissues having different drug specificities; these include our recent finding that paracetamol is four times more potent than aspirin against the synthetase from rabbit kidney[20] and the observations that aspirin is ineffective against a synthetase prepared from dog heart.[25]

8. SOME UNANSWERED QUESTIONS

Could the anti-synthetase action of the aspirin-like drugs account for any of the side effects of these compounds? Several studies suggest a link between the anti-synthetase action and the well-known side effect of gastrointestinal irritation (which often occurs even when the drug is administered parenterally). Prostaglandins are found in the stomach, and have a direct anti-secretory effect, as well as augmenting mucosal blood flow. Thus, there are at least two possible ways in which aspirin-like drugs could cause gastric irritation. Opinion is at present divided between these two possibilities and more evidence will be needed to establish which of the two—if either—is correct (see Ref. 26 for a brief discussion of this point). A second side effect shared by aspirin-like drugs is a tendency to produce kidney damage. Whether this is related to inhibition of prostaglandin biosynthesis remains to be seen. Prostaglandins may well have a role in the kidney and indomethacin certainly reduces blood flow in dog kidneys.[27,28]

There are a few observations in the literature which are in disagreement with (but do not contradict) our theory. Amongst these is the finding that large doses of prostaglandins actually *suppress* adjuvant-induced arthritis and some other acute inflammatory responses.[29–31] The doses required for this apparently paradoxical action are, however, certainly not within the "physiological" or even "pathological" range and are sufficient to cause other effects such as stimulation of the adrenal cortex; hence it is possible that the anti-inflammatory effects of protaglandins are entirely a secondary phenomenon.

On a more fundamental level, we should like to know a great deal more concerning the mechanisms which regulate the catalytic activity of the enzyme system *in vivo*. How is the prostaglandin production controlled; is substrate, co-factor, or molecular oxygen the limiting factor? Or is compartmentalization the most important control mechanism? How is prostaglandin synthesis triggered? Many observations from this laboratory, as well as those of others, suggest that distortion of cell membrane may be an important factor in initiating synthesis.

Another fascinating question as yet unanswered is whether intermediates or by-products of the enzyme system are important in inflammation, pain or fever. Malondialdehyde and lipid peroxides formed by the synthetase *in vitro* have a well-documented toxicity. Perhaps these compounds produce local molecular damage within the cell, whilst prostaglandins exert "pharmacological" actions extracellularly.

9. CONCLUSIONS

Although still largely unexplored, the field of synthetase inhibition promises to yield many interesting results in the clinic and in the laboratory. One important con-

sequence of this work is the recognition that we have in our possession an inexpensive and readily available group of drugs which should enable us to define more closely the role played by prostaglandins *in vivo*. Experiments of this nature have already been performed and implicate prostaglandins in (amongst other efffects) the maintenance of smooth muscle tone *in vitro*, ovarian function, control of sympathetic mediator release, control and autoregulation of renal blood flow and the contractile activity of the uterus.

The fact that such large quantities of aspirin are consumed without any appreciable ill effects might suggest either that prostaglandins are without significance to the healthy organism, or that their normal role can be adequately maintained in their absence by some other mechanisms. Another possibility to be considered is that the consequences of inhibiting prostaglandin synthesis over long periods of time have simply not been looked for. One example of this follows directly from the observations suggesting that prostaglandins play a part in the contractile activity of the uterus. The finding that the onset of parturition in rats[32] and of induced abortion in women is delayed by aspirin or indomethacin[33] suggests that the use of aspirin-like compounds as pain-killers during labour is contra-indicated. Other contra-indications may also come to light, as the resurgence in scientific interest in aspirin gains momentum. More hopefully, a better understanding of the individual synthetase enzymes from different tissues may also lead to more specific aspirin-like drugs in which the unwanted effects have been eliminated.

REFERENCES

1. J. R. Vane, *Nature, New Biol.* **231,** 232 (1971).
2. J. B. Smith and A. L. Willis, *Nature, New Biol.* **231,** 235 (1971).
3. S. H. Ferreira, S. Moncada and J. R. Vane, *Nature, New Biol.* **231,** 237 (1971).
4. D. H. Nugteren, R. K. Beerthuis and D. A. van Dorp, *Rec. Tav. Chim.* **85,** 405 (1966).
5. M. Hamberg and B. Samuelsson, *J. biol. Chem.* **242,** 5335 (1967).
6. R. J. Flower, R. Gryglewski, K. Herbaczynska-Cedro and J. R. Vane, *Nature, New Biol.* **238,** 104 (1972).
7. R. J. Flower, H. S. Cheung and D. W. Cushman, *Prostaglandins* **4,** 325 (1973).
8. E. A. Ham, K. J. Cirillo, M. Zanetti, T. Y. Shen and F. A. Kuehl, In *Prostaglandins in Cellular Biology*, (Eds. P. W. Ramwell and B. B. Pharris), p. 345. Plenum Press, New York (1972).
9. E. C. Ku and J. M. Wasvary, *Fedn. Proc.* **32,** 803A (1973).
10. W. E. M. Lands, P. R. Le Tellier, L. H. Rome and L. Y. Vanderhock, In *Advances in the Biosciences* (Eds. S. Bergstrom and S. Bernard), Vol. 9, p. 15. Pergamon Press: Vieweg, Braunschweig (1973).
11. C. Takeguchi and C. J. Sih, *Prostaglandins* **2,** 169 (1972).
12. R. V. Tomlinson, H. J. Ringold, M. C. Qureshi and E. Forchielli, *Biochem. Biophys. Res. Commun.* **46,** 552 (1972).
13. M. Hamberg, *Biochem. biophys. Res. Commun.* **49,** 720 (1972).
14. M. Hamberg and B. Samuelsson, *J. biol. Chem.* **247,** 3495 (1972).
15. A. G. A. Flores and G. W. G. Sharp, *Am. J. Physiol.* **233,** 1392 (1972).
16. S. H. Ferreira, *Nature, New Biol.* **240,** 200 (1972).
17. W. Feldberg and K. P. Gupta, *J. Physiol.* **228,** 41 (1973).
18. A. S. Milton and S. Wendlandt, *J. Physiol.* **218,** 325 (1971).
19. J. G. Collier and R. J. Flower, *Lancet* **ii,** 852 (1971).
20. G. Blackwell, R. J. Flower and J. R. Vane, manuscript in preparation.
21. E. J. Christ and D. A. van Dorp, *Biochem. biophys. Acta.* **270,** 537 (1972).
22. P. Bhattercherjee and K. E. Eakins, *Pharmacologist* **15,** Abs. 298 (1973).
23. Z. P. Horovitz, B. Beer, D. E. Clody, J. R. Vogel and M. Chasin, *Psychosomatics* **13,** 85 (1972).
24. R. J. Flower and J. R. Vane, *Nature, Lond.* **240,** 410 (1972).
25. C. J. Limas and J. N. Cohn, *Cardiovasc. Res.* **7,** 623, (1973).

26. A. BENNETT, I. F. STAMFORD and W. G. UNGER, In *Advances in the Biosciences*, (Eds. S. BERGSTROM and S. BERNARD), Vol. 9, p. 265. Pergamon Press: Vieweg, Braunschweig (1973).
27. A. J. LONIGRO, H. D. ISKOVITZ, K. CROWSHAW and J. C. MCGIFF, *Circ. Res.* **32,** 712 (1973).
28. J. W. AIKEN and J. R. VANE, *J. Pharmac. exp. Ther.* **184,** 678 (1973).
29. R. B. ZURIER and F. QUAGLIATA, *Nature, Lond.* **234,** 304 (1971).
30. M. E. GLEN and N. ROHLOFF, *Proc. Soc. exp. Biol. Med.* **139,** 290 (1970).
31. R. L. ASPINALL, P. S. CAMMARATA, A. NAKAO, J. JIU, M. MIYANO, D. E. BAKER and W. F. PAUTSCH, In *Advances in the Biosciences* (Eds. S. BERGSTROM and S. BERNARD), Vol. 9, p. 419. Pergamon Press: Vieweg, Braunschweig (1973).
32. J. W. AIKEN, *Nature, Lond.* **240,** 21 (1972).
33. R. WALTMAN, V. TRICOMI and A. PALOW, *Prostaglandins* **3,** 47 (1973).

Other Reviews

34. J. R. VANE, *Hospital Practice* **7,** 61 (1972).
35. J. R. VANE, In *Inflammation, Mechanisms and Control* (Eds. I. H. LEPOW and P. A. WARD) p. 261. Academic Press, New York (1972).
36. J. R. VANE, In *Advances in the Biosciences* (Eds. S. BERGSTROM and S. BERNARD), Vol. 9, p. 395. Pergamon Press: Vieweg, Braunschweig (1973).
37. J. R. VANE, In *Pharmacology and the Future of Man* (Proc. 5th Int. Congr. Pharmacology, San Francisco 1972), Vol. 5, p. 352. Karger, Basel (1973).
38. S. H. FERREIRA, *Medicine Today* **7,** 29 (1973).