Anti-Inflammatory and Anti-Rheumatic Drugs

Volume I: Inflammation Mechanisms and Actions of Traditional Drugs

Editor

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Chapter 4

SALICYLATES

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I. INTRODUCTION

The salicylates (or *ortho*-hydroxy-benzoic acids) are an immensely versatile group of relatively safe drugs. Proof of this is seen in the highly successful survival today of one of the oldest members in the series, aspirin or acetylsalicylic acid. Likewise, salicylate — the natural percursor of aspirin, as the acid or in the form of its salts or esters, is also used extensively for internal or external use. The relative safety and efficacy of these two compounds through centuries has provided a sound basis for continuing development in recent years, against severe competition, of a wide variety of more potent salicylate derivatives for the treatment of pain and various inflammatory states and thromboembolic conditions to name a few. Considerable effort has also been extended toward improving the safety of aspirin, most especially in the gastro-intestinal tract; recognition that by reducing or eliminating some of the untoward side-effects there are still great commercial and therapeutic possibilities for this cheap and effective drug. Indeed, the fact that this is the cheapest among the anti-inflammatory/analgesic drugs is powerful commercial argument for its retention in the therapeutic armamentarium.

It is, therefore, only appropriate that the opening chapter in the discussion of anti-inflammatory/analgesic drugs should be on the salicylates, since they not only represent the oldest members of the series, but they are a standard against which other drugs are measured experimentally, clinically, and in commercial viability. Other drugs must inevitably be graded or ranked against aspirin or other salicylates.

A chapter on this group of drugs with such an enormous literature cannot even pretend to be comprehensive. Indeed a more extensive coverage will be found in a book recently published entitled *Aspirin and the Salicylates*,[1] several valuable symposia, [2-7]

including one recent book with the challenging title *Acetylsalicylic Acid: New Uses for an Old Drug*[8] (which emphasized the anti-thrombotic and related actions of aspirin).

The reader should consult several earlier books for a deeper appreciation of the history and development of the salicylates, history having a habit of repeating itself; and it is often found in this field that workers have (re)-discovered earlier observations without apparently recognizing or appreciating for one reason or another the earlier published record. Moreover, as fashions change, so the value of the earlier observations becomes lost in the wealth of literature that has evolved in the salicylates. Amongst these earlier books, those by Smith and Smith (1966),[9] Gross and Greenberg (1948),[10] and Hanzlik (1927)[11] represent a rich and valued collection of references of the earlier work. Additionally several comprehensive reviews have been published over the years on the history,[12-14] chemistry,[15-18] pharmacology,[19-23] and pharmaceutical aspects[24] of aspirin and other salicylate compounds. There has even been at least one book published for the lay reader on aspirin.[25]

With this background it is appropriate that the coverage in this chapter should be restricted to: (1) an overview of the "state of art" of salicylate chemistry, pharmacology, and clinical uses, (2) new drug developments, (3) the current concepts of their mode of action and applications with comparisons being made where appropriate with other drugs, and (4) some future directions for research.

II. CHEMISTRY

A. Physical and Chemical Properties

Some basic physico-chemical properties of the more commonly used salicylates are listed in Table 1 (see also References 18 and 26). It is apparent overall that they embrace a wide variety of these properties. The pKa and liposolubility (log P) of the salicylic acids is a major determinant in their absorption from the gastrointestinal tract and subsequent biological responses.[1,26]

1. Solid States

Both aspirin and salicylic acid exist as dimers in the crystalline state, with intermolecular binding between carbonyl and hydroxyl groups of different molecules.[27,28] The dimeric structure has been shown by Laser Raman spectroscopy to be maintained when the solids are suspended in aqueous solution, but the dimers dissociate upon dissolution of the drug in buffered solutions of neutral pH, dimethyl sulfoxide or formamide.[26] Aspirin crystallizes to form a monoclinic space group $P2_{1/c}$ with dimensions $a = 11.446$, $b = 6.596$, $c = 11.388$Å, $\beta = 95°33'$.[27] The length of hydrogen bonds between each molecule of the dimer is 2.645Å.[27] Quantum chemical analysis has shown that the energy for this bond is derived from charge transfer and electrostatic interactions. The angle between the plane of the benzene group and that of the carboxyl groups is 2°2′, and correspondingly, that with the acetyl group is 84°45′ showing that the latter group is angled considerably out of plane to the benzene nucleus.[27] The crystal structure of salicylic acid is rather similar to that of this group in aspirin with the exception that (1) the ring of aspirin is somewhat more pulled out by the carboxyl group, so causing a slight bond angle distortion, and (2) the plane of the carboxyl group is inclined at 1.1° with respect to the benzene ring.[27,28] Salicylic acid has the same space group as aspirin with dimensions $a = 11.52$, $b = 11.21$, $c = 4.92$A, $\beta = 90°50'$, making it considerably different in crystal dimensions to that of aspirin.[28]

Six distinct polymorphs of aspirin have been identified by their respective melting points and density measurments, even though their UV spectra and x-ray powder diffraction patterns are identical.[32] This polymorphism may explain the high variability in the melting points for aspirin reported by various authors (Table 1).[29]

Table 1
PHYSICO-CHEMICAL PROPERTIES OF SOME SALICYLATES

Drug	Properties	Ref.
Aspirin (mol. wt. 180.15)	M.pt.: 135 — 137°C (variable, see Ref. 27) 139 — 143°C (boils with decomposition at 140°C)	18,27
	Solubility: (in 100 m*l* solvent): 0.33 g, H_2O (25°C); 20g, EtOH (25°C); 29g $(CH_3)_2CO$ (20°C); 5.9g, $CHCl_3$ (25°C)	18,27
	pK_a = 3.45 — 3.9	24,26
	Log P = 1.23 (*n*-oct-H_2O, pH 7.3)	
	Dipole moment (dioxane) 2.90D	30
	λ_{max}: 259 nm (ε = 1160) (molecular form, i.e. protonated at acidic pH)	35
	λ_{min}: 259 nm (ε = 760); no maxima or isobestic points in ionic form	
	M.Pt.: 159°C; b_{20} = 211°C; sublimation pt. = 76°C, d^{20}_4 = 1.443	18
Salicylic acid (mol. wt. 138.12)	Solubility: (in 100 m*l* solvent): 0.18g, H_2O (20°C), 38.46g, MeOH (21°C), 34.87g, EtOH (abs., 21°C), 1.56g, $CHCl_3$ (30°C), 23.4g, $(C_2H_5)_2O$ (17°C), 31.3g, $(CH_3)_2CO$ (23°C), 1g, C_6H_6 (30°C)	18
	pKa = 2.95 — 2.98	24,26
	Log P = 2.26 (*n*-oct/H_2O, pH 7.3)	
	λ_{max} (molecular form, i.e. protonated at acid pH): 236 nm (ε = 8350), 302 nm (ε = 3620); λ_{min}: 261.5 nm (ε = 300)	35
	λ_{max} (ionic form): 228.5 nm (ε = 6920), 296.5 nm (ε = 3520), 263.5 nm (ε = 590); isobestic points of ionic form: 229.5, 249, 298.5, and 327 nm	35
Diflunisal (mol.wt. 250.20)	M.Pt: 210 — 211°C	33
	pKa = 4.0 log P = 4.5 (*n*-oct/H_2O pH 7.3)	26
Salicyl salicylic acid (mol.wt. 258.22)	M.Pt: 148°C	33
Salicylazosulfapyridine (mol.wt. 398.39)	M.Pt: (decomp.) 240 — 245°C	33
	U.V.$_{max}$: 237 ($E^{1\%}_{1cm}$ 658) and 359	
Methyl salicylate (mol.wt. 152.14)	M.Pt: −8.6°C	33
	B.pt: 220 — 224°C	
	d_{25} 1.184; n = 1.535 — 1.538	33
	Solubility: 1g in 1.5l H_2O	

2. Solutions

One of the main aspects of importance about aspirin in solutions, both pharmaceutically as well as pharmacologically, is its ability to hydrolyze in aqueous and indeed alcoholic solutions.[1] The rate of hydrolysis depends on pH and, where applicable, the type of buffer system employed.[1] The rate is lowest at about pH 2,[35] which is about the pH of the stomach contents, most especially that in the milieu of the acid-secreting fundic zone. The rate of hydrolysis at pH 5 to 8 is about double that at pH 2, and above pH 10 the rate increases dramatically.[35] Various amines and α-amino acids, as well as urea and certain fatty acids, used as tablet excipients will stimulate the hydrolysis of aspirin,[1,36,37] but sorbitol appears to stabilize the drug from hydrolysis.[38] Clearly these aspects are important in the formulation of aspirin.

The mechanism of hydrolysis of aspirin (and its analogues) in aqueous media is by the intramolecular general base catalysis by the carboxylate group;[39,40] the interaction energies in this reaction have been calculated by molecular orbital (LCAO *ab initio*) methods.[41] The rate of hydrolysis is enhanced in aspirin analogues with halogens, methoxy or nitro substituents at the 4 position, and a nitro substituent at the 5 position, but not by halogen or methoxy substituents at the 5 position.[39] Fluorophenyl substi-

tuents at the 5 position [e.g. flufenisal = 2-acetoxy, 5-(4'-fluorphenyl)-benzoic acid], alkyl, or aryl substituents at the 3-position cause an appreciable reduction in the rate of hydrolysis.[42,43] Higher carbon-containing esters of salicylic acid generally have a slower rate of hydrolysis than that of aspirin.[44] Certain carboxylate esters [e.g. methyl, ethyl, 2-deoxyglucosyl, and (1'-ethoxy) ethyl] of aspirin with low steric hinderance of the 2-acetoxy group will slightly reduce its rate of hydrolysis.[43,45,46] These aspects are of theoretical interest in the development of less gastrotoxic, and/or selective or more bioeffective aspirin derivatives or formulations (see References 1 and 47 for detailed discussion), and some of these aspects will be considered later (Sections III.B., IV.E.).

Solutions of salts of salicylate specifically interact with cationic surfactants (with resultant increase in specific activity,[48] and the acid interacts with urea,[50] polyamides,[51] and polyvinyl pyrrolidones,[52] all of which may, by affecting solution viscosity, influence drug absorption[53] and even gastric irritancy of the salicylates.

Salicylate in solution can be induced to produce free radicals,[54] a factor which may be important in the biological activities of this and related salicylate drugs.[1] Additionally, the electron donor characteristics of salicylic acid may facilitate the formation of certain charge-transfer complexes,[55] including those with biomolecules; the latter may, in fact, contribute to a wider spectrum of activity in biological systems than previously supposed.

B. Major Synthetic Routes

1. Salicylic Acids

The classical carboxylation of phenol (as the alkaline phenoxide) to form salicylic acid developed by Kolbe and Lautermann over a century ago is still applied in the synthesis of this drug today[18] (Figure 1). Moreover, it is the basic procedure used for the preparation of most if not all (ring) alkyl, aryl, and phenoxyphenyl-substituted salicylic acids (e.g. see References 56 to 61).

2. Aspirin

Aspirin, the acetyl ester of the salicylic acid, other than substituted salicylic acid esters, are synthesized by reacting salicylic acid with acetic anhydride using pyridine or strong acids as catalysts[17,61,62] (Figure 1). Various patented procedures for the commercial production of aspirin are described (e.g. see Reference 63), and of these one which produces the drug in remarkably high yields (>99%) involves mixing salicylic acid with molar excess of acetic anhydride, then distilling the mixture to dryness *in vacuo* at elevated temperatures in the presence of acetic acid to prevent breakdown products being generated from acetic anhydride or aspirin.[64]

3. Carboxylate Esters

Much interest has been shown in recent years in the development of various carboxylate esters of aspirin, salicylic acid, and their congeners as pro-drugs to prevent the inherent gastrotoxicity of the parent acids.[1] The success of these ventures has been rather variable, since it critically depends on the rate of cleavage of the particular ester moiety and subsequent biodisposition of the parent acid to express its pharmacological activity (see Reference 1 for review). Among the oldest of these esters are the methyl, ethyl-, phenyl-, salicyl-, triglyceryl-, or salicoyl- and various other heteroaryl-esters of salicylic acid and aspirin. For reviews of the earlier synthetic procedures to prepare these esters, including those of the early German chemists, the reader is referred to References 65 to 68. Mostly the simple alkyl and aryl carboxylate esters of salicylic acids are somewhat weaker than their parent acids in anti-inflammatory and other potentially therapeutic activities and, apart from methyl salicylate, have low gastrotoxicity (for review see Reference 1). Apart from the identical twin salicyl salicylic acid

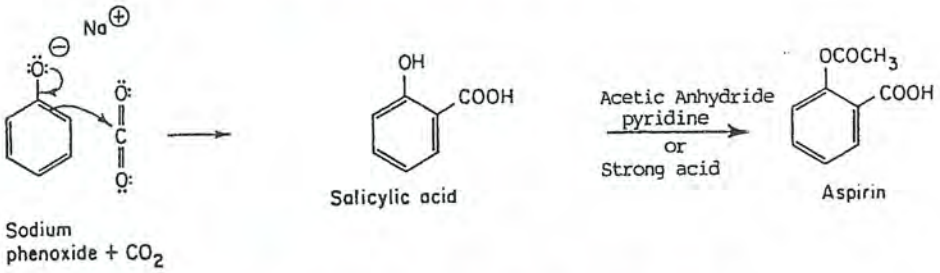


FIGURE 1. Synthesis of salicylic acid and acetyl salicylic acids.

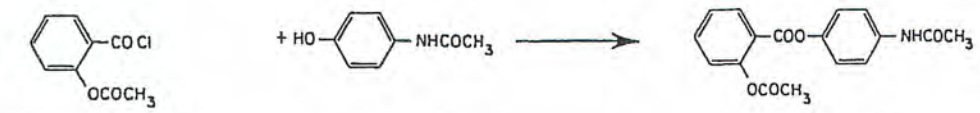
(diplosal, salsalate) and methyl or ethyl salicylate, most of the other esters of salicylic acid are of little commercial interest today. The methyl and ethyl esters of salicylic acid can be prepared by treating these acids with excess acidified (HCℓ or H_2SO_4) methanol or ethanol, respectively,[17] or, for small batches, with ethereal diazomethane.[69,70] The aspirin esters are these prepared by acetylation with acetic anhydride[69] (as for the preparation of aspirin itself). One patent describes the use of an alkali metal acetate as a catalyzing reagent for the production of aspirin methyl ester.[71]

There are several approaches which can be employed in the synthesis of the salicyl ester of salicylic acid.[72-74] An early Boehringer patent describes the production of this dimer by mixing salicylic acid, dimethyl aniline, benzene, and $PC\ell_3$ together for several days and precipitating out the acid by addition of HCℓ.[72] Phosphorus trichloride serves as a catalyst to overcome the inherent difficulties in self-condensation.[74] Other procedures described include condensation of benzyl salicylate with the acid chloride of salicylate benzyl ether to produce a benzyl-protected dimer, which is then debenzylated by catalytic hydrogenolysis.[74]

The other (acetyl) salicylate ester of major commercial interest is the paracetamol ester of aspirin, benorylate (Win 11,450), which was developed by Robertson in 1969 at the Sterling Winthrop Laboratories.[75] Commercial manufacture involves mixing paracetamol (acetaminophen *N*-acetyl-*p*-amino-phenol) with acetyl-salicoyl chloride in either sodium hydroxide solution or dry benzene at elevated temperature[63] (Figure 2). The acyl chloride procedure is another procedure used extensively for producing aspirin or salicylic acid esters, such as (1) the recently developed acetylamino phenoxyethyl ester of aspirin, eterylate (Alter S.A., Spain), which has an ethanol group between the bridge linking paracetamol and aspirin in benorylate,[77,78](2) the glyceryl-[78] and triglyceryl-[79] esters of aspirin, (3) the cyclic triglyceride esters of aspirin,[80] and (4) the glucitol-, or sucrose-esters of salicylic acid.[81] The reverse procedure, whereby the aspirin or salicylic acid is derivalized by an activated chloride, has been employed in the synthesis of (1) some acetoxy carboxylate esters of aspirin from their corresponding acetoxychlorides[82] (2) the phenylalanyl ethyl ester of aspirin,[83] (3) the 2-acetamido-2-deoxy-β-D-glucopyranosyl carboxyl-esters of salicylic acid from the α-glucosyl-chloride,[84] (4) the β-hydroxybutyryl cyclic ester of 5-chlorosalicylic acid, meseclazone [7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-b)-(1,3)-benzoxazin-9-one, W-2395, Carter Wallace Laboratories, Cranbury, N.J.], from the fatty acyl chloride,[85] and (5) polyethylene oxides esters through the carboxylate groups of aspirin and salicylic acids via the chlorides of the polymer.

4. Diflunisal

Diflunisal [5,-(2,4-difluorophenyl)-salicylic acid], a potent salicylate recently introduced by Merck, Sharp and Dohme (Rahway, N.J.) can be synthesized by first forming the 2,4-difluoro-biphenyl (I) from either reacting benzene with 2,4-difluoro-aniline or producing the 2-methoxy 2,4-difluoro-biphenyl compound by replacing benzene with



aspirinyl chloride paracetamol benorylate

FIGURE 2. Synthesis of benorylate.

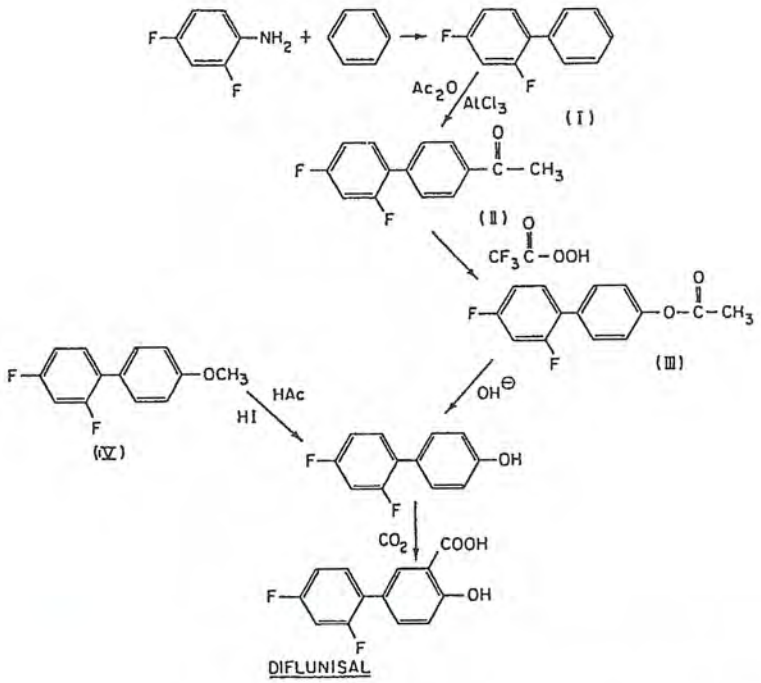


FIGURE 3. Synthesis of diflunisal. (Based on Rainsford, K.D., *Aspirin and the Salicylates*, Butterworths, London, 1984. With permission.)

2,4-difluoroanisole[61] (Figure 3). The 2,4-difluorobiphenyl from the first route is used to form the 4-acetyl derivative (II) by Friedal-Craft's acylation which is then converted to the acetoxy-derivative by employing the Baeyer-Villiger reaction. The phenol obtained following alkaline hydrolysis is then carboxylated by a modified Kolbe reaction to yield diflunisal. The 2-methoxy-2,4-difluoro biphenyl from the second route can be converted to the phenol by treatment with hydroiodic and acetic acids.

5. Salicylazosulphapyridine (= Salazapyrine, Sulphasalazine)

This drug was developed in 1946 by Askelöf, Svartz, and Willstaedt at the Pharmacia Laboratories in Sweden[87] and used by Svartz for the treatment of rheumatism. This drug subsequently found wide application in the treatment of ulcerative colitis and other inflammatory bowel disease, and has only been recently revamped as an antirheumatic agent.[1] It is essentially prepared by the diazotization of sulphapyridine with sodium nitrite in HCl and subsequent coupling of the diazonium salt with an alkaline solution of potassium salicylate[63,74,87] (Figure 4). Commercial synthesis involves mixing the salicylate and diazotized salt for 2 days and obtaining the azo compound by precipitation with excess HCl.[63]

6. Miscellaneous Compounds

Discussion of the chemistry of other salicylates of minor commercial significance at least for the present is found in Reference 1. Many of these salicylate compounds

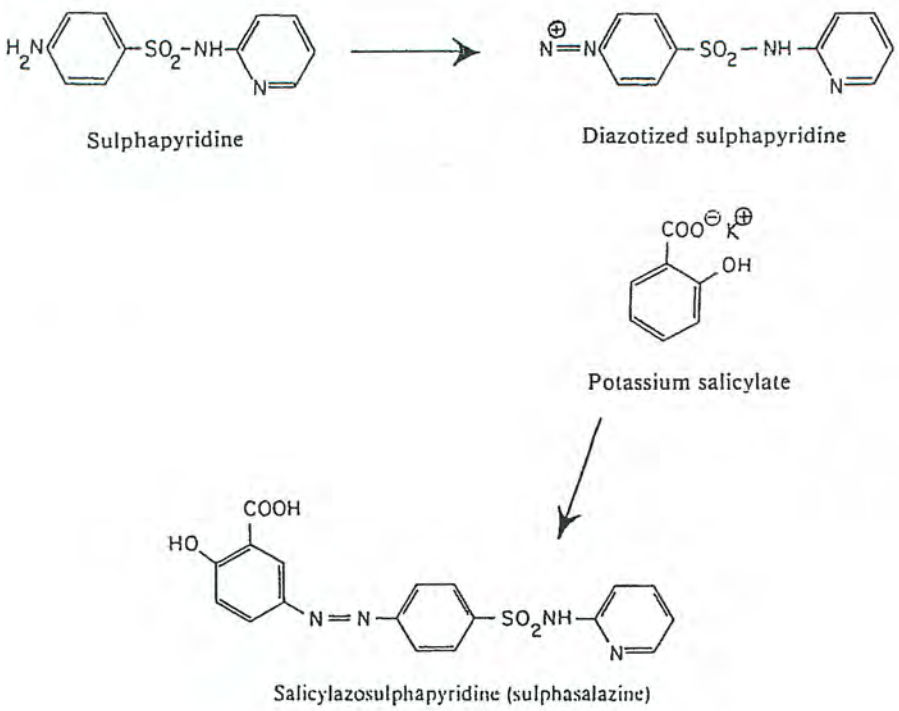


FIGURE 4. Synthesis of salicylazosulphapyridine.

developed previously are worthy of therapeutic development, as they may exhibit specific properties or safety features. As is often the case in drug development, it is worth reviewing some of their actions following progress in some area of biochemical or other area of fundamental medical research.[1] Thus, for example, the development of potent acetylators or sickle-cell hemoglobin (e.g., 3,5-dibromo aspirin) or platelets may help in control of sickle-cell anemia and cardiovascular conditions, respectively.[1] Some of these aspects will be discussed later; the further mention of aspects of their chemistry is not to diminish their status but is for the sake of brevity.

III. METABOLISM AND PHARMACOKINETICS

A. Aspirin, Salicylic Acids

Most, if not all, salicylic acids (except salicylazosulphapyridine) undergo biotransformation by (1) conjugation with glucuronic acid to form acyl or phenolic glucuronides, (2) conjugation with glycine to form salicyluric acids (except for diflunisal), and (3) hydroxylated by the liver P_{450} microsomal enzymes to yield 2,5-dihydroxybenzoic acids (also except for diflunisal), which may be subsequently conjugated with glycine to yield gentisurate[1] (Figure 5). The conjugation reactions occur primarily in the liver, intestinal tract, and in the case of salicylurate may also be formed in the kidney.[1] The glucuronide conjugates are essentially bioinactive, but the glycine conjugates may exhibit some effects, e.g. in displacement of urate from binding sites in circulating albumin.[1] Simple salicylic acids are, in general, strongly bound to circulating albumin and exhibit modest volumes of distribution (V_d) — approximately 10 to 20 ℓ — reflecting a relatively low degree of accumulation in deep body compartments. Salicylate kinetics fit well to a two-compartment model.[1]

1. Aspirin Esterases and Acetylation of Biomolecules

It is now, however, becoming clear that the original simple notions about aspirin metabolism, i.e. by enzyme-mediated (aspirin esterase) and spontaneous hydrolysis to

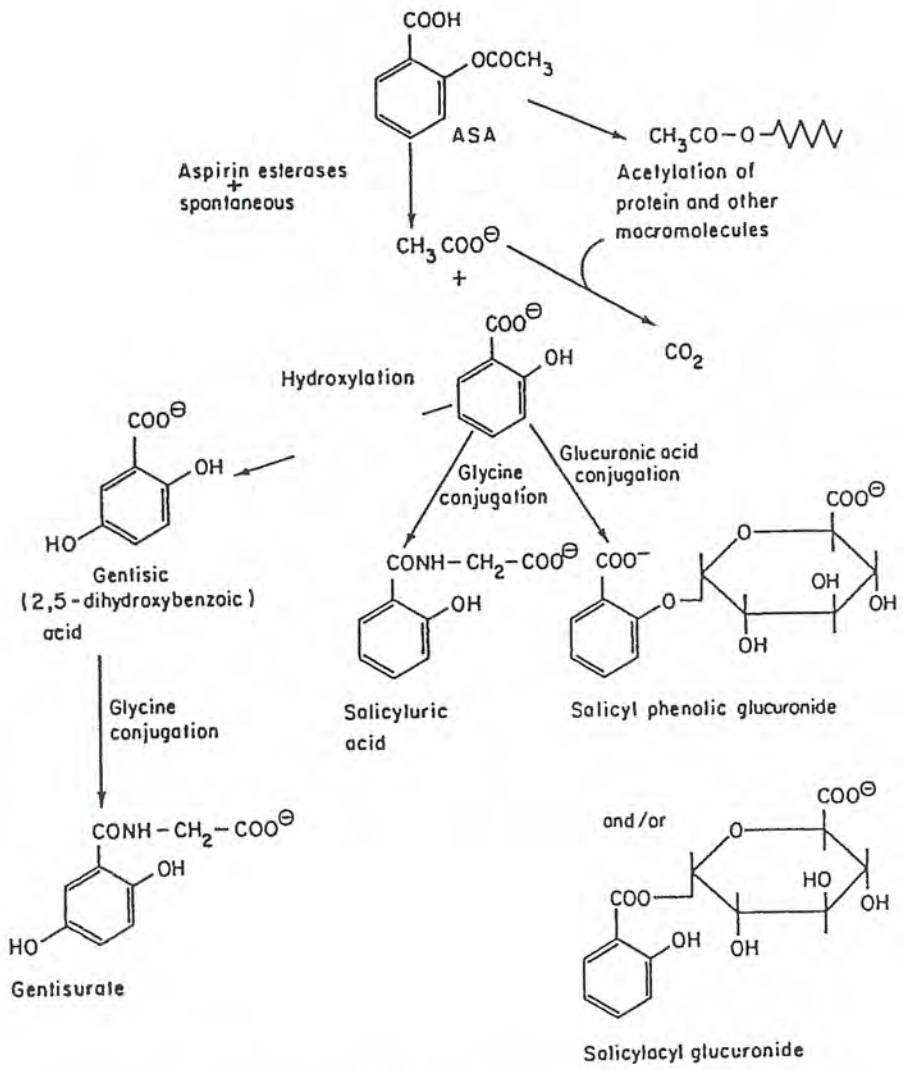


FIGURE 5. Biotransformation of salicylates. (From Rainsford, K. D., *Aspirin and the Salicylates*, Butterworths, London, 1984. With permission.)

yield the principal bioactive form of the drug (salicylate), are very much an understatement of the true situation. Recent studies following the fate of the acetyl group of aspirin have shown that it becomes covalently bound to a wide variety of proteins, glycoproteins, lipids, and possibly other biomolecules.[89] Studies in rats have shown that this acetylation is most evident in sites wherein side-effects are manifest (e.g., gastro-intestinal tract, kidney, bone marrow, but not the liver) and to a lesser extent in inflamed sites (the latter accumulation may in fact, be primarily due to acetylated albumin which has accumulated in these sites from damaged blood vessels).[89] While acetylation of prostaglandin cyclo-oxygenase has previously been considered a major part of the therapeutic actions of aspirin, and acetylation of albumin and hemoglobin may occur, it is clear from these most recent studies that there exists an enormous potential for aspirin to modify the actions of a much wider range of enzymes and other biomolecules. These acetylation reactions may have major consequences for the understanding of the mechanism of action of this drug. The balance between those hydrolytic reactions catalyzed by aspirin esterases in the gastro-intestinal tract, blood, liver, and possibly other organs in relation to the effectiveness of the remaining aspirin in the gastro-intestinal tract and other organs of the body to acetylate biomolecules in

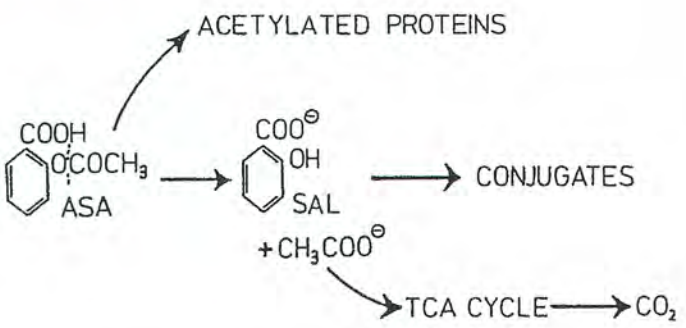


FIGURE 6. Modified concepts of aspirin metabolism. (From Rainsford, K. D., Schweitzer, A., and Brune, K., *Biochem. Pharmacol.*, 32, 1301, 1983. With permission.)

these sites, will clearly be a major factor determining the therapeutic and side-effects of this drug. Low aspirin-esterase activity in patients with alcoholic liver disease may predispose these patients toward gastro-intestinal intolerance from the drug.[1] Moreover, the possibility of extensive metabolism of the acetyl moiety of this drug through the tricarboxylic acid cycle having unique metabolic actions could also be important. Thus the concept of aspirin metabolism must now be modified to consider the fate of the acetyl moiety of aspirin (Figure 6). It is clear that aspirin is potentially a bifunctional drug with actions attributable to both its acetyl as well as salicyl moieties. The oft-quoted expression "aspirin-like" aligning the actions of aspirin with other non-steriodal anti-inflammatory drugs is obviously most inappropriate since these other non-acetylated drugs do not have the acetylating capacity of aspirin.

2. Absorption and Disposition

Oral aspirin and salicylic acid are relatively rapidly absorbed from the stomach and upper-intestinal tract, depending on the dosage form and dose.[1] The elimination half-time of aspirin (following oral intake of 1 to 3g) in the plasma is about 15 to 30 min, while that of salicylate is about 3 to 4.5 hr, indicating that the drug is rapidly eliminated from the body.[1] The low half-time of aspirin relative to that of salicylate reflects the extensive "first-pass" metabolic conversion of the drug through the gastrointestinal tract and liver, which can amount to 50 to 65% of the ingested dose in both man and laboratory animals.[1] Excretion of both aspirin and salicylic acid is primarily through the urine and is dependent on urine pH, especially if antacids are consumed concurrently.[1,90] Some biliary excretion of these drugs occurs in laboratory species and may also occur in man, and this may contribute to a small degree of entero-hepatic recirculation of aspirin and salicylate.[1]

Salicylate is excreted in the saliva of man following oral ingestion of aspirin.[91] Since good correlations are evident between plasma and saliva concentrations of salicylate,[1,92] the convenience of employing saliva collections to routinely determine salicylate levels, especially in patients receiving chronic aspirin therapy, has yet to be fully exploited.

Placental transfer of salicylate can lead to potentially toxic effects in the fetus following oral ingestion of aspirin.[93,94] Maternal protein deficiency may enhance placental transfer of salicylate; this not being a consequence of decreased maternal plasma protein binding, but of other physiological and pharmacokinetic factors.[95]

Orally administered salicylic acids and their esters will accumulate in rats at sites wherein an inflammatory lesion has been experimentally induced.[89,96-98] Transport of salicylates from the blood to synovial fluid assumes more complex dynamics than seen

in the simple experimental inflammation in rats. Thus peak levels in the synovial fluid are lower and are somewhat delayed compared with plasma levels in patients with arthritic conditions.[99,100] This may be influenced by the histological complexities of the inflamed synovium and adjacent tissues[101] as well as the propensity of the synovial fluid proteins to bind salicylate.[102] Synovial proteins of rheumatoid arthritis patients have a much lower capacity to bind salicylate than does the plasma,[102] so that this low synovial binding may represent a limit to the persistence of the drug in inflamed joints. Much has yet to be learned about the fate of the acetyl moiety of aspirin in the rheumatoid joint as well as the kinetics of drug uptake into inflamed synovium and connective tissues of man. These are certainly critical factors determining the bioeffectiveness of the salicylates.

3. Factors Influencing Drug Bioavailability

The dissolution characteristics of aspirin drug formulations are well known as a major factor governing its oral bioavailability,[1,24] and efficacy.[91,103] Furthermore, the marked variability in content of impurities (notably salicylic acid and the putative immunogen, acetyl-salicylsalicylic acid) and excipients in commercial tablets and suppositories of aspirin, will influence or even reflect physical stability and thus dissolution characteristics and effectiveness of the drug.[24,104,105] The purity and dissolution characteristics of commercially available enteric-coated aspirin preparations may vary considerably.[104,105] The dissolution rates of these are particularly poor, and while these do not appear to adequately reflect their bioavailability in vivo[104] must still indicate the potential for variable and incomplete absorption of these formulations. However, it must also be said that urinary recoveries of some enteric-coated as well as sustained-release aspirin formulations can approach 90%,[106,107] so that the problem of bioavailability of these preparations may depend very much on their formulation. Enteric-coated formulations do have the advantage that sustained salicylate levels can be achieved by twice-daily dosage.[108]

Crystal forms of aspirin have been mentioned as varying considerably (Section IIA), and these are known to markedly influence the dissolution behavior of aspirin.[109] Low concentrations of sodium bicarbonate relative to aspirin enhance the dissolution of aspirin and promote its absorption, providing the intragastric pH is not raised such that an appreciable amount of the drug becomes ionized.[1,24] High concentrations of bicarbonate ionize the drug, markedly reducing its absorption (i.e., reducing plasma levels) and enhance the urinary excretion of the salicylate ion.[1,24,90,110] The same problem exists with other antacids taken with aspirin.[111]

Concurrent oral ingestion of paracetamol (acetaminophen) with aspirin elevates the plasma salicylate, acetylsalicylate and paracetamol levels.[112,113] Caffeine can complex with aspirin,[114] and this may retard absorption of aspirin.[115] Corticosteriods depress plasma salicylate levels by enhancing renal clearance of salicylate.[116] Codeine phosphate, dextropropoxyphene, several alkaloid opiates, hydroxychloroquine, cimetidine, propanolol, and alcohol inhibit aspirin esterase in serum and so may enhance plasma levels of aspirin relative to salicylate.[117] Concurrent administration of NSAI drugs with aspirin does not, except with diclofenac, lead to an alteration in plasma salicylate levels.[1] However, aspirin frequently depresses the plasma levels of the other NSAI drugs, mostly by displacing the latter from their binding sites on cirulating albumin.[1]

B. Salicylic Acid Esters

Orally ingested carboxylate esters of aspirin or salicylic acid release these acids following absorption through the gastrointestinal mucosa and liver metabolism.[1,46,62,70,76,77,79,85,96] The rate of this biotransformation determines their bioeffectiveness and probably their diminished gastrotoxicity.[1,69,70,96]

C. Diflunisal

Diflunisal, in contrast to aspirin and salicylic, has a much slower rate of absorption in both man and laboratory animals.[118,119] Thus peak levels of this drug in plasma occur at 3 hr following oral ingestion of 250 mg of the drug (c.f. 30 to 40 min following 640 mg aspirin).[119] The drug shows dose-dependent increases in plasma levels after single or repeated oral ingestion in man with values of 14.4 to 60 μg/mℓ present after 125 to 375 mg of the drug b.i.d.[119]It is strongly bound to plasma albumin(>99%).[119] The half-life of the drug after repeated ingestion is 11 to 12 hr, which is appreciably longer than that of aspirin or salicylate.[119] This long half-life is a major advantage in long-term therapy with this drug (compared with conventional aspirin preparations) because of the convenience of twice daily dosage.[1]

Concurrent ingestion of the drug with food reduces the diflunisal plasma levels by about 16% but does not diminish its total bioavailability.[120] Co-ingestion of the antacid, aluminum hydroxide, with diflunisal will decrease the bioavailability of the drug in the fasted state but not under non-fasting conditions.[120] However, ingestion of magnesium hydroxide with diflunisal by fasted (but not non-fasted) individuals will increase both peak plasma levels and bioavailability of the latter.[120] These studies show the importance of appropriate choice of antacid and advice on the timing of drug intake especially in individuals taking antacids for the relief of epigastric distress.[1]

D. Salicylazosulphapyridine

This drug has a much more complex metabolism than those salicylates mentioned previously. In both man and the rat the orally-administered drug is split via azo reduction by the gut bacteria into its two half-components, 5-aminosalicylic acid (5-ASA) and sulphapyridine (SP).[121-125] The former is absorbed by the colon and considered with principally bioactive form of the drug in relation to inflammatory effects of SASP in the treatment of inflammatory bowel diseases, though recent studies suggest that the parent drug may also have some effects on the prostaglandin-generating system.[1] The major toxic reactions of SASP have been ascribed to SP.[126] Both 5-ASA and SP undergo hepatic *N*-acetylation reactions. The rate of *N*-acetylation determines the detoxification of both 5-ASA and SP, and this genetically determined polymorphism causes enormous intersubject variations in the plasma levels of SASP, 5-ASA and SP, with consequent effects on the therapeutic and side-effects of SASP.[126-128]

Steady state serum levels of SASP *circa* 25 μg/mℓ) and its metabolites (*circa* 33μg/mℓ) are observed about 5 to 10 days after repeated daily ingestion of 4g SASP.[123] The drug is almost completely eliminated in the urine of both man and rats.[123]

IV. PHARMACOLOGY

The pharmacology of aspirin has probably been the most extensively studied compared with that of the other NSAI/analgesic drugs. The understanding of its mode of action attracted much attention following the suggestion by Vane in 1971[129] that the therapeutic actions could be explained by the inhibition by this drug of the release of inflammatory prostaglandins. Later work by this author and his colleagues,[130-134] as well as by others (see review, Reference 23) has attempted to unify this concept and reinforce it with certain arguments and evidence (e.g., see Reference 134). This concept has not been without its serious criticisms,[135-137] and critics of it have emphasized that while in essence the inhibition of prostaglandins *does* explain *some* of the actions of aspirin and related drugs,[1,135-137] that this is by no means the total concept of their actions. As succinctly stated by Bonta and co-workers:[136] "The concept (of Vane's) was attractive enough to seduce some investigators into believing that the pharmacological control of a pathological condition as complicated as inflammation could be explained on the basis of a single, albeit extremely important, mechanism."

The problems at present are to determine:

1. What relationship exists between the inhibition of prostaglandin production and the therapeutic actions of the salicylates?
2. Given that there may be alternative modes of action of aspirin, what contributions are made by the inhibition of prostaglandin production to the therapeutic actions of this drug and its congeners, compared with its other biochemical/cellular effects?

This section will consider these two questions and provide an overview of some of the other biochemical actions of the salicylates which are known to be important for their actions.

A. Anti-Inflammatory Activities

1. Inhibition of Prostaglandin Production

Aspirin is well known to inhibit the cyclo-oxygenase enzyme, most likely as a consequence of it acetylating sites in or near the active site of the enzyme.[138,139] Its principal metabolite salicylate, is a much weaker inhibitor of the isolated enzyme system[131,135,140] in vitro, although it is equipotent with aspirin in reducing the content of prostaglandins in inflamed tissues.[141,142] However, salicylate may not always inhibit the prostaglandin cyclo-oxygenase system in vivo, for thromboxane A_2 production in rat platelets is unaffected by even high doses (200 mg/kg) of the drug,[143] and gastric mucosal prostaglandin I_2 content in rats has been reported to be unaffected by high oral or parenteral doses of salicylate.[142]

It has been suggested that the cyclo-oxygenase enzyme system might have different activities in inflamed tissue to that in the gastric mucosa;[142] this might account for the differences in sensitivity of the effects of salicylate on in vivo prostaglandin production in different systems. While the activity of this enzyme system might be higher than normal in inflamed tissues (possibly as a consequence of there being more enzyme present) this might at first sight account for the lack of effects of salicylate, since on first principles the more active the enzyme system the greater the possibility of expressing inhibitory effects of the drug.[1] Another possibility is that a hypothetical drug metabolite produced by metabolism of salicylate has been invoked to explain the paradoxical inhibitory effects of salicylate in vivo compared with those in vitro.[144] However, neither gentisic (= 2,5-dihydroxybenzoic) or salicyluric acids, two principal metabolites of salicylate, have been found to inhibit platelet thromboxane A_2 production in vivo.[143] The in vivo effects on prostaglandin production of the acyl- and phenyl-glucuronides as well as the gentisurate metabolites have still to be determined, but the former two are generally regarded as being biologically inert, and the latter might well have the (in)activity of salicyluric acid. Still this aspect remains somewhat unresolved.

Smith[135] has pointed to the fact that salicylate has a much lower plasma binding capacity in the rat than in man,[146] so that more of the drug would be expected to accumulate in inflamed sites in rats compared with that in man. However, exudation of plasma proteins carrying bound drug into inflamed tissues does occur.

Also, aspirin is bound to a much lesser extent to circulating albumin than salicylate,[146,147] so the amount of bound aspirin actually arriving at inflamed tissues by plasma exudation would be expected to be less than salicylate. Conversely, the amount of free aspirin available for uptake into inflamed tissues would be higher than that of free salicylate. Since relatively much less aspirin actually reached inflamed tissues compared with salicylate in these tissues of both man and rats, because of hydrolysis of aspirin and acetylation reactions in other body sites,[89,97,141,148] this implies that the major part of the local anti-inflammatory and analgesic effects of aspirin in inflamed

tissues must be due to its metabolite, salicylate.[135] Thus the cyclo-oxygenase inhibitory effects of the small amount of aspirin which actually reaches the inflamed tissues would be expected to be relatively small. Furthermore, salicylate is known to prevent the aspirin-induced inhibition of the cylco-oxygenase enzyme system in vitro and in vivo,[149-151] so that salicylate would appear to have the dominant effect in any observed effects of aspirin on prostaglandin production in inflamed tissues. The question remains as to how the prostaglandin content of inflamed tissues is reduced by salicylate or aspirin.

a. Leukocyte Migration

Several authors[152-156] have reported that aspirin and salicylate when given orally in modest doses (usually about 50 mg/kg) to rats inhibits the migration, notably of monocytes and to a lesser extent of polymorphonuclear leukocytes, into various sites and types of inflamed tissues elicited by inflammagens (e.g. carrageenan). Against this there have been some few reports of studies in rats showing no effect of aspirin,[134,157] but in these the dose of aspirin was appreciably higher than employed in the earlier studies. In one brief report by Ferriera[134] the dose employed was 300 mg/kg which is well within the toxic range for the drug in rats. These higher doses could have resulted in sufficiently high concentrations of aspirin in the inflamed tissues so as to cause potent cyclo-oxygenase inhibition, with consequent diversion of arachidonate through the lipoxygenase pathway to form appreciable quantities of the potent chemoattractant leukotriene B_4.[158,159] This effect could override the separate effects of aspirin and salicylate on leukocyte emigration. Low doses of aspirin would only cause weak inhibition of prostaglandin cyclo-oxygenase in inflamed sites, and that effect would be overridden by excess salicylate present, so there would be no appreciable diversion of arachidonate to lipoxygenase products in these tissues. The equi-effective reduction in prostaglandin content induced by both salicylate and aspirin could, therefore, be a reflection of reduced numbers of prostaglandin-generating leukocytes.

The relationship of these drug effects on vascular permeability, and blood flow to leukocyte emigration in immune complex inflammation and the actions of chemotactic factors has been recently investigated by Issekutz and Bhimji.[160] They found that 150 μg aspirin locally injected (subcutaneously) into rabbits reduced vascular permeability (i.e., ^{125}I-albumin accumulation), blood flow (i.e., ^{86}Rb accumulation), and leukocyte infiltration (i.e., from ^{51}Cr labeled leukocytes) in sites wherein a reverse Arthus reaction had been elicited.[160] Interestingly, these authors found that the reduced leukocyte infiltration induced by aspirin (150 μg, sc) in response to zymosan-activated plasma could not be reversed by locally applied prostaglandin E_2 (0.5 μg), indicating that correcting for a prostaglandin deficiency created by the drug has no influences on its response to this inflammagen. Previously, leukocyte emigration into skin windows of rabbits (in the absence of any immunogenic or inflammagenic agents) had been shown to be inhibited by local administration of aspirin and salicylate.[161] Thus, it appears that these drugs may affect leukocyte emigration in response to immuno-inflammagenic agents or "passively" (i.e., without any local agent) by combined effects on blood flow, and vascular permeability. Actions of these drugs on the intrinsic migratory responses of leukocytes (i.e., in vitro) have not been satisfactorily resolved. Some authors have reported inhibitory effects of these drugs on random migration and chemotaxis of polymorphs[162,163] and monocytes[164] in vitro, while others have reported no effects or stimulation on one or either of these cells[165,166] (see also review[167]). Stimulatory responses (in vitro) in cells obtained following ingestion of these drugs in man have been reported,[164,165] making it very difficult to resolve these in vivo effects with those from the in vitro studies, even in man. It appears, therefore, that the reduced leukocyte emigration observed in vivo must be due to these drugs influencing vascular

permeation of leukocytes; possibly effects on the complement system could also be of importance, but these aspects have yet to be fully established.

b. Responses in Essential Fatty Acid Deficient Rats

Bonta and co-workers[136] showed that subcutaneous administration of 125 or 250 mg/kg aspirin caused an equal suppression of carrageenan edema in essential fatty acid (EFA) deficient rats (i.e., deprived of biosynthetic precursors necessary for prostaglandins, or other eicosanoids), compared with that in normal rats. Moreover, the anti-inflammatory response to both doses of aspirin in these EFA groups was 60% of that in control animals, suggesting that the prostaglandin- (or eicosanoid) independent inflammatory response was a dominant part of the total inflammatory reactions affected by this drug.

c. Inhibition of Prostaglandins Related to Anti-inflammatory Actions of Different Salicylates

In Figure 7 the relationship is shown of the inhibitory effects of a variety of salicylates on prostaglandin E_2 production by mouse macrophages[140,168] to the acute anti-inflammatory activities of these drugs in the carrageenan paw edema induced in rats.[1,69] This data shows a possible trend, but much of this depends on the effects of diflunisal, for the other drugs show a wide scatter and no obvious correlation exists. The evidence is, therefore, at best tenuous.

In another approach, Glenn and co-workers[170] have shown that no relationship is evident between effects of some salicylates on serum levels of $PGF_{2\alpha}$ or platelet prostaglandin production, and their anti-edemic activities in the rat carrageenan edema assay. Moreover, the anti-inflammatory effects of aspirin could not be reversed by local administration of 5 to 15 μg of PGE_2 or PGE_1,[160] thus ruling out the effects of a prostaglandin deficiency in the actions of aspirin in established inflammation.

d. Other Effects on Eicosanoid Metabolism

Aspirin and other salicylates can, under some conditions, exert some paradoxical effects on the lipoxygenase pathways in some cellular/organ systems. For aspirin, therefore, the view that this drug is a pure cyclo-oxygenase inhibitor is questionable. Indeed in light of the foregoing discussion (Sections 1 and 2, above), to consider the effects of this drug on the prostanoid pathway alone in inflammation may depend very much on the cell system and its location. Thus, it is conceivable that these drug effects on lipoxygenase pathways in vivo will largely involve cells in the blood, as they would come in contact with much higher concentrations of aspirin, i.e., from direct contact of these cells within the gastro-intestinal circulation. Thus the inhibition of platelet 12-HPETE peroxidase by low-to-moderate concentrations of both aspirin and salicylate[171] and other lipoxygenase products in neutrophils from rats[171] and rabbits[172] might be due to drug effects on these cells as they pass through the gastrointestinal circulation and not due to effects on the local (i.e., non-mobile) cellular components in the inflamed tissues.

In addition to inhibiting the cyclo-oxygenase and lipoxygenase enzymes, aspirin has been shown (in higher concentrations than required for these latter effects) to inhibit the acyl-coenzyme A:lysolecithin acyltransferase, an enzyme which catalyzes the reacylation of lysolecithin which is produced following cleavage of arachidonate by phospholipase A_2.[173] This might have two consequences: (1) reducing the availability of arachidonate in its esterified form (i.e., attached to phosphatidyl choline, and (2) promoting the inhibition by lysolecithin of cyclo-oxygenase activity.[174]

More subtle influences of aspirin and related drugs on the regulation of lipoxygenase activities via cyclic AMP production have been suggested recently by Ham and co-

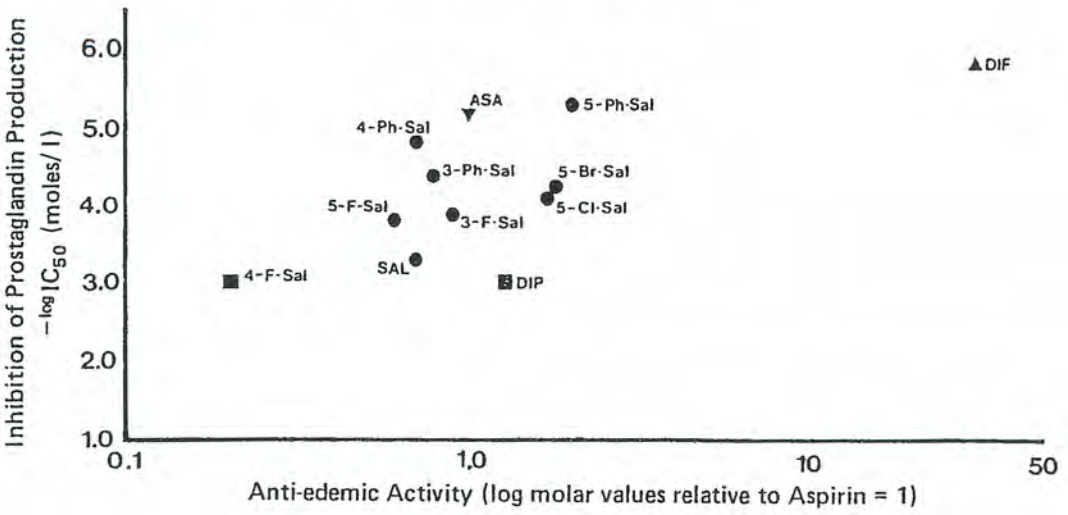


FIGURE 7. Relationship between inhibitory effects of various salicylates on prostaglandin E_2 production by mouse macrophages[140-168] to their relative anti-edemic activities.

workers.[175] These authors observed that PGE_2 or PGE_1 inhibited the production of LTB_4 in polymorphonuclear leukocytes elicited by the chemoattractant agent, *N*-formylmethionylleucylphenylalanine (fMet-Leu-Phe), coincident with increased levels of cyclic AMP.[175] They suggested that the enhanced production of lipoxygenase products by cyclo-oxygenase inhibitors (e.g., aspirin, indomethacin) could be due to the reduction in the inhibitory actions of cyclic AMP because of low PGE_2 levels. Additionally, it is possible that the drug-induced inhibition of PGE-dependent cyclic AMP production[176] might also be important in the control by cyclic AMP of lipoxygenase activities. Clearly, these subtle effects should be examined in detail before pronouncements can be made about the role of inhibiting prostanoid or leukotriene production in the actions of the salicylates and related drugs.

e. Reconciliation of Anomalies of the PG System

The arguments above suggest that inhibition by salicylates of the production of inflammatory prostaglandins may not entirely account for its effects on even the acute, so-called "prostaglandin component or phase", of the inflammatory responses.

It does seem that the role of inhibition of prostaglandin production by salicylates might be confined to the vascular component of the inflammatory responses, especially that involving co-participation of bradykinin.[178,179] The reduced content of prostaglandins in inflamed sites might, as previously suggested, effect reduced leukocyte numbers.

It also is most likely that the long-lasting irreversible inhibition of platelet thromboxane and PGG_2-dependent aggregation by contact with high concentrations of aspirin in the gastrointestinal circulation, might contribute to reduced accumulation of the drug in inflamed tissues. This would have the effect of reducing another cellular source of inflammatory prostanoids.

In comparison with aspirin, diflunisal is a much weaker, reversible, inhibitor of platelet aggregation and does not covalently modify the cyclo-oxygenase enzyme as observed with aspirin. Thus, the contribution of platelets to the anti-inflammatory actions of the drug might be considered much less than observed with aspirin.

2. Effects on Early Phase Amines and Kinins

The co-participation of bradykinin in expressing (with PGE_2) enhanced vascular permeability[178,179] offers another site of action of the salicylates. Aspirin blocks release

of kinins, possibly by affecting kininogen synthesis (see review, Reference 1). Aspirin also affects the bradykinin-induced prostaglandin production, but there is also some, albeit indirect, evidence that it could suppress bradykinin receptors.[181] Additionally, this drug might inhibit the production of histamine and serotonin (5-hydroxytryptamine),[1] so it is conceivable that salicylates could have multiple actions on the production of these early phase mediators.

3. Oxyradical Production

The production of tissue-destructive superoxide (O_2^-) radicals, especially from leukocytes, has been considered an important mediator in the genesis of inflammation.[82] Aspirin, along with other NSAI drugs, has been found in pharmacological concentrations to inhibit superoxide production in elicited peritoneal guinea pig and phorbol myristate acetate-stimulated rat pulmonary macrophages.[183,184] These effects could be particularly important in the expression of inflammagenic actions of macrophages and polymorphs which have migrated into inflamed sites.

4. Lysosomal Enzyme Release

Tissue and leukocyte lysosomal organelles serve as the focus for a variety of actions of inflammatory mediators and/or cellular controls affected by the latter, some of which are affected by aspirin and other salicylates.[1] Likewise, release of lysosomal enzymes (e.g., phospholipase A_2) promotes formation of prostaglandins.[185] Salicylates directly affect the stability of the lysosomal membrane,[1] thus preventing release of lysosomal enzymes. Aspirin can also indirectly inhibit the activities of the acid phosphatases, cathepsins, and possibly hyaluronidases of these organelles depending on the source (see review, Reference 1), thus being direct effects of the drug. Indirect influences of these drugs on inflammatory mediators/cell regulators can be summarized as follows:[1]

1. Inhibition of the production of PGE_2 relative to that of $PGF_{2\alpha}$ causes reduced cAMP/cGMP, thus favoring release of leukocyte lysosomal enzymes.[186] Likewise, since LTB_4 can stimulate lysosomal enzyme release both directly and possibly also through its stimulation of adenylate cyclase production, there may be additional effects of aspirin in diverting arachidonate to cause production of this nucleotide, which are counterbalanced by effects of the drug on adenylate cyclase activities. Thus, there are several sites for the actions of the salicylates on PG-dependent and cyclic nucleotide events controlling lysosomal enzyme secretion. These aspects have yet to be resolved, but it is important to appreciate these sites for potential intracellular controls of lysosomal enzyme secretion from leukocytes.
2. Effects of salicylates on secretion of some but not all lysosomal enzymes are Ca^{++}-dependent.
3. Free oxyradicals cause breakdown of the lysosomal membrane and release of autolytic enzymes, so that inhibitory effects of salicylates on production of superoxide would be expected to affect this aspect of tissue destruction.

The consequences of these drug effects on lysosomal stability of leukocytes invading connective tissues has yet to be fully determined, but at present there is evidence suggesting that degradation of sulfated proteoglycans and possibly collagen fibers may be prevented.[1]

5. Connective Tissue Metabolism

In addition to the possibility of preventing breakdown of connective tissue proteoglycans and collagen fibers (but see References 187 to 189), the salicylates inhibit the

biosynthesis of these components and may even attenuate the growth of proliferating cells in inflamed tissues.[190,191] These aspects have been reviewed recently.[1] To summarize, the principle actions of salicylates (chiefly aspirin and salicylate) on connective tissue metabolism are

1. To produce the amount of ATP for biosynthetic reactions by uncoupling of oxidative phosphorylation[1,192] and possibly inhibition of the mitochondrial respiratory dehydrogenases
2. To inhibit many of those enzymes (transferases) involved in the transfer of oligosaccharides, acetate, and sulfate to acceptor peptides/glycopeptides[1,193,197]
3. To inhibit the turnover of collagen proteins[193,197]
4. To reduce the secretion of glycosamino-glycans by inhibiting the PGE-dependent synthesis of cyclic AMP, the synthesis of prostaglandins, and the activities of cyclic AMP-dependent protein kinases.[199]

The possible beneficial effects of aspirin in inhibiting the synthesis of sulfated proteoglycans of cartilage have recently been challenged by the observations of Palmoski and Brandt.[200] These authors observed that feeding of 120 mg/kg/d aspirin to groups of 3 dogs (per treatment group), in whom joint immobilization had been induced surgically and a treadmill exercise program maintained through the experiment, caused an adverse effect on cartilage proteoglycan composition.[200] Although the authors made much of this, it is noteworthy that they reported that the aspirin treatment did not cause any deterioration in cartilage or synovial gross morphology or histology.[200] It is difficult to reconcile these observations with their observed decrease in proteoglycan-hyaluronate interactions following aspirin treatment or their published claims that the aspirin treatment was in any way adverse in its effects. If anything, the biochemical observations failed to have any relevance whatsoever to the histological status of the joint. It is therefore questionable whether this particular model has any relevance to understanding of joint pathology and reaction of the salicylates in man or in induced inflammatory or joint degradative states.

6. Effects on the Immune System

Aspirin has been reported to inhibit lymphocyte transformation in vitro in response to mitogens.[1] Sodium salicylate (200 mg/kg twice weekly) was found to suppress the enhanced lymphocyte levels in rats infected with *Mycoplasma arthritidis* but did not influence the susceptibility of rats to (re)infection.[201] These results are of particular interest in view of the (somewhat controversial) suggestions that mycoplasmas may be involved in the etiology of rheumatoid arthritis in man.

The stimulation by the interferon inducer, poly (I)·poly(C), of prostaglandin E and hyaluronic acid production from human synovial fibroblasts is inhibited by 1 m*M* aspirin.[202] Despite this drug-induced influence on the actions of interferon, aspirin does not affect the antiviral or antiproliferative actions of interferon.[203] Other influences of this drug on the cellular immune system are relatively minor and may in fact be accounted for by its effects on prostaglandin biosynthesis.[1]

B. Analgesic Activity

1. Peripheral Compared with Central Actions of Aspirin and Salicylate

The classical studies of Lim and co-workers[204,205] using an elegant cross-perfused spleen preparation in dogs, and recordings of pain perceived in the central nervous system of cats and dogs[206] emphasized the peripheral mode of aspirin in the pain evoked at chemoreceptors by bradykinins and some other noxious stimuli. Furthermore, their results suggested that aspirin was some four times more potent than sali-

cylate as an analgesic agent where bradykinin was used to evoke vocalization response to intrasplenic injections of bradykinin.[205] Later studies by Collier and Schmieder[207] and Ferriera[130,132-134,208] (comprehensively reviewed by Ferreira[209]) showed that the inhibition of the production of pain evoking prostaglandins (stimulated by bradykinin and other agents) constituted a major role for the actions of aspirin. There is definitely an anti-inflammatory action of salicylates which is important for expression of their analgesic effects. More recent evidence[209] indicates that a central prostaglandin-dependent effect of aspirin is also important, but clearly this is quite distinct from the actions of morphine-like drugs on their respective opiate receptors. The evidence for a minor central action of aspirin has also been supported by (1) neurophysiological studies showing that salicylate protects against the nociceptive responses elicited by electrical stimulation of the lateral hypothalamus in rats,[210] and (2) that aspirin and salicylate enhance serotonin (5,hydroxytryptamine) production or turnover,[211-212] such as seen in morphine analgesia where activation of serotoninergic pathways has been invoked as part of the central actions of these latter drugs.[214-216]

2. Enhanced Serotonin Turnover

Since (1) salicylates displace tryptophan from its binding sites on serum albumin,[217] (2) the free tryptophan displaced from albumin exchanges with brain tryptophan,[218] and (3) the salicylate-displaced tryptophan (as well as the drug effects on inhibiting the tryptophan degrading enzyme, tryptophan pyrrolase), enhances brain tryptophan levels in rats, it has been suggested that the tryptophan displaced by salicylate might be the cause of the enhanced serotonin turnover by the drug in the brain[211-213,220] and so be responsible for the pain relief by the drug.[220,221] It is also possible that the anti-inflammatory effects of salicylates and other NSAI drugs, which also express the pain-relieving properties of these drugs, might also be accounted for by displacement of tryptophan. While enhanced serotonin turnover by salicylates has been noted in certain areas of the brain,[212] it should be noted that these effects have not been correlated with effects on serotoninergic activation. Indeed the principle changes in response to aspirin (500 mg/kg) in rats are increased levels of 5HT in the hippocampus and increased 5-hydroxy-indole acetic acid levels in the hypothalamus, brain stem, and cerebellum. Some of these changes cannot be reconciled with actions on specific CNS pathways of serotoninergic activation as seen with morphine. It may be that enhanced levels of 5HT in the hypothalamus by aspirin could reflect antipyretic activities induced by this drug[1] (see later).

3. Effects on Nerve Impulse Transmission

In addition to influencing the inflammatory component of pain response and possibly CNS effects on serotonin production, there is evidence to suggest that aspirin and salicylates can depress the activities of certain nerves[222-228] involved in pain transmission,[224,227,228] possibly by influencing membrane permeability involving decreased Cl^- and increased K^+ conductances.[223,226,229-232] There may also be effects on ATP production and activities of those ATPases involved in ion transport.[233,234]

Among the most recent studies of analgesic effects of aspirin in laboratory animals relevant to that in man is the study in adjuvant arthritic rats by Guilbaud and coworkers.[228] These authors observed that a dose of 50 mg/kg of aspirin depressed the number of spikes in discharges recorded in the ventro-basal region following mobilization or pressure application to inflamed joints.[228]

4. Effects on Kinin Release

In addition to aspirin inhibiting the bradykinin-induced stimulation of prostaglandin production, it appears the drug directly affects release of bradykinin.[1,235,236] Thus, In-

oki and co-workers[235,236] found that aspirin (200 μg/kg i.p.) inhibited the release of bradykinin-like substances and of kinin-forming enzyme in the perfused rat paw upon stimulation with heat, pressure, or sciatic nerve stimulation. They contrasted these results of the drug inhibiting kinin release from all forms of noxious stimuli with the effects of morphine (5 mg/kg i.p.) which only inhibited kinin release mediated by nervous stimulation.[235,236]

5. Other Biochemical Actions of Aspirin/Salicylate

Among these, (1) reduction in noradrenaline turnover, (2) alterations in the ratio of the excitatory neurotransmitter glutamate to its inhibitory analogue, GABA (γ-amino*iso*butyric acid), and (3) reduced activity of pseudo-cholinesterases have all been invoked to explain the actions of aspirin or salicylate on nerve function.[1] The relevance of these effects to the analgesic actions of aspirin/salicylate has, however, still to be determined.

It is of interest that administration of leukocyte pyrogen or exposure to infrared radiation has been shown to cause an approximate two-fold increase in the concentrations of aspirin in the brain of rabbits.[237] Thus, some of the influences of the febrile state might well involve alterations in drug pharmacokinetics, and this may have importance in interpreting dose-responses to various drugs.

6. Comparison of Analgesic Actions of Aspirin and Salicylate With Other Drugs

The analgesic potency of aspirin/salicylate relative to that of other salicylates or analgesic/anti-inflammatory drugs varies considerably, according to the choice of noxious stimuli and species employed.[1] Generally, aspirin is slightly more potent than paracetamol, and both of these are appreciably less potent than diflunisal, indomethacin, *dextro*-propoxyphene and morphine in the hyperalgesia from injection of Freund's adjuvant to rats and manipulation of the injected hindpaw[238] (see Table 2). This model may be particularly useful for its relevance to arthritic pain in man.

C. Antipyretic Activities

The well-established antipyretic effects of aspirin have been considered by some to involve inhibition by this drug of the synthesis of E-type prostaglandins presumably induced by the endogenous pyrogens, but the evidence for inhibition of prostaglandin production alone being the mode of action of aspirin is rather doubtful.[238-240] Several lines of evidence mitigate against the prostaglandin theory including (1) the negative effects of prostaglandin antagonists,[240,241] (2) lack of effects of hydrocortisone (which should also inhibit PG production) in fever induced by endotoxin,[242] (3) the equipotency of salicylate and aspirin in inhibiting endogenous pyrogen-mediated fever in rabbits despite the fact that salicylate is a much weaker inhibitor of prostaglandin production than aspirin,[243] and (4) that diflunisal is only 1.5 times more potent than aspirin as an antipyretic;[244] yet of the two the former is relatively much more effective as an inhibitor of prostaglandin synthesis.[140] Endogenous pyrogen and endotoxins act in fever by altering the "set-point" for temperature regulation at sites in the pre-optic and anterior hypothalamic regions of the central nervous system.[240] Several studies have shown that salicylate or aspirin have direct effects when applied in the hypothalamic region[243,245] indicating that these drugs are capable of acting directly on the hypothalamus without influencing production of prostaglandins from peripheral sites, though no doubt this might form a contributory action. It is possible that the salicylates might exert their effects in the CNS by enhancing serotonin levels in the hypothalamus,[212,246] noradrenalin metabolism,[1] and/or influences on cyclic AMP actions which is known to be influenced by salicylate.[247]

Table 2
COMPARISON OF ANALGESIC POTENCY[a]

Drug	ED_{50} (mg/kg)
Aspirin	180
Diflunisal	7.18
Glatenine	26.4
Indomethacin	2.56
Morphine	2.42
Nalorphine	28.3
Paracetamol	200
D-Propoxyphene	20.4
Zomepirac[b]	16.6

[a] Comparison of analgesic potency of aspirin and diflunisal with other analgesics in rats with adjuvant hyperalgesia.
[b] Now withdrawn.

Data from Winter, C. A., Kling, P. J., Tocco, D. J., and Tanabe, K., *J. Pharmacol. Exp. Ther.*, 311, 678, 1979.

D. Side-Effects and Toxicology

The principal side-effects of the salicylates include the following:[1,248]

1. Gastro-duodenal hemorrhage and ulceration, generalized epigastric pain, dyspepsia, diarrhea, and constipation manifest especially in arthritic patients consuming relatively large quantities of the drugs over long periods of time
2. Nephrotoxicity, especially from combinations of aspirin with other NSAI/analgesic drugs
3. Asthma and hypersensitivity conditions involving the skin
4. Hepatotoxicity, most evident in patients with systemic lupus erythematosus and possibly rheumatoid arthritis
5. Rarely, blood dyscrasias, teratogenicity, and reduced birth weight

Of these, the first is by far the most frequent and potentially very serious.[1,248] For many of these conditions there is strong evidence of drug-disease and/or drug-drug interactions, so that in some instances these side effects may be preventable simply by recourse to avoiding drug/disease potentiation of salicylate-induced toxic reactions, adjusting the therapeutic regimen, or adopting certain preventative/adjunctive therapies.[1]

There is a belief that the side-effects of aspirin, especially those involving the gastrointestinal tract, are much more frequent than experienced with other NSAI drugs. For comparisons of GI intolerance with some of the latter drugs this might be true.[1,248,249] However, it is as well to note that aspirin has been used far more extensively than any other NSAI drug since its introduction at the turn of this century. Hence, the frequency of reports and attention devoted to their study has been much greater relative to that for other drugs. Furthermore, many of the newer NSAI drugs have unique, and in some cases more severe, side-effects[250,251] which means, in effect, that aspirin and some of the salicylates may be relatively safer when the side effects of all these drugs are compared.

1. Upper Gastrointestinal Tract

a. Factors Influencing Gastric Injury

The major factors involved in the development of gastric mucosal injury and bleed-

ing in man have been reviewed and compared with studies in laboratory animals (mostly rats).[1,249,254] There is general agreement that the same factors are implicated in these species, the main features of which are that gastric injury is (1) dependent upon acidity of the gastric contents and the presence of the acidic moiety in the drug, (2) influenced by particle size and tablet formulation, (3) exacerbated by exposure to alcohol, stress, and vitamin C deficiency, and (4) reduced by certain foodstuffs, certain buffering agents, and nutrients. There is some confusion about the role of sex and blood group status.

Although there is a pronounced systemic effect in the gastric ulcerogenesis of aspirin, there is no doubt that direct contact of the drug and/or some irritant with the gastric mucosa is essential for the development of lesions/ulcers.[1,252-255] Thus, parenteral aspirin or salicylic acid reduces the gastric mucosal electropotential without causing lesions.[252]

While even high (single) doses of these drugs given parenterally do not induce gastric lesions,[253-255] concurrent exposure to cold, physical (restraint) or swim stressing procedures (which are alone insufficient to cause lesions) does cause expression of mucosal irritancy of aspirin.[253,254] Likewise, peroral administration of the "barrier breaker" taurocholate in rats dosed parenterally with sub-ulcerogenic doses of aspirin causes lesion development. Presumably, the inhibition of mucosal prostaglandin production — a factor considered important in the genesis of mucosal damage by NSAI drugs — is not *per se* a requirement for lesion development. However, topical irritation either by the drugs or other agents, as well as exposure to stress, is the key to the initiation of mucosal injury presumably potentiated by inhibition of mucosal prostaglandin production.[255]

It is through variations in the systemic availability of aspirin/salicylate that ulceration may be potentiated in individuals with alcoholic liver disease, in the elderly, or by other plasma protein bound NSAI drugs.[1,256-258] This occurs from the reduced detoxification of aspirin (to salicylurate),[256] lower fraction of plasma protein bound salicylate[256] (from reduced albumin levels[256,257]) and reduced plasma[257] and mucosal[258] aspirin esterase activities in alcoholics. While normal elderly patients may not be presented with these problems,[256] those with rheumatoid arthritis and other related conditions will be expected to have lower levels of plasma albumin and decreased detoxifying capacity, so that aspirin may be particularly ulcerogenic in these individuals as a consequence of these systemic factors.[1]

Tablet formulations of aspirin are particularly important in determining the intrinsic ulcerogenicity of this drug.[1] Soluble or buffered formulations are well-known to be less ulcerogenic than "plain" compressed aspirin tablets.[1] Certain tablet excipients or cationic buffer systems may exert variable effects on either the ulcerogenicity or bioefficacy of aspirin.[259] For example, the aluminum oxide formulation of aspirin (Aloxiprin®) or the heavily buffered ($NaHCO_3$-containing) aspirin preparations (Alka-Selzer®) may be less ulcerogenic in rats, but they are also less effective in their antipyretic or anti-inflammatory activities.[259,260] Another aluminum oxide formulation of aspirin (Bufferin®) is actually more ulcerogenic than aspirin. While certain cationic polymers have no effect,[259] carboxymethyl cellulose, methyl cellulose, and the detergent Tween-80® noticeably enhance the gastric ulcerogenicity or gastric bleeding induced by aspirin.[260] The possibility that enteric-coated formulations of aspirin may be less ulcerogenic than compressed tablets of aspirin is debatable, since there is a disparity between the results obtained at post-mortem in pigs compared with observations using less-invasive procedures in man.[1]

b. Mechanisms of Gastric Damage

Some insights into the mechanisms of gastric mucosal injury by aspirin and related drugs have recently been provided from (1) observations of the time-sequence of cel-

lular damage from electron-microscopic studies, (2) the relationship of this or other morphologic observations to inhibition of the synthesis of cytoprotective prostaglandins and a variety of other biochemical effects, and (3) the modification of aspirin effects by various pharmacological agents.[1,254,254,261-279]

These studies have shown a sequence of events which can be briefly summarized as follows:

1. Sloughing of the mucus protective barrier, with enhanced drug absorption to underlying mucous cells,[1] destruction of the hydrophobic protective barrier comprising surface active phospholipids[280] associated with surface membrane damage and loss of the permeability barrier[1,252,254]
2. Selective destruction of acid-secreting parietal cells resulting from drug accumulation (a consequence of the pH dependence of drug absorption), lysosomal membrane disruption, and mitochondrial/secretory canniculi damage[262]
3. Disruption of endothelial cell junctions of capillaries causing loss of blood into the interstitial tissue[62] aided by a drug-induced inhibition of platelet aggregation from inhibition of thromboxane/PGG_2 production. Hypothetically, this might induce localized ischemic reactions and perhaps release of lysosomal enzymes and tissue destructive oxyradicals.[1,254]
4. Inhibition of the synthesis of cytoprotective prostanoids[261,263] might favor impaired blood flow with reduction in removal of back-absorbed H^+ ions (from both deprotonation of the drug and that from acid back-diffused into the mucosa),[1] reduction of bicarbonate secretion[66] through to the mucus barrier, and effects on mucus discharge.[1]
5. Acid back-absorbed into the pepsin(ogen) containing chief cells together with drug favors pepsin production, so aiding autodigestion[1]
6. Stimulation of acid production by histamine release from (?degranulated) mast cells,[1,273,274] coupled with inhibition of phosphodiesterase activity[274] (so causing increased levels of cyclic AMP), enhanced vagal-parasympathetic activity,[1,267] and possibly stimulated gastrin secretion[1]
7. Inhibition of ATP production by salicylate, especially in parietal cells (which have abundant mitochondria to supply large quantities of ATP for acid secretion) might lead to enhanced AMP levels, and activation of xanthine oxidase to produce tissue destructive superoxide radicals.[1,254] Acid secretion would be subsequently reduced following lowering of ATP levels.
8. Secondary changes include reduction in mucus biosynthesis from lowered ATP levels, direct inhibition of enzymes involved in mucus glycoprotein biosynthesis and effects on prostaglandin-release and cyclic nucleotides which regulate mucus synthesis.[1]
9. Long-term effects of the drug might involve reduced mucosal detoxification reactions,[269] effects on cell regrowth and, following extensive exposure to the drug (possibly with other ulcerogenic agents and stress), localized immunological reactions and chronic ulcer formation. The extent of long-term injury from the drug[281] will depend upon the extent of natural adaptation, which is known to be quite profound in uncomplicated medication with the drug (i.e., no stress or alcohol).

The above is a hypothetical presentation of events; there is evidence for the existence of certain of these events but the sequence and interactions in some cases may not be completely understood at present. It is intended to give the reader notional ideas of the concepts of gastric mucosal damage as considered today.

c. Procedures to Reduce Mucosal Injury

Based on the concepts of gastric mucosal damage by aspirin, many procedures have been explored to prevent this injury. For instance, the recognition that the carboxylic acid moiety of aspirin is largely responsible for its ulcerogenic activity has led to the development of various esters of aspirin (e.g., alkyl-, triclyceryl-, paracetamol-, and aryl-derivatives) and even some other acidic NSAI compounds). Many of these show less gastric mucosal damage in animals and even in some cases in man, compared with aspirin.[1,69,70,76-83] These are essentially pro-drugs, since upon absorption they undergo hydrolysis to slowly release the acid itself.[96] However, some such as the paracetamol ester of aspirin, benorylate, have such a slow rate of aspirin release that they have to be administered in proportionately larger doses for full anti-inflammatory and analgesic activity.[1] Certain alkyl- (e.g., C1 to C5) esters of aspirin release this drug and its metabolites more rapidly[67,70,96] and so do not show the disadvantages of those more bulky esters (i.e., by showing any reduction in therapeutic efficacy).[1]

Other successful approaches have been to correct (1) the biochemical "deficiencies" caused by aspirin, e.g. reduced production of cytoprotective prostanoids, and impaired ATP production, or (2) the overt actions of histamine in exacerbating injury by promoting acid secretion in the stomach. Extensive studies in laboratory animals and clinically in man[1] have shown that prostaglandin E_2 or its long-acting methylated derivatives will protect the gastric mucosa against injury from aspirin.[270,276-279] Likewise, histamine H_2-antagonists (cimetidine, ranitidine) also exert potent protective actions against these drugs.[268,271,272,278] Both could be regarded as an expensive way of preventing gastric damage, and thus it would seem it should be confined only to extreme cases, e.g. elderly arthritic patients who have ulcers and who may have experienced severe bleeding from the GI tract. Furthermore, those individuals prone to bleeding by aspirin or related drugs would appear excellent candidates for employing the cheaper approach simply to add certain nutrients to the aspirin formulation to correct the ATP deficit induced by this drug (i.e. from inhibition of mitochondrial enzymes), and at the same time promote solubilizing of the drug. Clinical studies show this is a very effective means of preventing aspirin injury at relatively low cost and with the full therapeutic potential of aspirin being retained.[12]

2. Nephropathy

Aside from the renal injury which develops from abuse of analgesic mixtures, the major concern in arthritis therapy is the impairment of renal function and infrequent nephropathy from aspirin in some arthritic states.[1]

Aspirin causes a reversible decline in renal function in patients with rheumatoid arthritis, manifest as a fall in glomerular filtration with associated mild water, sodium, potassium, and urate retention. This damage is mild and may not constitute a reason for drug withdrawal except in patients especially prone to its effects, or where specific damage has occurred from drug combinations. However, patients with systemic lupus erythematosus (SLE) appear at particular risk. It appears, therefore, that the particular arthritic condition plays a major factor in the etiology of more serious renal pathology.[1] Also, there is a very real possibility that as a consequence of prescribing more than one NSAI drug, patients may be exposed to an excessive risk of renal nephropathy.[1]

a. The Role of Drug Distribution

The pharmacokinetics of aspirin/salicylate are important in understanding development of renal damage by these drugs, especially in combination with other NSAI/analgesic drugs. High concentrations of salicylate accumulate in the kidney, especially in the outer cortical region.[89,97] The mechanism of this accumulation may include the

involvement of the normal renal mechanism for concentrating organic anions. Salicylate has been shown to be ultrafiltered at the glomeruli, then secreted by the proximal segments of the tubules to undergo back-diffusion. This back-diffusion of salicylate which is pH-sensitive, is not evident with its metabolite, salicylurate. Hence, it appears that the rate-limiting metabolism of salicylate to the nontoxic salicylurate could be a major factor in determining the renal toxicity of salicylate and aspirin.[1]

Aside from this disposition of the salicyl moiety, aspirin extensively acetylates proteins, lipids, and glycoproteins in the kidney, especially in the cortex.[89,97] High concentrations of acetylated proteins have been observed in renal microsomal fractions, and this may reflect the acetylation of the prostaglandin endoperoxide synthetase enzyme system. Other proteins are also acetylated, and this could be a basis for drug-induced inhibition of enzymes — so contributing, with salicylate, to the cellular damage in the kidney. Aspirin may be a bifunctional drug in its nephrotoxicity because of the specific accumulation and subsequent effects of both its salicylic and acetyl moieties. The mechanisms of renal injury are therefore linked to the distribution of these moieties.

b. Mechanisms of Renal Damage

The inhibition of the production of renal prostaglandins has been advocated as a major factor in the pathogenesis of aspirin-associated nephropathy.[1] However, the influence of aspirin on the production of prostaglandins appears to be one of a priming function, i.e. as a prelude to the subsequent development of renal damage. One theory with considerable evidence is that aspirin and indeed other NSAI/analgesic drugs induce ischemic reactions in the kidney as a result of the inhibition of vaso-dilator prostaglandins E_2 and I_2. Reduction in the prostaglandin-regulated glomerular filtration and in the excretion of sodium and water in the urine could potentiate the drug-induced influences on prostaglandin metabolism and renal blood flow. The effects of Na^+ excretion appear to be related to reduced renin release (from inhibition of prostaglandin E_2) for activation of angiotensin I (to angiotensin II).

The impairment by aspirin and salicylate of the production of ATP and related high energy products necessary for normal cellular metabolism and synthesis from the drug-induced inhibition of mitochondrial enzymes contributes to renal damage.[1,248] Inhibition also occurs of renal glycoprotein and protein biosyntheses from decreased ATP for the cellular synthetic reactions as well as direct inhibitory effects of the salicylates on enzymes involved in the biosynthesis of these macromolecules.[1] These drug effects are added to those of stimulation of tissue-destructive lysosomal enzymes, possibly initiated by ischemia — or free oxygen radical generation.[1,248]

3. Asthma and Hypersensitivity Reactions

Aspirin is a frequent cause of hypersensitivity reactions — manifestations comprising skin rashes and eruptions, angioedema, vasomotor disorders, rhinitis, purpura, asthma, and nasal polyps.[1,248] Symptoms of aspirin hypersensitivity are somewhat anaphylactoid in character and include difficulty in breathing, sudden weakness, fainting, sweating, and collapse. In the case of skin conditions, the symptoms range from the simple rash to large urticarial weals and swellings. The incidence of these side-effects has been variously estimated at between 0.001 to 0.02% of the population consuming aspirin.

Cross-reactions occur frequently in aspirin-sensitive individuals, especially with other NSAI drugs and the food-coloring agent tartrazine. Tartrazine and a number of NSAI/analgesic drugs may themselves induce hypersensitivity reactions. These observations suggest that there may be a common mechanism of action of these drugs. These effects involve either perturbations of eicosanoid metabolism and/or immunological reactions. However, no one mechanism can explain the cross-reactions that occur in some individuals between aspirin and the NSAI drugs and tartrazine.

There is one popular concept at present that inhibition of prostaglandin synthesis by aspirin causes diversion of arachidonate to produce large quantities of leukotrienes, which mediates hypersensitivity reactions.[1,248] This has some evidence to support it, but it cannot alone account for the etiology of complex hypersensitivity reactions. It is most likely that an intrinsic sensitivity of some individuals towards these drugs, perhaps as a consequence of abnormalities of their immune system, could be important. This could explain why patients with SLE so frequently develop hypersensitivity reactions with aspirin and other NSAI drugs. Activation of the complement system and hypocomplementemia have also been implicated in hypersensitivity reactions to aspirin and some other NSAI drugs. De Weck and Reinhardt have been proponents of a concept that aspiryl antibodies may be initiated during hypersensitivity reactions and, more recently, that lymphocytes may mediate these and related hypersensitivity reactions.[248]

Acetylation by aspirin of various proteins, especially the plasma proteins, could induce structural changes in these proteins (especially albumin) which may induce antibody formation or hypersensitivity reactions against these self-proteins.[1] This could, in part, form the basis of some of the postulated immunological reactions. It has been found that the nasal polyps from aspirin-sensitive individuals have a prostaglandin-synthesizing system which is uniquely sensitive to aspirin, suggesting that there is an intrinsic sensitivity in the capacity of aspirin to divert arachidonate to produce broncho-constrictor leukotrienes. If this sensitivity is immunologically derived, it could represent the link between the eicosanoid and immunological systems.

4. Hepatotoxicity

Aspirin is rarely implicated as a cause of hepatotoxicity, but there have been sporadic reports of hepatic injury following salicylate therapy in arthritic patients, most especially in patients with SLE.[1,248] Initially there is evidence of hepatitis preceded by liver dysfunction (e.g., elevated levels of serum transaminases). It appears that the incidence of abnormal liver function is relatively low in patients with rheumatoid- or osteo-arthritis, even in those patients taking large quantities of aspirin for long periods of time. However, in patients with SLE and some with severe rheumatoid arthritis, it appears that certain complications arising from abnormal liver metabolism predispose these individuals to liver injury.

Aspirin and salicylate cause only minor structural damage to the liver of animals when given orally or parenterally in relatively high doses. These changes involve (1) an increase in the number and size of hepatic microbodies, (2) an increase in smooth endoplasmic reticulum, and (3) changes in mitochondrial morphology.[1] The changes in (1) and (2) could reflect increased activity of those liver enzymes responsible for metabolizing aspirin or salicylate (i.e., the oxidases in microbodies and those of the P-450 system). While there are not any extensive pathological changes following administration of large doses of aspirin to animals, there are some minor fine structural and biochemical changes which could be a prelude to damage in those individuals or animals with chronic inflammatory conditions or with severe liver dysfunction.[1] In those few instances where there have been pathological studies in man of salicylate-induced liver injury, it appears that structural changes develop, reflecting reduction in the activities of the protein synthetic machinery in the endoplasmic reticulum and the energy-producing system in mitochondria.[1] There is also evidence of enhanced autolytic activity of the liver. These correlate with in vitro and in vivo evidence in laboratory animals of direct inhibitory effects of salicylate, and in some cases aspirin, on protein biosynthesis and mitochondrial ATP production, together with enhanced lysosomal activity.[1] Salicylate and aspirin also cause leakage of glutathione from the livers of rats.[1] Since ethanol and paracetamol can depress liver glutathione levels, it is possible that those

drugs such as paracetamol, which depend upon this tripeptide for conjugation reactions in their detoxification, could be more hepatotoxic when ingested in subtoxic quantities with aspirin, salicylate, or ethanol.[1,248]

5. Other Side Effects

Other side effects have been reviewed elsewhere,[1] and include teratogenic effects and reduced birth weights,[93,94,282] for which the incidence is debatable. Recent studies in rats[282] confirm earlier published literature on the embryotoxicity of high doses of aspirin, with abnormalities of the fetal skeletal system predominating.

Concern about aspirin being involved in the development of Reye's syndrome in children[1,284] has continued to be expressed.[283] Hepatic pathology and cerebral pathology have recently been reported with this syndrome.[284] Proposals that warning labels should be placed on aspirin packages in the U.S. have been criticized,[283] and this procedure would seem to be of little value in overcoming this rare and occasionally fatal condition. Only research will help in understanding the involvement of aspirin in its etiology.

Salicylates continue to be implicated in pulmonary edema, but associated factors (e.g., cigarette smoking) also contribute to this syndrome, which obviously has a multifactorial basis.[285] Alterations in vascular permeability in the lungs constitute the central feature of this disorder.[285]

V. NEW CLINICAL USES

General aspects of the uses of the salicylates in treatment of arthritic conditions are discussed elsewhere in these volumes by Brooks et al. and have also been recently reviewed.[1] Here some recent developments with salicylate derivatives will be considered which could be of interest in the therapy of arthritic diseases.

While aspirin and some related salicylates (e.g., choline magnesium trisalicylate, benorylate, salicyl salicylic acid, (diplosal and salsalate) continue to serve a fundamental role in the treatment of many arthritic conditions, there has been a recent revival of interest in the use of salicylazosulfapyridine (= SASP, sulfasalazine) for these conditions.[1]

SASP was originally introduced by Svartz in 1948 for treating rheumatic conditions in the belief that this sulfa drug derivative would control the intestinal infective basis of rheumatic conditions — a popular belief at that time.[1] It soon fell into disuse following reports of its variable efficacy and was subsequently successfully employed for the treatment of ulcerative colitis, Crohn's disease and other inflammatory bowel disorders, where it is now well established as an effective treatment.[1] It was revived for therapy of rheumatoid arthritis by McConckey and co-workers.[1] This drug has novel effects on prostaglandin metabolism in that the parent drug and its 5-aminosalicylate (5-ASA) metabolite inhibit the breakdown of PGE_2 by the 15-hydroxy-prostaglandin dehydrogenase.[86,287]

These drugs also exert variable effects in vitro on other aspects of arachidonate metabolism, depending on enzyme sources/preparations and substrate availability (see Reference 286 for review). However, it appears that 5-ASA can inhibit prostaglandin formation in human colonic tissue and both 5-ASA and SASP may inhibit 5-lipoxygenase activity.[286] The latter actions might be related to some of the drug effects on polymorph function.[1] The SASP has immunosuppressant properties, so that it is of particular interest in treatment of some of the cellular-immunological events underlying the development or maintenance of rheumatoid disease.

In clinical practice, SASP produces some quite severe side effects (e.g., dyspepsia, megaloblastic anemia, infertility, hepatotoxicity),[1] and no doubt this has limited use

of this drug. Since the sulfapyridine moiety (formed on azo-link reduction of SASP) is responsible for most of these side effects, there has been much interest in developing analogues without this group, or in the use of 5-ASA itself. The interest here has been more for therapy of inflammatory bowel disorders, but the applications could extend to their use in rheumatoid and related arthropathies. To this end Berry, Hoult, and coworkers[287] have investigated the effectiveness of a number of SASP analogues (produced by Pharmacia AB Uppsala, Sweden) and established that one of the SASP analogues, homosalazine (which is devoid of the pyridine aminosulfonate group and has a CH_2COOH in place of $-COOH$ in the salicyl moiety) is equipotent with SASP as an inhibitor of 15-hydroxyprostaglandin dehydrogenase. This drug may serve as a type for safer treatment for both inflammatory bowel disorders (IBDs) and rheumatoid arthritis, and clinical trials are currently underway with this drug. Likewise, four other SASP analogues, salicylozo-*p*-aminohippurate, disodium azodisalicylate,[288,289] ipsalazide [5-(carboxymethylcarbamoyl-4-phenylazo)-salicylic acid disodium salt],[290] and balsalazide [5-(carboxyethyl-carbamoyl-4-phenylazo)-salicylic acid disodium salt],[290] have been developed and are currently undergoing clinical trials in IBDs. These, together with 5-ASA, could equally find places in the therapy of arthritic conditions.

Copper complexes of aspirin and other NSAI drugs continue to attract interest for their novel biochemical effects (e.g., effects on oxygen radical production and leukocyte functions[291-293] which are potentially interesting for exploitation in the therapy of inflammatory diseases. Recent studies have shown the low chronic toxicity of copper aspirinate administered orally to rats and dogs, associated with low copper retention.[294] One interesting feature that emerged from these studies was that there was an increase in Kupffer cell activity in animals receiving copper aspirinate for 3 months.[294] This may be of particular relevance in the therapy of rheumatoid diseases where liver functions are compromized and where macrophage phagocytic activity might be usefully modified.

VI. CONCLUSIONS

This brief review has focused on novel biochemical and therapeutic properties of the salicylates. Previously, the mode of therapeutic actions and side effects of drugs under the blanket term "the aspirin-like drugs" or "salicylates" has been ascribed to the inhibitory effects of these drugs on prostaglandin production. While this is one important aspect of the actions of these drugs, it is clear that a whole range of biochemical and cellular actions are affected by the salicylates and that these actions vary considerably with different drugs.

The salicylates have been explored for therapeutic uses in a wide variety of conditions, and the development of diflunisal and some novel salicylazasulfapyridine (or sulfasalazine) analogues represent some interesting and important advances in the therapy of arthritic conditions. Likewise, the reduction of gastrotoxic and other side effects of aspirin has given new hope for the safer use of this valuable drug.

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