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DISTRIBUTION OF THE ACETYL COMPARED WITH THE SALICYL MOIETY OF ACETYLSALICYLIC ACID

ACETYLATION OF MACROMOLECULES IN ORGANS WHEREIN SIDE-EFFECTS ARE MANIFEST

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Abstract—The distribution in rats of the acetyl group of aspirin has been compared with that of the salicyl moiety with the objective of establishing if: (1) there are differences in their biodisposition which might be important in the development of side- or therapeutic effects of aspirin, and (2) the range of organs and biomolecules therein which are acetylated by aspirin. Using whole-body autoradiography and liquid scintillation counting techniques it was found that the acetyl group of ^{3}H- or ^{14}C-acetyl-labelled aspirin became bound to a wide variety of proteins, glycoproteins and lipids of the glandular and non-glandular region of the stomach, kidney, liver and to a lesser extent bone marrow, i.e. organs in which side-effects are frequently encountered. It is suggested that: (1) the acetylation of biomolecules may be a major factor in the development of side-effects in these organs, and (2) in addition to acetylation of prostaglandin synthetase, the acetylation of enzymes and other biomolecules may have a much wider bearing on the biochemical changes underlying the development of these side-effects. Acetylation of the protein/macromolecular components was especially pronounced in inflamed (c.f. control) hindpaws of carrageenan-injected rats. This could be a result of acetylation of the drug-carrier protein, albumin, and other proteins carried into inflamed tissues and this acetylation could have marked consequences for the functions of these proteins.

The biodistribution of aspirin (acetylsalicylic acid) and related drugs is a major factor in their therapeutic actions and side-effects [1–3]. The accumulation of high concentrations of these drugs in the inflamed tissues, stomach and kidney (i.e. relative to other tissues) [1–4] has been attributed to the physico-chemical properties of these drugs, since they are moderately strong acids with high lipophilic properties. Aspirin is, however, rather unique amongst the non-steroid anti-inflammatory (NSAI) drugs in its capacity to form covalent derivatives with proteins and other macromolecules in the body by acetylation [5–8]. While the exact relevance of these acetylation reactions has yet to be established, it is known that the presence of the acetyl moiety on many salicylate derivatives enhances their gastric ulcerogenic [9, 10] and possibly nephrotoxic [11] side-effects. Acetylation of platelet proteins (including prostaglandin cyclo-oxygenase) accounts for long-lasting (over 5 days) effects of aspirin on platelet aggregation [7, 12]. Acetylation of prostaglandin cyclo-oxygenase [12] and albumin [6] might be expected to have profound effects in inflammatory and immunological reactions. Also acetylation of certain haemoglobin types (e.g. sickle cell) has been reported to alter the oxygen-carrying capacity of this protein [13, 14].

We have, therefore, compared the distribution in rats of the acetyl and salicyl moieties of selectivity labelled aspirin, using whole-body autoradiographic [15] and liquid scintillation counting techniques [4]. The object of this study was to: (1) compare the gross distribution of the acetyl group from [^{3}H]- or [^{14}C-*acetyl*]salicylic acid with that of labelled salicylic acid, and (2) to identify the components in various organs that are acetylated by acetylsalicylic acid.

MATERIALS AND METHODS

Radioactively-labelled drugs

^{14}C- *or* ^{3}H-acetyl-*labelled aspirin.* (See Fig. 1 for position of labelling.) These were prepared by the acetylation of a 2 M excess of salicylic acid dissolved

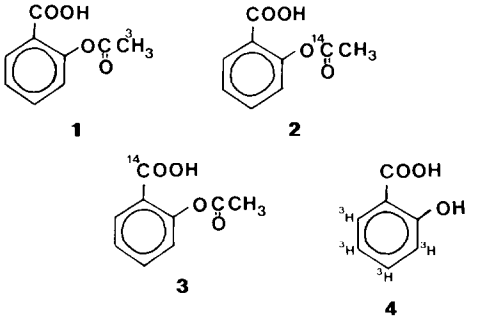

Fig. 1. Positions of labelling of aspirin used in these studies. The ^{3}H-*acetyl*- and 1-^{14}C-*acetyl*-labelled aspirin (i.e. **1** and **2**) were employed in autoradiographic and quantitative scintillation counting studies respectively to follow the fate of the acetyl moiety of aspirin. The ^{14}C-*carboxy*-labelled aspirin and [^{3}H]salicylic acid (i.e. **3** and **4**) were used to follow the fate of the salicyl group.

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in benzene with either [1-^{14}C]- or [^{3}H]acetic anhydride respectively (Radiochemical Centre, Amersham, U.K.) at room temp for 4 hr in the presence of pyridine as a catalyst [16, 17]. The products of all these reactions were twice purified by TLC on Merck F_{254} preparative plates developed in petroleum ether (40–60° b.p.)/propionic acid (10:1) [18]. Appropriate dilutions were subsequently made for dosing with non-active aspirin (as described in the tables and Results). In all cases the acetyl-labelled aspirin samples were finally purified by preparative TLC and diluted samples checked by analytical TLC on the day of the experiment to ensure that no hydrolysis of aspirin had occurred.

[^{14}C-Carboxy]*salicylic acid and* [1-^{14}C]*acetate (sodium salt).* These were obtained from the Radiochemical Centre. [^{3}H]Salicylic acid was purchased from New England Nuclear (Boston, MA). All these labelled compounds were diluted with non-active drug to give the concentrations described in the tables and Results.

Whole-body autoradiography

Male Sprague–Dawley rats (Tierfarm, Tuttlingen, F.R.G.) weighing 100–150 g were fasted for 24 hr and allowed water *ad lib.* before the experiments. The animals were given an oral dose of 1 ml of the radioactively-labelled drugs which were prepared immediately beforehand as fine suspensions in distilled water followed by 1 ml containing the aqueous drug residues. Blood samples (50–200 μl) were drawn from the tail veins of each animal at time intervals of 10, 60 and 180 min for determination of radioactivity following total combustion.

At 3 hr after dosing the prior-shaven animals were anaesthetized with diethyl ether, final blood samples were collected and the animals were then bled as near as possible to completion. The carcasses were immediately immersed into a hexane–dry ice mixture, stored at −70° and sectioned to approximately 100 μm by a cryo-microtomy as described previously [2, 15]. These sections were freeze-dried and subsequently exposed by direct contact for periods of 1–12 weeks to either Ultrofilm ^{3}H® autoradiography film (LKB, Bromma, Sweden) (^{3}H-labelled drugs) or Kodak X-ray film (^{14}C-labelled drugs).

Determination of radioactivity

For determination of the total radioactivity present in tissue homogenates or protein fractions, the samples were first digested in Protosol® tissue solubilizer (Packard Instrument Co.), then neutralized with acetic acid before counting in Instagel® (Packard) scintillation fluid. In cases where the protein content was also determined the tissues or protein fractions were first briefly dissolved in 1 N NaOH; the samples for protein and radioactive analysis were taken subsequently. The radioactivity was determined in a Packard liquid scintillation spectrometer and the data were corrected for quenching by the internal standardization technique [19].

Extraction of drugs and protein fractions

Tissue homogenates were prepared in 0.3 M sodium citrate (pH 3) (1:10 or 1:50 w/v) and then extracted twice with chloroform (1:10 or 1:50 by vol.). The pooled chloroform layers were evaporated to dryness by rotary evaporation at 20° (to minimize hydrolysis of aspirin) and the extracts were dissolved in 2 ml ethyl acetate (stored at −20°) for subsequent radioactive assay (following dissolution in Instagel as earlier) and TLC.

An equal vol. of 10% trichloroacetic acid (TCA) was added to the aqueous layers (after extraction with chloroform) and the protein/macromolecular fractions separated by centrifugation (5000 *g* for 10 min at 4°). The TCA-insoluble protein fractions were dialyzed in Visking dialysis bags for 4–7 days against 0.9% NaCl with 0.01% NaN_3 added, then distilled water with 0.01% NaN_3 for a further 4–7 days. The dialysates were then freeze-dried, weighed and the content of radioactivity determined as earlier. Protein assays on these fractions were performed according to the Lowry procedure [20], after first dissolving samples of the fractions in 0.1 N NaOH.

The glycoprotein components present in the protein/macromolecular fractions were isolated by pronase digestion and dialysis. Approximately 100–200 mg dry wt of the freeze-dried fractions were treated with 2 ml of 5 mg/ml pronase (Serva) in 0.2 M ammonium citrate buffer (pH 6.5) for 24 hr at 37° [21]. The digests were dialyzed against 0.9% aqueous NaCl for 3 days, then distilled H_2O for 1 day. The dialyzed fractions were subsequently freeze-dried prior to weighing and determination of radioactivity.

Distribution of [^{3}H-acetyl]*salicylic acid in the subcellular fractions of the glandular mucosa, liver and kidney*

Fasted female rats (140–150 g body wt) were dosed orally with 100 μCi (3.7 GBq) [^{3}H-*acetyl*]salicylic acid (10 mg/kg) freshly prepared and dissolved in H_2O (2 ml/rat). The rats were anaesthetized with diethyl ether 1 hr after dosing, blood samples were collected from the carotid arteries for radioactive assay and the animals were then bled to termination. Homogenates of the glandular mucosa (2% by vol.), liver (20% by vol.) and kidney (20% by vol.) were prepared in 0.25 M sucrose in 0.05 M Tris–HCl (pH 7.4) (4°) and centrifuged at 500 *g* for 5 min (in an International centrifuge) to obtain the nuclear-membrane fractions in the precipitate (= P-500) and also the post-nuclear supernatant (= SN-500) fractions. A 1-ml aliquot of both fractions was taken for radioactive assay and the remainder of the SN-500 fraction was centrifuged (in a Spinco–Beckman ultracentrifuge) at 100,000 *g* for 1 hr at 4°. The supernatant (cytosol) and precipitate (microsomal and mitochondrial) fractions thus obtained were designated SN-100,000 and P-100,000 respectively.

The lipids present in these centrifuged fractions were isolated by extraction twice with chloroform/methanol (2:1 v/v) and evaporation of the organic phase to dryness under vacuum. The dried extracts were dissolved in 2 ml ethyl acetate and separated aliquots were taken for determination of radioactive content by scintillation counting and TLC. Neutral and phospholipids were separated by TLC on Merck F_{254} silica gel plates developed in petroleum ether (60–80° b.p.)/diethyl ether/acetic acid (90:10:1 v/v/v) [22], while cholesterol and its esters were

separated by developing the plates in chloroform/ethyl acetate (80:20 v/v) [23]. The identity of the lipids was ascertained by comparison with standards of cholesterol, cholesterol acetate, phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, arachidonic acid, linoleic acid, linolenic acid and oleic acid. The radioactivity present in the various lipids was determined using a Berthold Radiochromatogram Scanner.

The proteins in the aqueous phase following lipid extraction were obtained by TCA precipitation, subsequent dialysis and freeze-drying (as earlier).

TLC

Isolation of acetylsalicylic acid and salicylate metabolites was performed by spotting 30 μ aliquots of the ethyl acetate solutions (following chloroform extraction as earlier) on Merck F_{254} silica gel TLC plates (0.25-mm) and developing these in the following solvents: (1) petroleum ether (40–60° b.p.)/propionic acid (10:1 by vol.) for identification of acetylsalicylic and salicylic acids [18], (2) benzene/acetic acid/H_2O (2:1:1 by vol.) for identification of 2,5-dihydroxy-benzoic acid (= gentisic acid) [18], and (3) benzene/diethyl ether/acetic acid/methanol (120:60:18:1 by vol.) for identification of salicyluric acid [18]. Standards of 2–10 μl of 10 mg/ml drugs were applied to each plate. The zones corresponding to the standards were scraped off and the radioactivity present was determined (as earlier) after addition of Instagel® scintillation mixture.

$^{14}CO_2$ evolution from [^{14}C-acetyl]salicylic acid

Rats were housed individually in glass metabolism flasks which were connected in line to two flasks each filled with 1 N KOH so that the expired air was bubbled through the solution to collect the $^{14}CO_2$. The input line was plugged with cotton-wool and the output line was operated under slight negative pressure from a water vacuum pump to ensure an adequate flow through of air; this being confirmed in appropriate control experiments.

The $^{14}CO_2$ collected in KOH was neutralized with an equal vol. of 1 N HCl and 1-ml aliquots were taken for scintillation counting.

RESULTS

Blood levels and $^{14}CO_2$ production from [^{14}C-acetyl]salicylic acid

Blood levels of all the labelled compounds showed an initial rapid rise to peak at 10–30 min after oral administration, followed by a progressive decline (Fig. 2). The blood levels of [^{14}C-*acetyl*]salicylic acid and [^{14}C]acetate were significantly lower at 10–30 min than those of [^{3}H]salicylic acid (Fig. 2).

As shown in Fig. 3 an appreciable proportion of $^{14}CO_2$ appeared in the breath of rats from orally-administered [^{14}C-*acetyl*]salicylic acid in comparison with that obtained with an equimolar dose of [^{14}C]acetate. No $^{14}CO_2$ was present in the breath of rats given [^{14}C-*carboxy*]acetylsalicylic acid (data not shown).

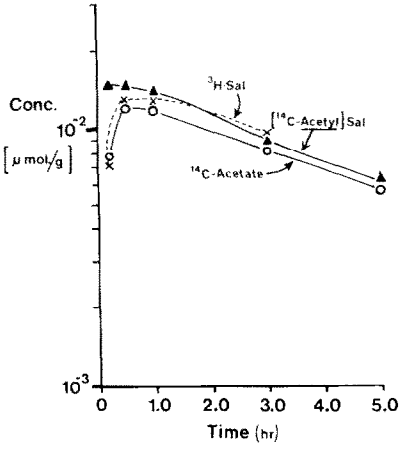


Fig. 2. Concentrations of salicylates, the ^{14}C-*acetyl* group from [^{14}C-*acetyl*]salicylic acid and [1-^{14}C]acetate orally administered in equimolar concentrations equivalent to 100 mg/kg aspirin to rats. There is an apparent similarity in the blood levels of these three labelled compounds except at the earliest time of 10 min where there is a striking difference between the levels of ^{14}C-*acetyl* from labelled aspirin and the levels of [^{14}C]acetate or [^{3}H]salicylic acid. This difference may reflect acetylation reactions of blood components specific to ^{14}C-*acetyl*-labelled aspirin.

Autoradiographic distribution of ^{14}C-acetyl and ^{14}C-carboxy-labelled aspirin, [^{14}C]acetate and [^{3}H]salicylic acid

Examination of the whole-body autoradiographs of rats given a single oral dose of these labelled compounds (Fig. 4a–c) reveals the following points concerning their biodistribution:

(1) High concentrations of the acetyl group of either ^{3}H-*acetyl*- or ^{14}C-*acetyl*-labelled aspirin were present in the glandular and to a lesser extent in the non-glandular mucosa of the stomach (Fig. 4a–c). The pattern of labelling was the same with the ^{3}H-*acetyl* compared to that of the ^{14}C-*acetyl*-labelled drug (Fig. 4a and b, cf. Fig. 4c). Lower concentrations were found in the upper intestinal tract, bone marrow, kidney cortex, liver, lungs, spleen, salivary

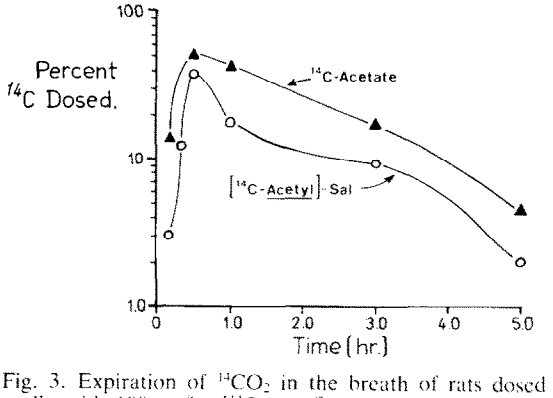
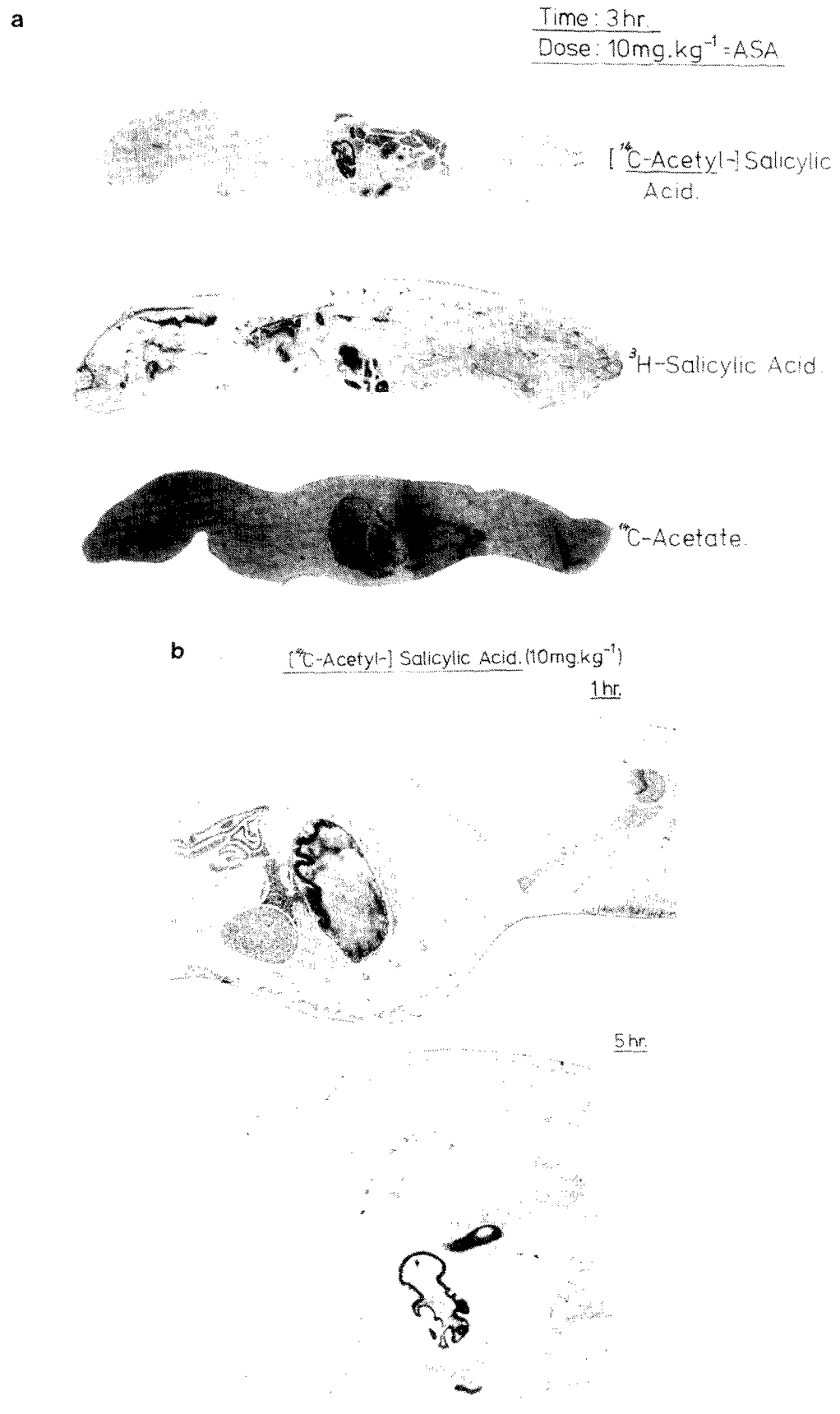


Fig. 3. Expiration of $^{14}CO_2$ in the breath of rats dosed orally with 100 mg/kg [^{14}C-*acetyl*]salicylic acid compared with an equimolar concentration of [1-^{14}C]acetate. These results show that (relative to [^{14}C]acetate) an appreciable proportion (about 60%) of the ^{14}C-*acetyl* group from labelled aspirin is metabolized to CO_2.


a
Time: 3 hr.
Dose: 10 mg.kg^{-1} = ASA
[^{14}C-Acetyl-] Salicylic Acid.
^{3}H-Salicylic Acid.
^{14}C-Acetate.
b
[^{14}C-Acetyl-] Salicylic Acid. (10 mg.kg^{-1})
1 hr.
5 hr.

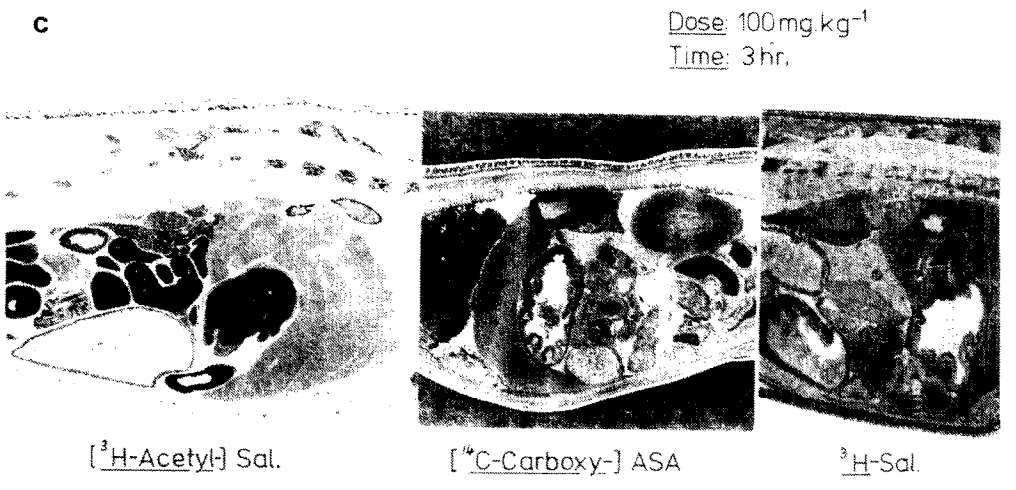


Fig. 4. Autoradiographic distribution of ^{14}C-*acetyl*- or ^{3}H-*acetyl*-labelled aspirin (100 and 10 mg/kg p.o.) compared to that of [1-^{14}C]acetate, [^{14}C-*carboxy*]aspirin and [^{3}H]salicylic acid (given orally in equivalent doses to aspirin for 3 hr (a and c), and for comparison with a, 10 mg/kg [^{14}C-*acetyl*]salicylic acid given for 1 and 5 hr (b). Following the rapid absorption of [^{14}C-*carboxy*]aspirin or [^{3}H]salicylic acid at 3 hr there is very little radioactivity present in the glandular mucosa of the stomach, whereas there is high residual labelling from the ^{14}C-*acetyl* or ^{3}H-*acetyl* label of aspirin in this region, which persists for at least 5 hr following dosage. Appreciable radioactivity from ^{14}C-*acetyl*- or ^{3}H-*acetyl*-labelled aspirin was present in the bone marrow, but no such activity was evident in rats dosed with [^{14}C-*carboxy*]aspirin, [^{3}H]salicylic acid or [^{14}C]acetate (a–c). Considerable radioactivity from the acetyl-labelled aspirin was present in the kidney cortex, intestinal tract, liver and spleen, though the distribution was not as specific as observed in the glandular mucosa and bone marrow (a–c).

glands and dermal skin layer (Fig. 4a). The pattern of distribution of [^{14}C-*acetyl*]salicylic acid in these organs was essentially similar over the 1–5-hr period following oral administration of the drug (Fig. 4b). Also, the distribution appeared to be the same (at 3 hr) following dosing with 10 and 100 mg/kg of the acetyl-labelled aspirin (Fig. 4a, cf. Fig. 4c).

(2) In contrast to that obtained with the acetyl-labelled aspirin, very little [^{3}H]salicylic acid was present at 3 hr in the glandular mucosa although very high concentrations were present in non-glandular mucosa and the lumen of the stomach (Fig. 4a and c). Appreciable concentrations of [^{3}H]salicylic acid were also present in the liver, intestinal tract, salivary glands, kidneys, lungs and dermal layers (Fig. 4a and c).

(3) Orally administered [^{14}C]acetate accumulated in the glandular region of the stomach similarly to that observed following dosing with ^{3}H-*acetyl*- or ^{14}C-*acetyl*-labelled aspirin (Fig. 4a). A sharp zone of radioactive labelling was present in the outer layer of the non-glandular mucosa of rats given [^{14}C]acetate (Fig. 4a). Appreciable labelling was also evident in these animals in the liver, kidneys and upper intestinal tract (Fig. 4a).

(4) The distribution of ^{14}C-*carboxy*-labelled aspirin was essentially the same as that obtained with [^{3}H]salicylic acid (Fig. 4c).

^{3}H-*Acetylation of proteins, glycoproteins and lipids*

Quantitative analysis showed that, on a relative basis, the greatest quantity of radioactivity from [^{3}H-*acetyl*]salicylic acid was found in the protein/macromolecular components of the glandular mucosa, whereas much less was present in the non-glandular mucosa of the stomach (Fig. 5). Appreciable radioactivity was also present in the kidney (Fig. 5). More radioactivity was found in the subplantar tissues of the inflamed compared with that in the non-inflamed (control) hindpaws (Fig. 5). The protein/macromolecular components of the bone marrow also had considerable radioactivity

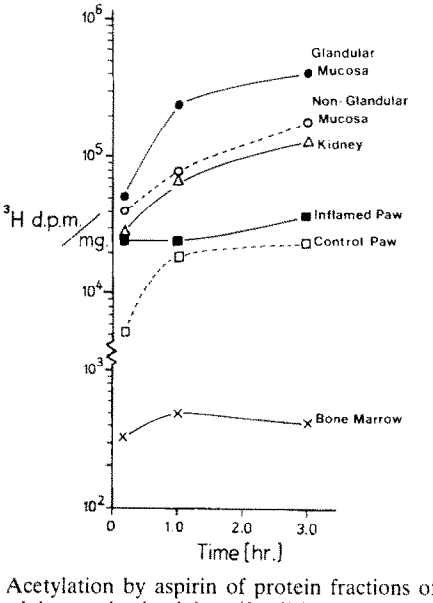


Fig. 5. Acetylation by aspirin of protein fractions of the non-glandular and glandular (fundic) regions of the stomach, kidney, inflamed and non-inflamed (control) paws and bone marrow of rats dosed orally with 100 mg/kg ^{3}H-*acetyl*-labelled aspirin.

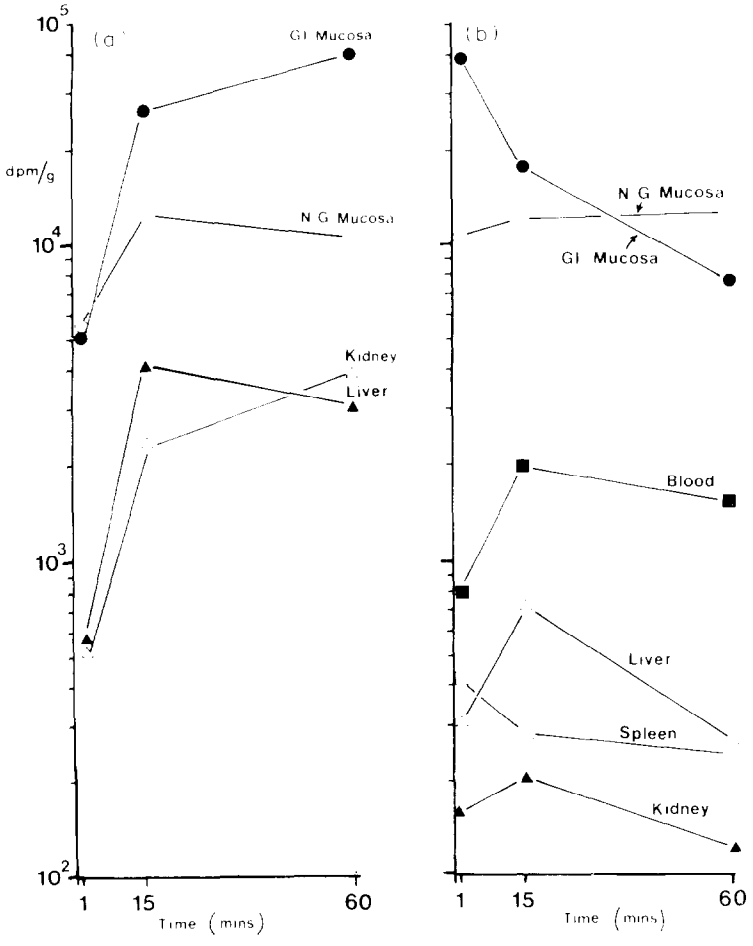


Fig. 6. Acetylation of the glycoprotein (a) and lipid (b) fractions of the glandular and non-glandular mucosa, kidney, liver, blood and spleen from rats dosed orally with 100 mg/kg ^{3}H-*acetyl*-labelled aspirin.

though this was less than observed in the aforementioned organs (Fig. 5).

The glycoproteins of the glandular mucosa were also extensively labelled; the radioactivity increasing progressively over 1–60 min following oral dosing of [^{3}H-*acetyl*]salicylic acid (Fig. 6a). The glycoproteins of the non-glandular mucosa had about 1/4 of the radioactivity overall compared with those in the glandular mucosa (Fig. 6a). The liver and kidney glycoproteins had about 1/10 the radioactivity of these components of the glandular mucosa (Fig. 6a), but there was no detectable labelling of glycoproteins from the spleen.

The lipids of the glandular and non-glandular mucosa were also extensively acetylated, the radioactive content being about the same as in the glycoproteins of these tissues (on a wt basis) (Fig. 6b). Interestingly, the radioactivity in the lipids of the glandular mucosa declined over the period of 1–60 min, whereas that in the non-glandular mucosa increased slightly over this period (Fig. 6b). This pattern of labelling of the glandular mucosal lipids contrasts with the general trend of increasing labelling with time for all other organ components (Figs 5, 6a and b). By comparison with the labelling in stomach lipids, very little radioactivity was present in the lipids of the liver, kidney and spleen (Fig. 6b).

No radioactivity was detected in any of the protein, glycoprotein or lipid fractions in the organs of rats dosed orally with [^{14}C-*carboxy*]acetylsalicylic acid (data not shown) showing that the labelling from [^{3}H-*acetyl*]salicylic acid specifically involved the ^{3}H-*acetyl* moiety.

Thin-layer chromatographic analysis of the lipid fractions of the gastric mucosa and liver revealed that at least five major and a further three minor lipid components were labelled, although their exact identity could not be discerned accurately because these components did not have the same mobilities as the lipid standards employed. This suggests that derivatization by acetylation had occurred so changing the mobility of these compounds on TLC.

In Tables 1 and 2 it can be seen that there was generalized labelling of the protein and lipid components of all the subcellular fractions of the glandular mucosa, livers and kidneys of rats given [^{3}H-*acetyl*]salicylic acid p.o. There were some variations in the specific activities in some of the subcellular fractions. Most noticeable were the higher values of the lipids in the SN-500 and P-100,000 fractions of

Table 1. Distribution of radioactivity in protein fractions of subcellular components 1 hr following oral administration of [^{3}H-*acetyl*]salicylic acid

Organ	Subcellular fraction	Total dpm	dpm/mg dry wt
Glandular mucosa	P-500	18,917	3386
	SN-500	5318	3826
	P-100,000	6527	2738
	SN-100,000	5464	2344
Liver	P-500	77,296	304
	SN-500	71,308	363
	P-100,000	21,498	545
	SN-100,000	22,823	291
Kidney	P-500	2440	297
	SN-500	8791	801
	P-100,000	1285	646
	SN-100,000	2492	659

Groups of five female rats were dosed orally with 100 μCi [^{3}H-*acetyl*]salicylic acid (10 mg/kg). One hour later the animals were killed under ether by bleeding and subcellular fractions prepared from excised glandular mucosa, liver and kidney. The respective fractions are denoted by those obtained on centrifugation, i.e. P-500 (nuclear-membrane fraction), SN-500 (post-nuclear supernatant), P-100,000 (microsomal + mitochondrial fraction) and SN-100,000 (cytosol). The radioactive content was determined following isolation of the TCA-precipitated fractions.

the glandular mucosa, the P-100,000 fraction of the liver and kidney (Table 2). No outstanding differences were observed in the distribution of radioactivity in the proteins of the stomach, liver and kidney subcellular fractions (Table 1) even though the gross distribution in these organs differed markedly.

DISCUSSION

Overall the results show that the acetyl moiety of aspirin has two principle fates. First, that relative to an equimolar dose of acetate there is appreciable catabolism of acetate release from aspirin by hydrolysis (spontaneously and from the aspirin esterases—see Refs 17 and 24–26) which yields CO_2 (Fig. 3). In fact the rate of catabolism would appear to be about 60–70% that of an equimolar concentration of acetate judged by the area under the curve of Fig. 3.

Second, there is considerable acetylation of proteins, glycoproteins, other macromolecular components and lipids in the glandular and non-glandular mucosa of the stomach, inflamed tissues, liver, kidney and bone marrow (Figs 5, 6a and 6b). Traces of the lipids in the spleen were acetylated, but no other major components were acetylated at least within detection limits. The acetylation was specific to the acetyl group of aspirin since no radioactivity was present in any of these organ components after isolation (i.e. proteins etc.) after administration of ^{14}C-*carboxy*-labelled aspirin or [^{14}C]acetate. Acetylation was not specific to any one particular protein or lipid fraction (Tables 1 and 2 and Results). Apart from the lipids of the microsomal/mitochondrial frac-

Table 2. Distribution of radioactivity in lipid fractions of subcellular components 1 hr following oral administration of [^{3}H-*acetyl*]salicylic acid

Organ	Subcellular fraction	Total dpm	dpm/mg dry wt
Glandular mucosa	P-500	386	693
	SN-500	190	1370
	P-100,000	323	1244
	SN-100,000	35	147
Liver	P-500	4963	181
	SN-500	2411	132
	P-100,000	1704	433
	SN-100,000	449	57
Kidney	P-500	513	597
	SN-500	471	416
	P-100,000	195	1016
	SN-100,000	92	222

Conditions of experiment and abbreviations of fractions as described in Table 1. The radioactive content of the lipids was determined following their isolation by chloroform/methanol (2 : 1 v/v) extraction (described in Materials and Methods).

tions of the glandular mucosa and liver (Table 2), there was no acetylation of any one of the macromolecular or lipid components of subcellular fractions.

These results suggest that the acetylation by aspirin of subcellular components is relatively non-specific. However, in gross distribution certain organs (where it is known that aspirin accumulates) showed high overall residual acetylation of the tissues. This can be seen especially in the glandular region of the gastric mucosa where there is residual acetylation of tissues long after the salicyl moiety has been absorbed (i.e. at 3 hr) (see Figs 4a–c, 5, 6a and b). In agreement with the present results, the relatively non-specific acetylation of biomolecules by aspirin has also been previously demonstrated in the kidneys of mice and rabbits [11]. The predominant acetylation of proteins in the microsomal (P-100,000) fraction, in the kidney (Table 2) may be a reflection of acetylation of the prostaglandin synthetase enzyme system present in this fraction [12].

Thus from these results it appears that the overall metabolism of aspirin should now be modified to include particular emphasis on the metabolism (to CO_2) and acetylation reactions involving a wide variety of biological molecules (Fig. 7).

The high degree of acetylation of biomolecules in the glandular mucosa, kidneys, liver and bone marrow may be related to the development of side-effects that frequently appear in these organs following a high intake of aspirin [1, 11, 27–31]. The irreversible inhibition of prostaglandin cyclo-oxygenase [12] is just one of the biochemical factors which could be implicated in the development of these side-effects. However, the fact that such a wide variety of biomolecules are acetylated raises the possibility that other biochemical systems could be affected by aspirin through acetylation of their components. This may be of major significance in the development of side-effects especially in view of the current concern about alkylation by drugs of biomolecules being related to toxicity by these drugs. Clearly, acetylation of enzymes and other molecules should be examined further for relationship to the biochemical changes underlying the development of side- and therapeutic effects of aspirin.

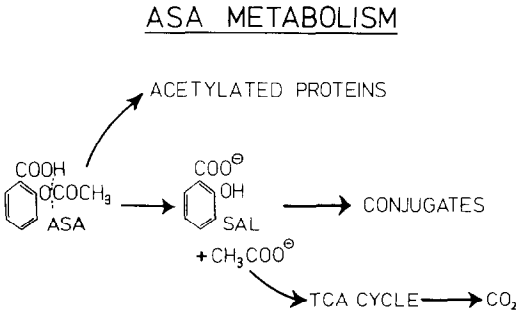


Fig. 7. Modification of the concept of aspirin metabolism emphasizing the competing fates of the acetyl moiety of aspirin. The acetyl moiety of aspirin can either directly acetylate a wide variety of proteins, other macromolecules and lipids or, following aspirin esterase-catalyzed or spontaneous hydrolysis, yield acetate which can enter the tricarboxylic acid (Krebs) cycle to be metabolized to CO_2.

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