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INHIBITORY EFFECTS OF SOME ANTI-INFLAMMATORY AND OTHER ANALGESICS AND NITROFURANS ON THE INDUCTION OF β-GALACTOSIDASE SYNTHESIS IN *KLEBSIELLA AEROGENES*

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Abstract—The effects of the following drugs were studied on the induction of β-galactosidase synthesis by and the growth of *Klebsiella aerogenes:* acetylsalicylic acid (I), salicylic acid (II), salicylamide (III), diacetylpyrocatechol-3-carboxylic acid (Movirene, IV), ibufenac (V), ibuprofen (VI), paracetamol (VII), phenacetin (VIII), morphine (IX), nalorphine (X), nitrofurazone (XI), nitrofurantoin (XII), furazolidone (XIII), oxine (XIV), nitroxoline (XV), niridazole (XVI). The antimicrobial compounds XI–XVI inhibited enzyme induction at low concentrations and were even more active against growth which was preceded by a lag. The analgesics I–X reduced the growth rate but did not give a lag and were more active against enzyme induction. The anti-inflammatory antipyretic analgesics I–VI inhibited enzyme induction at concentrations of the same order as those which inhibit nociception and inflammation in mammals. The non-anti-inflammatory antipyretic analgesics VII and VIII inhibited enzyme induction at higher concentrations and the narcotic analgesic IX and its antagonist X inhibited enzyme induction at concentrations about 100 times those which inhibited nociception. IX did not antagonise the inhibitory effects of X and *vice-versa*. The inhibitory effects of I can be ascribed to II produced by hydrolysis. There is a general parallelism between inhibition of enzyme induction and anti-inflammatory activity, which is discussed and related to current theories of anti-inflammatory action. Inhibition of protein synthesis is suggested to be a primary biochemical effect leading to anti-inflammatory properties. Testing for the ability to inhibit induced enzyme synthesis by bacteria may be useful in screening drugs for anti-inflammatory properties.

THE PHARMACOLOGICAL effects of a drug must arise from some initial interactions of the drug with certain molecules that take part in an important biochemical process. The primary and even the subsequent biochemical interactions that lead to the relief which the anti-inflammatory antipyretic analgesics bring about are still not properly understood and are still open to debate.[1] The important suggestion by Adams and Cobb[2] that the ability of these drugs to uncouple oxidative phosphorylation accounts for the reduction of inflammation has been supported by the extensive researches of Whitehouse.[3] On the other hand, Smith[4] has suggested that the uncoupling action may play no such part or merely a supporting role to other sites of interaction. Among several mechanisms[5] proposed to explain the anti-inflammatory action of the drugs is the inhibition of the biosynthesis of kinins, of other proteins and of mucopolysaccharides.

In an attempt to throw a little more light on the biochemical mode of action of the

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anti-inflammatory analgesics, the effects of some of these drugs were investigated using a very simple biological system. The organism chosen was *Klebsiella aerogenes* NCTC 418, a bacterium whose biochemistry is fairly well known and upon which the effects of drugs have been extensively studied.[6] The induced biosynthesis (induction) of β-galactosidase is here found to be a biochemical process which is inhibited by suitable concentrations of certain anti-inflammatory antipyretic analgesics. Other drugs included for comparison were the non-anti-inflammatory antipyretic analgesics, paracetamol and phenacetin, the narcotic analgesic morphine, and its antagonist, nalorphine which also has analgesic properties, and some antibacterial compounds, including nitrofurans.

MATERIALS AND METHODS

The experimental details are described elsewhere.[7]

A strain of *Klebsiella aerogenes* (*Aerobacter aerogenes*) NCTC 418 which had previously been thoroughly adapted to grow in glucose-mineral salts medium was maintained on "Oxoid" nutrient agar slopes.

The pH of all chemically defined media was 7·12 and did not change during the time in which measurements were made. Any change in pH caused by the addition of a drug was nullified by the addition of sodium hydroxide or hydrochloric acid. In referring to the acidic drugs as their anions, which are the predominant forms at pH 7·12 (>99 mole %), there is no intention of implying that the anions are the active species. Indeed, the unionised molecule of a weak acid is often more active biologically than the corresponding anion. Since the media were buffered to the same pH value in all experiments, the concentration of the active species of a drug was always a constant fraction of the total concentration of drug added.

Acetylsalicylate was slowly hydrolysed to salicylate and acetate under the conditions of growth and of β-galactosidase induction. The hydrolysis was catalysed by the intact bacteria. After precipitating the bacteria in a test sample by centrifugation, salicylate was detected by the characteristic purple coloration on addition of ferric chloride solution and by the ultraviolet absorption spectrum of the solution using a Unicam SP 800 recording spectrophotometer. Salicylate was determined at 296 nm where it absorbs maximally and obeys Beer's law and where unhydrolysed acetylsalicylate and other constituents of the medium do not interfere. Diacetylpyrocatechol-3-carboxylate (2,3-diacetoxybenzoate; Movirene is the acid form) was also slowly hydrolysed under the conditions of growth but the products and the rate of hydrolysis were not investigated. The other drugs were apparently stable and so the concentrations of their active species were assumed to be constant throughout the range of measurements.

Growth curves in glucose-mineral salts medium with and without the drug at suitable concentrations were plotted after at least five subcultures in drug-free medium, in which the lag was negligible and the mean generation time 30 ± 2 min. The time (T) taken for a standard inoculum of dry weight concentration 1·56 μg/ml ($\log_{10}$ μg/ml = 0·19) to multiply by a factor of 100 (i.e. to $\log_{10}$ μg/ml = 2·19) was determined. The percentage inhibition of growth (I_{gr}) was calculated as follows:

$$I_{gr} = 100 - \frac{100\,T_o}{T_d}$$

where T_d is the value of T in the presence of drug and T_o is that for the drug-free control whose growth was logarithmic during this time.

The effects of the drugs on the induction of β-galactosidase synthesis were studied using two modifications[7] of Creaser's[8] induction system. After at least five subcultures in "Oxoid nutrient broth No. 2", the organism was inoculated into a mineral salts medium with or without drug and containing *either* lactose as inducer (and substrate) of the enzyme and as sole source of carbon and energy *or* a mixture of maltose as sole source of carbon and energy and methyl-β-D-thiogalactopyranoside (MTG) as non-metabolized (gratuitous) inducer. In these drug-free media the bacteria were capable of growing and dividing but only after a lag of at least 250 min during which they adapted to the new source of carbon and energy. The β-galactosidase activity (G) was constant and small for the inoculum but increased linearly with time for 150 min and at the same rate in either of the drug-free media. In each case no growth was found to take place during this time. G was determined after 60 min and 120 min and the percentage inhibition of induced enzyme synthesis (I_{es}) was calculated at each time as follows:

$$I_{es} = 100 - \frac{100(G_d - G_i)}{G_o - G_i}$$

where G_d is the β-galactosidase activity in the presence of drug, G_o is that for the corresponding drug-free control and G_i is the initial activity at the moment of inoculation.

In simple bacterial systems such as these the results are very reproducible.

RESULTS

None of the drugs studied inhibited the actual activity (G) of β-galactosidase when present in the enzyme assay solution or when present in the solution during both the ultrasonic disintegration and the assay at the highest concentrations used in the induction experiments. This shows that the observed effects of each drug on the induction system do really apply to the induced synthesis of β-galactosidase and not to the actual activity of the enzyme.[7]

The effects of the antibacterial drugs on the overall growth of *Klebsiella aerogenes* was to increase the lag and to reduce the growth rate. Nitrofurazone also increases the lag of other bacteria including *Staphylococcus aureus* and *Escherichia coli*.[9,10] Niridazole caused *K. aerogenes* to give growth curves similar to those in the presence of the sulphonamides[11] or ethidium bromide;[12] slower growth was followed by faster growth and was preceded by a lag at sufficiently high concentrations.

The minimum inhibitory concentrations of the nitrofurans over 24 hr for the present organism are about the same as those for another strain of *Aerobacter aerogenes*;[13] these concentrations (μg/ml) respectively are: nitrofurazone, 10–50, 13; nitrofurantoin, 10–100, 100; furazolidone, 1–10, 5. 5-Nitro-8-hydroxyquinoline (nitroxoline) inhibits more strongly than does 8-hydroxyquinoline (oxine) the growth of *K. aerogenes* (Table 1) and, in general, other bacteria.[14,15] Niridazole, although used in the treatment of schistosomiasis and severe forms of amoebiasis, is very active against the growth of the present organism. The nitrofurans, oxine, nitroxoline and niridazole are not only very active against the growth of *K. aerogenes* but also readily inhibit induced enzyme synthesis (Table 1). Gale[16] found that nitrofurazone also inhibits induced enzyme synthesis in *Mycobacterium butyricum;* the enzyme system used was that involved in benzoate oxidation.

TABLE 1. INHIBITION BY VARIOUS DRUGS OF GROWTH (I_{gr} %) AND OF β-GALACTOSIDASE SYNTHESIS (I_{es} %) WITH *Klebsiella aerogenes* NCTC 418

Drug	(μg/ml)	Growth inhibition: MIC* during 24 hr	Growth inhibition: MIC* during 96 hr	I_{gr} (%)	Inhibition of β-galactosidase synthesis: I_{es} in lactose medium, 60 min	I_{es} in lactose medium, 120 min	I_{es} in maltose + MTG† medium, 60 min	I_{es} in maltose + MTG† medium, 120 min
				Anti-inflammatory antipyretic analgesics				
Acetylsalicylic acid	100	> 500	> 500	7	0	14	1	5
	200			70–100	1	30	—	—
	500			70–100	9	38	15	34
Salicylic acid	77	> 1000	> 1000	0	26	27	4	21
	383			70–100	42	55	69	72
Salicylamide	10	> 500	> 500	0	15	3		
	100			13	45	17		
	500				51	61		
Movirene	10	> 1000	> 1000	5	3	4		
	100			5	11	13	5	3
	500			10	81	56	34	55
Ibufenac	10	> 500	> 500	0	17	2		
	100			13	43	39		
	500			21	63	51		
Ibuprofen	10	> 1000	> 1000	9	27	13	27	10
	100			10	60	64		
	500			16	66	83		
				Non-anti-inflammatory antipyretic analgesics				
Paracetamol	1000	> 5000	> 5000	12	15	21		
	2000			29	49	55	63	62
	5000			69	75	89		
Phenacetin	500	> 1000	> 1000	24	23	33	52	25
	1000			54	60	76	62	59
				Narcotic analgesics				
Morphine	10	> 500	> 500	4	12	2		
	100			4	14	22	5	1
	500			9	40	40	14	10
Nalorphine	10	> 500	> 500	6	11	0		
	100			8	16	28	15	13
	500			10	45	73	48	29
Morphine+ Nalorphine	10,10				9	10		
	50,50				8	16		
	100,100				37	39		
	500,500				64	78		
	500,100				46	59		
	100,500				57	70		

TABLE 1. *cont.*

Drug	(μg/ml)	Growth inhibition: MIC* during 24 hr	Growth inhibition: MIC* during 96 hr	I_{gr} (%)	Inhibition of β-galactosidase synthesis: I_{es} in lactose medium, 60 min	I_{es} in lactose medium, 120 min	I_{es} in maltose + MTG† medium, 60 min	I_{es} in maltose + MTG† medium, 120 min
				Antibacterial drugs				
Nitrofurazone	0·3	10–50	>50	3	0	0		
	1			6	17	12		
	10			90	49	42		
	50			95	87	95		
Nitrofurantoin	1	10–100	10–100	80	0	4		
	10			89	71	70		
Furazolidone	0·3	1–10	10–100	83	5	3		
	1			>85	20	25		
Oxine‡	1	10	10–100	8	3	11		
	10			88	53	69	43	36
	100			100	74	72	65	73
Nitroxoline	0·1	1–10	1–10	9	13	1		
	0·3			17	51	57	27	21
	1·0			83	76	89	74	83
Niridazole§	1	10–50	10–50	53	28	13		
	10			96	81	78		
	50			100	87	92		

* MIC = minimum inhibitory concentration (μg/ml).
† MTG = methyl-β-D-thiogalactopyranoside.
‡ Oxine (8-hydroxyquinoline) has anti-inflammatory properties.
§ Niridazole is used for the treatment of schistosomiasis and amoebiasis.

The antibacterial drugs exerted a greater inhibitory action against growth than against β-galactosidase synthesis (Table 1). Since these drugs were designed or selected for their action against growth, this is to be expected. Nitroxoline which inhibited the synthesis of the enzyme more strongly than growth of the bacteria was the exception.

The analgesics at suitably high concentrations reduced the growth rate of *Klebsiella aerogenes* without affecting the lag. This behaviour gives rise to a fan-shaped set of growth curves as shown with paracetamol (Fig. 1). The organism behaves similarly in the presence of ethylenediaminetetraacetate.[17] The solubility and antibacterial activities of the other analgesics were not high enough to give widely separated growth curves.

With acetylsalicylate at <200 μg/ml or salicylate at <100 μg/ml growth proceeded at the same rate as for the drug-free control. With acetylsalicylate at $\geqslant 200$ μg/ml or salicylate at $\geqslant 100$ μg/ml growth was at first uninhibited but suddenly slowed down at a bacterial concentration of 52 μg/ml, i.e. after about 150 min or five generations. Growth was then so slow that a considerable time elapsed until the bacterial concentration reached 156 μg/ml, so that I_{gr} had a high value of 70–100 per cent. The

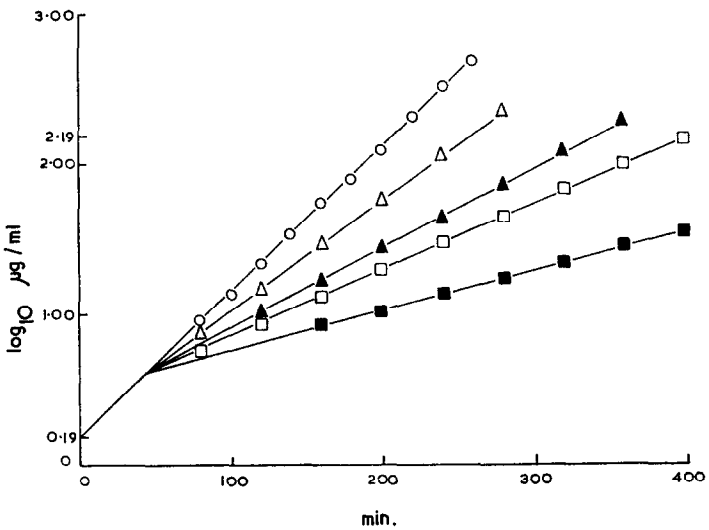

FIG. 1. Growth curves of *K. aerogenes* in glucose-mineral salts medium containing the following concentrations of paracetamol: 0 (○), 2000 (△), 3000 (▲), 4000 (□), 5000 µg/ml (■). The other analgesics give similar behaviour. The ordinate, $\log_{10}$ µg/ml, is the logarithm of the bacterial concentration in terms of dry weight.

minimum molar concentration of acetylsalicylate required to cause a slowing of growth was less than that of salicylate or salicylate plus acetate. By the time growth had slowed, a considerable amount of the acetylsalicylate originally present had undergone hydrolysis to salicylate.

In an induction medium at 40° containing acetylsalicylic acid at 500 µg/ml the hydrolysis was of the first order and its specific rate and half life were as follows: in the presence of normal living bacteria of dry weight concentration 1·0 mg/ml, 0·00147 min^{-1}, 473 min; in the presence of heat killed bacteria (100° for 15 min) at the same concentration, 0·00089 min^{-1}, 778 min; in the absence of bacteria, 0·00089 min^{-1}, 778 min. The bacteria therefore catalyse the hydrolysis enzymatically. In the presence of bacteria the concentrations of (ionized) salicylic acid at 60 and 120 min were therefore 71 and 128 µg/ml respectively. Comparison of I_{es} for salicylic acid at these concentrations with I_{es} for acetylsalicylic acid at 500 µg/ml and the very marked increase in I_{es} with time in the presence of salicylic acid (Table 1) suggest that the inhibition of β-galactosidase synthesis by acetylsalicylate is caused mainly by salicylate produced by hydrolysis. The presence of an equivalent concentration of acetate was found neither to inhibit growth nor the synthesis of β-galactosidase nor to increase the inhibitions in the presence of salicylate. Thus the inhibitions by acetylsalicylate can be ascribed to its hydrolysis to salicylate which not only inhibits bacterial growth,[18,19] but also induced enzyme synthesis in *Pseudomonas fluorescens*;[18] the enzymes studied were those of the Krebs tricarboxylic acid cycle.

In general, the analgesics inhibited β-galactosidase induction more than they inhibited growth (Table 1). With the anti-inflammatory antipyretic analgesics, salicylic acid, salicylamide, ibufenac and ibuprofen, the concentrations (µg/ml) which produced 50 per cent inhibition of induced β-galactosidase synthesis (Table 1) are of the

same order as those which caused 50 per cent inhibition (ED_{50} in mg/kg) of nociception and inflammation in mice, rats and other small mammals (Table 2). The concentrations and the percentage inhibitions of induced β-galactosidase synthesis (Table 1) and of inflammation (Table 2) for these anti-inflammatory antipyretic analgesics are very similar despite differences of biological system and other conditions. Ibuprofen was the strongest inhibitor of induced enzyme synthesis and has the greatest anti-inflammatory activity. Movirene also inhibited enzyme synthesis (Table 1) and is believed to have anti-inflammatory properties as will be discussed, but no quantitative anti-inflammatory data is available. The parallelism between inhibition of enzyme synthesis

TABLE 2. PUBLISHED DATA OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF VARIOUS DRUGS *in vivo*

	Analgesic activity ED_{50} (mg/kg)						
Route* and reference	p.o.[59]	s.c.[60]	s.c.[60]	s.c.[61]	s.c.[62]	p.o.[62]	i.p.[63]
Acetylsalicylic acid	160	61	100	190	—	55	—
Salicylic acid (as Na salt)	—	216	450	860	140	268	—
Salicylamide	—	170	165	310	—	—	—
Paracetamol	—	220	800	—	71	124	—
Phenacetin	260	132	210	—	—	105	—
Morphine (s.c. or i.p.)	1·15	—	—	1·5	0·52	3·82	0·45
Nalorphine (s.c. or i.p.)	—	—	—	—	1·96	—	0·92

	Dose (mg/kg)	Analgesic activity (%)	Dose (mg/kg)	Anti-inflammatory activity (%)	Route* and reference
Acetylsalicylic acid	30	26	100	45	p.o.[64,65]
	60	55	300	73	
Ibufenac	15	43	100	40	p.o.[64,65]
	30	61	300	52·5	
Acetylsalicylic acid	30	20·5	100	23	p.o.[65]
	60	69	300	64	
Ibuprofen	1·88	50	18	38·5	p.o.[65]
	3·75	79	54	47	
Salicylamide	135	43	215	33	i.p.[47]

	Anti-inflammatory dose mg/kg		
	ED_{50}	ED_{50}	Therapeutic plasma level *in vivo* in man and guinea pigs[3]
Route* and reference	p.o.[66]	p.o.[41]	
Acetylsalicylic acid	100–200	80	
Salicylic acid (as Na salt)	170	100	130 (u.v. erythema) 170–260 (rheumatic fever)
Salicylamide	>400	>320	
Paracetamol	>400	>240	
Phenacetin	400	>240	

* p.o. = oral, s.c. = subcutaneous, i.p. = intraperitoneal.

(Table 1) and analgesic activity (Table 2) was not marked. The non-anti-inflammatory antipyretic analgesics, paracetamol and phenacetin, produced 50 per cent inhibition of β-galactosidase synthesis only at much higher concentrations (1000–2000 μg/ml) than did the anti-inflammatory antipyretic analgesics (100–500 μg/ml) (Table 1) and at much higher concentrations than those which gave 50 per cent inhibition (ED_{50}) of nociception (100–500 mg/kg) (Table 2).

With the narcotic analgesics, morphine and nalorphine, the concentrations (μg/ml) which caused 50 per cent inhibition of β-galactosidase synthesis (Table 1) are of the order 100 times greater than those which produced 50 per cent inhibition (ED_{50}) of nociception (Table 2). Although nalorphine antagonises the analgesic and narcotic effects of morphine, it does not antagonise the inhibition of β-galactosidase synthesis by morphine.

Thus, although all the analgesics tested inhibited the induced synthesis of β-galactosidase, the degree of the inhibition corresponded most closely to the anti-inflammatory properties of the anti-inflammatory antipyretic analgesics.

DISCUSSION

The induced synthesis of an enzyme such as β-galactosidase involves several different stages. Some stages involve the inducer, such as its uptake and binding, and others, such as the synthesis of messenger-RNA, the synthesis of aminoacyl-*t*RNA and finally the building of the polypeptide chain at the ribosomes, are also involved in general protein synthesis. Each drug may act at one or more of these stages and the sites of action and the stages affected may not be the same for the different drugs. Salicylate, its derivatives and other anti-inflammatory drugs at concentrations of the same order (1–3 mM) as those which are active against bacterial enzyme induction (Table 1) and against inflammation and nociception in mammals (Table 2) have been found to inhibit general protein synthesis and some of its constituent stages. For example, salicylate inhibits the incorporation of labelled amino acids into the proteins of rat isolated diaphragms and of rat liver microsomal preparations.[20] Salicylate and other anti-inflammatory drugs act similarly on human lymphocytes.[21,22] Salicylate, acetylsalicylate, salicylamide and salicylaldehyde were found to inhibit the messenger-RNA-directed incorporation of labelled amino acids into proteins in rat liver ribosomal preparations.[23] Smith and co-workers working with rat liver preparations have found that salicylate preferentially inhibits the formation of certain aminoacyl-*t*RNA species and does not affect the transfer of an aminoacyl-*t*RNA to a polyribosome fraction[24] and have evidence that salicylates may preferentially inhibit the biosynthesis of messenger RNA in mouse tissue *in vivo*.[25]

In the light of the above results the correlation between the inhibition of induced enzyme synthesis in the present work and anti-inflammatory activity of the anti-inflammatory antipyretic analgesics is readily explicable if the anti-inflammatory action of the drugs is due to the direct or indirect inhibition by the drugs of the biosynthesis of an enzyme or other protein. Possible proteins or enzymes whose synthesis may be inhibited in mammals are polypeptides of the kinin type such as bradykinin and kallidin, which are well-known as intermediate factors in inflammation and pain[1,3,5] and the proteases, such as kallikrein and plasmin, that produce the kinins. There is evidence for[26] and against[27–29] the hypothesis that anti-inflammatory drugs inhibit the synthesis of kinin by proteases. Other possible macromolecules whose synthesis

may be inhibited are the mucopolysaccharides and various peptides that constitute connective tissue in which the major lesions in rheumatic disease appear and whose synthesis is important in inflammation.[1,3,5] Many anti-inflammatory drugs inhibit mucopolysaccharide biosynthesis in various connective tissues *in vitro*[30–35] and *in vivo*.[36,37] Some drugs without anti-inflammatory activity, however, also inhibit mucopolysaccharide biosynthesis.[1]

The anti-inflammatory antipyretic analgesics may inhibit the synthesis of the above macromolecules either directly by acting, say, on the synthesizing enzymes,[24,25] or indirectly by inhibiting a reaction, such as oxidative phosphorylation,[2] which yields the free energy necessary for biosynthesis. Whitehouse and co-workers found a general parallelism between anti-inflammatory activity and the uncoupling and inhibitory effect on oxidative phosphorylation (and sulphation reactions) in connective tissue.[3,33,35,38] The inhibition by salicylic acid of induced enzyme synthesis in *Ps. fluorescens* has also been attributed to an uncoupling of oxidative phosphorylation.[18] Slater and co-workers, however, found that oral or perenteral administration of salicylate and 2,4-dinitrophenol to rats alters the distribution but not the overall content of ATP in the liver.[39,40] The latter fact suggests that significant uncoupling of oxidative phosphorylation in the liver *in vivo* does not occur.

The analgesic antipyretics can be divided into two groups: those with and those without anti-inflammatory activity.[5,41] Those with anti-inflammatory activity also possess antirheumatic properties and uncouple oxidative phosphorylation *in vitro;* concentrations of ≥ 1 mM reduce the P/O ratio to below 50 per cent. Those without anti-inflammatory activity lack antirheumatic properties and do not uncouple oxidative phosphorylation.[42] Since Movirene is a successful drug against various rheumatic conditions,[43–46] it belongs to the anti-inflammatory group. Salicylamide also has anti-inflammatory properties,[47] but these are not very pronounced in certain instances[5] (see Table 2).

The results of the present work with bacteria and work with rat liver ribosomes[22] are consistent with the view that biochemically acetylsalicylate itself exerts a minor effect but is hydrolysed to acetate which is inactive and to salicylate which exerts a major effect. Similarly, the primary biochemical mode of action of acetylsalicylate on mammals may be a result of its hydrolysis to salicylate which gives the action. In mammals the greater anti-inflammatory and anti-nociceptive activities of acetylsalicylate in the plasma in comparison with that of salicylate in the plasma,[1,48] may result from differences of absorption and distribution.[49] Acetylsalicylate is more lipophilic and less hydrophilic than salicylate and consequently will diffuse faster from the plasma through certain cell membranes and become more concentrated in the mammalian cells at the true sites of action. This point was made by Martin[49] and is often misunderstood. Once acetylsalicylate is inside the cell it may be hydrolysed by esterases which are active, abundant and relatively unspecific. The salicylate liberated inside the cell will exert the major biochemical effect.

The narcotic analgesic morphine and its antagonist nalorphine inhibit oxidative processes in brain tissue[50] and the bacterial synthesis of β-galactosidase in the present work only at much higher concentrations than those which are present in the brain or which are used clinically. The biochemical mode of action of these drugs is not understood.[51]

Oxine[52] and nitroxoline[53–55] exert their antibacterial effects by chelating certain

transition metals of variable valence, e.g. Fe^{2+}, Fe^{3+}, Cu^{2+} to form lethal complexes. The 5-nitro group increases the antibacterial activity of the complex. Many anti-inflammatory compounds including salicylate and the glucocorticoids also form chelate complexes with metal ions.[34,56,57] This fact formed the basis of the chelation theory of anti-inflammatory action. Oxine has anti-inflammatory activity and inhibits the biosynthesis of cartilage mucopolysaccharide *in vitro*[34] (but not *in vivo* in acute experiments[36]) and the synthesis of bacterial β-galactosidase in the present work. Since nitroxoline is also a chelating agent and affects *K. aerogenes* like the anti-inflammatory compounds, it might be well worth assaying for possible anti-inflammatory activity in a number of different biological systems.

There is no reason to suggest that the nitrofurans might have anti-inflammatory activity, since they are more active against bacterial growth than against the synthesis of β-galactosidase in the present work and do not uncouple oxidative phosphorylation.[58] The nitrofurans inhibit the anaerobic steps of pyruvate metabolism.[13]

The present work suggests that testing for the ability to inhibit induced enzyme synthesis by bacteria may be useful in screening drugs for anti-inflammatory activity.

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