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BIOCHEMICAL PROPERTIES OF ANTI-INFLAMMATORY DRUGS–II.

SOME EFFECTS ON SULPHATE-^{35}S METABOLISM *IN VIVO**

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Abstract—The following anti-inflammatory drugs which inhibit the metabolism of cartilage and other connective tissues *in vitro*, also inhibit the biosynthesis of polysaccharide sulphates by rat rib cartilage *in vivo*: Salicylate, phenylbutazone, oxyphenbutazone, hydrocortisone, cinchophene, glycyrrhetic acid and flufenamic acid. 2,4-Dinitrophenol was also a potent drug *in vivo*, but 2,5-dinitrophenol was not. Drug action on cartilage metabolism could not be attributed to competition by the drug for available 'active sulphate' (PAPS). Salicylate, phenylbutazone, oxyphenbutazone, flufenamic acid and 2,4-dinitrophenol all depressed the excretion of sulphate esters in the urine. It is concluded that the latter drugs uncouple oxidative phosphorylation in the whole animal both in peripheral tissues such as cartilage and in those visceral tissues such as kidney and liver, which are concerned with the biosynthesis of sulphate esters. This property of the drugs *in vivo* may underlie their anti-inflammatory activity.

Chloroquine and 8-hydroxyquinoline had no activity on cartilage metabolism *in vivo* in acute experiments. Evidence was obtained that nearly all the compounds listed in Table 1 were metabolised in the rat and excreted in the urine, in part at least—as ester sulphates.

The urinary excretion of sulphated metabolites of phenylbutazone and hydrocortisone by rats was dependent on strain and sex.

MANY anti-inflammatory (antirheumatic) drugs inhibit the *in-vitro* biosynthesis of mucopolysaccharide sulphates in connective tissues such as cartilage, cornea and heart valves.[1–6] These drugs would appear to act on processes generating adenosine triphosphate (ATP), either by inhibiting cellular respiration or by uncoupling oxidative phosphorylation. Anti-inflammatory steroids and certain antimalarials such as chloroquine (resochin) currently used as antirheumatic agents, are known to inhibit mitochondrial oxidative reactions.[4, 7] Anti-inflammatory drugs which can uncouple oxidative phosphorylation include salicylates, phenylbutazone (Butazolidine), oxyphenbutazone (Tanderil) and cinchophene (Atophan).[4, 5] However, these conclusions are based on studies of drug action *in vitro* with connective tissue slices and with subcellular liver or heart muscle fractions.

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We have now conducted some experiments to find out whether or not these drugs really do have the same action in whole animals as they have upon isolated tissues or subcellular fractions *in vitro*. Evidence has been obtained that all those antirheumatic drugs which inhibit the biosynthesis of cartilage polysaccharide sulphates *in vitro*, do so *in vivo* as well. We extended our observations to include (a) other drugs not currently employed as antirheumatic drugs but which are known to uncouple oxidative phosphorylation e.g. 2,4-dinitrophenol, (b) some chemical analogues of known antirheumatic drugs, and (c) some potential antirheumatic drugs such as glycyrrhetic acid (3β-hydroxy-11-oxo-18β-olean-12-ene-30-oic acid)[8, 9] and N-(ααα-trifluoro-m-tolyl)-anthranilic acid (CI-440, flufenamic acid)[10] which exhibit anti-inflammatory properties in experimental animals and also uncouple oxidative phosphorylation *in vitro*.[5]

We found that many of these drugs were metabolised *in vivo* and excreted in the urine as ester sulphates.

EXPERIMENTAL

The sources of drugs used for these studies were: J. R. Geigy AG, Basel, Switzerland (phenylbutazone, oxyphenbutazone, sulphinpyrazone); Smith and Nephew Research Ltd., Hunsdon, Herts. (di-*p*-carboxyphenylbutazone); Mann Research Laboratories, Inc., New York, U.S.A. (cinchophene). Imperial Chemical Industries, Manchester (chloroquine diphosphate); Parke, Davis and Co., Hounslow, Middlesex (flufenamic acid). Other compounds were obtained from British Drug Houses, Ltd., Poole, Dorset; L. Light and Co., Colnbrook, Bucks, Kodak Ltd., Liverpool, and Kebo AB, Stockholm.

The glycyrrhetic acid used (Light) m.p. 286–288° contained at least three components even after recrystallisation from aqueous ethanol (m.p. 288–290°). It contained not more than 84% glycyrrhetic acid calculated from its light absorption at 250 mμ in ethanol, compared with that of a chromatographically pure sample of 18β-glycyrrhetic acid, donated by Professor E. E. Turner, F.R.S. and Dr. S. Gottfried (Biorex Laboratories, London, E.C.1).

Groups of four male white rats (Wistar strain), each animal weighing 150–200 g, were injected i.p. with 20 or 100 mg (as indicated in the tables) per kg of a drug dissolved in either distilled water or 1 per cent Tween 20 solution. 10 mc/kg of carrier-free sodium ^{35}S-sulphate* was injected simultaneously. Control groups of four animals received injections of ^{35}S-sulphate and drug vehicle alone. The animals were kept without food in metabolic cages (with drinking water *ad libitum*) for 24 hr. During this period, the urine from each animal was collected in flasks cooled in dry ice so that each urine sample was frozen immediately after urination. All urine specimens were kept at −15° until analyzed as follows:

5- and 25-μl samples from each urine specimen were subjected to two-dimensional paper chromatography in two pairs of solvent systems,[11]

(i) phenol–ammonia followed by *n* butanol–ammonia

(ii) phenol–ammonia followed by *n* butanol–acetic acid.

The radioactive zones on these chromatograms were visualized by auto-radiography as described elsewhere,[11] to obtain the pattern of the urinary sulphate ester excretion.

* Obtained from the Radiochemical Centre, Amersham, Bucks, England: $^{35}S_i$ is used as an abbreviation for radioactive inorganic sulphate (i.e. $^{35}SO_4''$) hereafter.

The total ^{35}S excreted in the urine was measured by Geiger-Müller counting with mica end-window tube (1·9 mg/cm^2) after plating and drying 100-μl samples of urine which has been diluted 1:100 with distilled water. Inorganic sulphate-^{35}S was precipitated from this diluted urine, together with added carrier inorganic sulphate, with barium ions. 10 ml of the diluted urine (1:100) was mixed with 0·5 ml 0·1 M HCl, 1 ml 1 M $BaCl_2$ and 1 ml 0·5 M Na_2SO_4 in a centrifuge tube. The tubes were stoppered, vigorously shaken for 30 sec and stood at 4° overnight. After removal of the precipitated barium sulphate by centrifugation, 50-μl samples of the supernatant were assayed for radioactivity as described above, to obtain a measure of the sulphate esters-^{35}S in urine.

Rib cages from rats which had received $^{35}S_i$ 24 hr previously, were freed of muscle and extraneous tissue and stored at −15° until processed further. Rib cartilage was isolated by dissection, dried and defatted in acetone and ethanol for 24 hr at room temperature, weighed and then further dried at 80° for 30 min. Polysaccharide sulphates were isolated from the rib cartilage after digestion with papain[4] and assayed for radioactivity as described.[3] The specific radioactivity of the polysaccharide sulphates-^{35}S was computed as cpm/mg cartilage. In every experiment, cartilage from the control animals was always processed and assayed for radioactivity simultaneously with cartilage from the corresponding drug-treated animals.

RESULTS AND DISCUSSION

Drug action on cartilage metabolism

With the exception of chloroquine diphosphate, all the other anti-inflammatory drugs examined in these experiments and representing six different types of chemical compounds (i.e. salicylate, phenylbutazone, hydrocortisone, cinchophene, glycyrrhetic acid, flufenamic acid) reduced the incorporation of inorganic sulphate-^{35}S ($^{35}S_i$) into the rib cartilage polysaccharide sulphates (Table 1)*. This indicates that the drugs probably do affect cartilage metabolism in the whole animal in the same manner as they affect cartilage metabolism *in vitro*.[4, 5] The total ^{35}S excreted in the urine by any group of drug-treated animals was not significantly greater (more than 12%) than the ^{35}S excreted by the control group. So it would seem that these particular drugs do in fact act directly upon cartilage tissue *in vivo* to depress ^{35}S metabolism therein, and not merely appear to do so by channeling the $^{35}S_i$ away from this tissue, as for example by lowering the renal threshold for inorganic sulphate.

Effect of individual drugs

(i) *Salicylate* (*2-hydroxybenzoate*). Table 1A shows the relative specificity of salicylate action; neither benzoate nor 4-hydroxybenzoate had any effect on cartilage metabolism *in vivo*. Although *ortho* hydroxynaphthoates are more potent inhibitors than salicylate (either weight for weight or mole for mole) of cartilage metabolism *in vitro*[5] or of oxidative phosphorylation,[12] they appear to be much less effective *in vivo* on a weight basis. This is corroborated by their relative ineffectiveness, compared with salicylate, in suppressing the erythrema elicited in small animals by u.v. irradiation (Dr. S. S. Adams, private communication).

* The absolute values for the specific activities of the polysaccharide sulphates-^{35}S in Table 1 are not comparable from one type of drug experiment to another, *e.g.* between each series of controls, because the radioactive assays were carried out at different time intervals after the animals were killed; no corrections have been made for radioactive decay.

TABLE 1. EFFECT OF DRUGS ON (a) URINARY ESTER SULPHATE EXCRETION, AND (b) INCORPORATION OF $^{35}S_i$ INTO RIB CARTILAGE POLYSACCHARIDE SULPHATES (PS).

Mean values from groups of 4 rats, with standard error of the mean.

Section	Drug	Dose (mg/kg)	New sulphate ester(s) in urine	Ester sulphates-^{35}S as % of total ^{35}S in urine			Cartilage PS-^{35}S (cpm/mg)		
				with drug	control	% of control	with drug	control	% of control
A	Sodium salicylate	100	+	100 ± 3·7	15·8 ± 2·1	63	76 ± 31	142 ± 10	54
	Sodium benzoate	100	—	13·7 ± 4·5	12·6 ± 1·7	109	80 ± 22	81 ± 10	99
	4-Hydroxybenzoic acid	100	+	27·2 ± 13·0	19·6 ± 3·2	138	175 ± 42	185 ± 78	94
	2,5-Dihydroxybenzoate	100	+	33·6 ± 2·8	27·5 ± 4·5	122	192 ± 26	229 ± 29	84
	2,6-Dihydroxybenzoic acid	100	—	27·1 ± 5·2	27·5 ± 4·5	99	253 ± 37	229 ± 29	110
	1-Hydroxy-2-naphthoic acid	100	—	23·0 ± 3·7	26·0 ± 4·2	88	143 ± 12	197 ± 25	73
B	Phenylbutazone	100	·	10·2 ± 1·2	16·4 ± 6·5	62	21 ± 3·7	52 ± 4·1	40
	Oxyphenbutazone	100	—	15·8 ± 1·8	25·6 ± 3·0	62	121 ± 10	231 ± 20	52
	Sulphinpyrazone	100	—	18·8 ± 1·6	25·6 ± 3·0	73	168 ± 23	231 ± 20	73
	Di-*p*-carboxyphenylbutazone	100	—	14·8 ± 0·5	17·2 ± 1·7	86	118 ± 15	159 ± 7	74
	Antipyrine	100	+	33·5 ± 4·6	16·6 ± 3·6	200	24 ± 3·5	27 ± 2·5	89
	1,3-Diphenylpyrazol-5-one	100	·	29·0 ± 8·1	27·0 ± 1·8	107	332 ± 24	280 ± 16	118
C	2,4-Dinitrophenol (sodium salt)	20	(+)	8·9 ± 1·8	19·9 ± 2·0	45	67 ± 11	120 ± 5·0	56
	2,5-Dinitrophenol	20	·	50·1 ± 5·0	26·0 ± 4·2	193	189 ± 9	197 ± 25	96
	Dicoumarol	20	+	14·5 ± 0·5	13·6 ± 1·6	107	182 ± 9	173 ± 23	105
D	Hydrocortisone	5	—	15·6 ± 5·0	16·0 ± 1·6	97	73 ± 23	112 ± 10	65
	ACTH	5IU. per animal	·	15·2 ± 1·6	14·4 ± 4·2	106	37 ± 3·7	49 ± 9·2	76
E	Cinchophene	100	·	27·5 ± 0·1	21·8 ± 2·7	126	177 ± 15	238 ± 10	74
	Glycyrrhetic acid	84	(+)	17·4 ± 0·6	14·7 ± 0·8	118	82 ± 13	97 ± 15	85
	Oleanolic acid	100	—	25·6 ± 0·4	25·3 ± 2·6	101	217 ± 16	198 ± 17	110
	Flufenamic acid	100	+	12·0 ± 0·8	16·6 ± 5·6	72	58 ± 5	78 ± 39	74
	N-Methyl anthranilic acid	100	·	18·2 ± 6·8	18·4 ± 2·7	99	209 ± 34	235 ± 10	89
	Chloroquine phosphate	100	+	26·7 ± 8·0	27·0 ± 1·8	99	316 ± 13	280 ± 16	113
	8-Hydroxyquinoline	100	+	39·4 ± 7·7	19·6 ± 3·2	200	208 ± 21	185 ± 7·8	112
	2-Hydroxyquinoline	100	+	72·3 ± 4·2	21·8 ± 2·7	332	209 ± 2·5	238 ± 10	88

6-Hydroxy-2-naphthoate was virtually without effect on ^{35}S incorporation into cartilage polysaccharides but considerably increased the excretion of ester sulphates: this compound is not an uncoupling agent.[12] 2·5- and 2·6-dihydroxybenzoates (gentisate, γ-resorcylate) which are of questionable status as antirheumatic drugs and do not uncouple oxidative phosphorylation[12, 15] had no significant salicylate-like effects on cartilage metabolism *in vivo* or urinary ester sulphate excretion.

(ii) *Phenylbutazone and related pyrazolones.* Both phenylbutazone and its relatively long-lived phenolic metabolite formed in humans, oxyphenbutazone (Metabolite I)[13] strongly inhibited cartilage metabolism *in vivo* (Table 1B). Phenylbutazone was found to be more potent drug *in vivo* than salicylate. This is in good agreement with its greater efficacy as an antirheumatic drug, its greater effect on cartilage metabolism *in vitro* and the fact that phenylbutazone is a more powerful uncoupler of oxidative phosphorylation than salicylate.[5] Oxyphenbutazone and sulphinpyrazone which both have less effect than phenylbutazone on oxidative phosphorylation and upon cartilage metabolism *in vitro*,[4, 5] were also less potent *in vivo*. The di *p*-carboxy(phenyl) derivative of phenylbutazone (1:2 di (4'-carboxyphenyl)-4-*n*-butyl-pyrazolidine-3·5-dione, HP 358) was much less potent than phenylbutazone *in vivo*. It is also less potent than phenylbutazone as an anti-inflammatory drug.[14] *In-vitro* assays had shown it to be inactive[4, 5] which may indicate that HP 358 is metabolised to physiologically active compounds *in vivo*. Antipyrine (phenazone) which is sometimes claimed to be an antirheumatic agent as well as being an analgesic drug, had a rather questionable activity *in vivo*: the apparent inhibition of cartilage polysaccharide sulphation in this case might actually have been due to the preferential sulphation of antipyrine metabolites and enhanced excretion of urinary ester sulphates (compare with 2-hydroxyquinoline). Antipyrine has no effect on cartilage metabolism *in vitro*[4] nor does it uncouple oxidative phosphorylation in liver mitochondria at 2 mM.[15] 1,3-Diphenylpyrazol-5-one was also inactive towards cartilage metabolism *in vivo* even though it is a potent drug *in vitro*[4] and completely uncouples oxidative phosphorylation in liver mitochondria at 5×10^{-4} M (Whitehouse, unpublished observations). This compound also fails to inhibit the u.v. erythrema in guinea pigs (Dr. S. S. Adams, private communication).

(iii) *Other known uncoupling agents.* Like salicylate and phenylbutazone, both 2,4-dinitrophenol and dicoumarol can uncouple oxidative phosphorylation in liver and heart muscle mitochondria. Only 2,4-dinitrophenol had any effect on cartilage metabolism *in vivo* (Table 1C). Its isomer, 2,5-dinitrophenol had no effect on cartilage metabolism *in vivo*. 2,5-Dinitrophenol fails to uncouple oxidative phosphorylation in liver mitochondria at 5×10^{-5} M and has little or no effect on cartilage metabolism *in vitro* at 10^{-4} M.[12] At these same concentrations 2,4-dinitrophenol completely abolishes oxidative phosphorylation in liver mitochondria and profoundly depresses cartilage metabolism *in vitro*.[5]

(iv) *Hydrocortisone and ACTH.* (Table 1D). There are many reports that hydrocortisone and cortisone inhibit the synthesis of polysaccharide sulphates in rat connective tissues.[1, 16–18] The results of our experiments with hydrocortisone and ACTH are included here merely to allow a comparison with the other drugs. It is of interest that ACTH also produces a hydrocortisone-like effect even though the rat adrenal cortex secretes corticosterone, not hydrocortisone, as the major corticosteroid in response to endogenous and exogenous ACTH.[19]

(v) *Other anti-inflammatory compounds.* (Table 1E). Cinchophene, glycyrrhetic acid and flufenamic acid which suppress inflammation in experimental animals, were all active to some degree in modifying cartilage metabolism *in vivo.* Glycyrrhetic acid was the least effective of these three in our experiments with rats. Oleanolic acid and N-methylanthranilic acid were less potent *in vivo* than their respective congeners, glycyrrhetic acid and flufenamic acid, in agreement with their properties *in vitro.*[5]

It has been claimed that 8-hydroxyquinoline (oxine) has some anti-inflammatory activity in rats[20] but this compound had no consistent effect on cartilage metabolism *in vivo. In vitro,* it is a potent inhibitor of cartilage metabolism[4, 21] but at 5×10^{-4} M oxine had no appreciable effect upon oxidative phosphorylation by liver mitochondria (Whitehouse, unpublished experiments). Its isomer, 2-hydroxyquinoline (carbostyril appeared to have some effect on cartilage polysaccharide sulphation *in vivo* but in view of the enormously enhanced excretion of urinary sulphates by animals receiving this compound, this may perhaps only reflect a reduction in the circulating $^{35}S_i$ available for incorporation into the cartilage polysaccharides.

Chloroquine is a slow-acting antirheumatic drug clinically and also in *in-vitro* experiments. There is a distinct time lag before it has any effect upon the biosynthesis of polysaccharide sulphates in cartilage slices.[4] It is therefore not surprising to find it has no effect in an acute experiment *in vivo.*

Excretion of sulphated drug metabolites

Greiling and Schuler[22] have theorised that antirheumatic drugs are able to depress cartilage polysaccharide sulphation by competing for the supply of S_i or active sulphate (3′-phospho-5′-adenosinephosphosulphate, PAPS). This theory does not explain how these drugs could also depress the incorporation of acetyl or glucose fragments into these cartilage polysaccharide, observed in *in-vitro* experiments.[4] However it was possible to put this theory to the test by measuring the fraction of the ^{35}S excreted in the urine as ester sulphates by animals dosed with these drugs, compared with that excreted by the controls. According to Greiling and Schuler's theory, increases in the ester sulphate fractions due to the drug, should be accompanied by less incorporation of the ^{35}S into the cartilage polysaccharides. Both quantitative and qualitative evidence was obtained that many of the compounds given were excreted as sulphated metabolites (see Table 1 and Figs. 1, A–P). There was however no direct correlation between urinary ester sulphate formation and the *in-vivo* inhibition of cartilage ^{35}S metabolism except in two instances, when antipyrine or 2-hydroxyquinoline were administered. Neither of these compounds is considered to be a useful antirheumatic drug. On the other hand, urinary ester sulphates were increased when 2,5-dinitrophenol and 4-hydroxybenzoate were administered, although these compounds are without effect on cartilage metabolism *in vivo* and *in vitro.* Thus no convincing evidence was found to support this competition theory.

Salicylate, phenylbutazone and their pharmacologically active congeners (*o*-hydroxynaphthoate, oxyphenbutazone) together with 2,4-dinitrophenol all depressed urinary ester sulphate excretion (Table 1) even though they were all metabolised *in vivo* to yield ester sulphates. It would seem that these drugs, which all uncouple oxidative phosphorylation in cartilage and in liver tissue *in vitro,*[5] also uncouple hepatic (or renal) oxidative phosphorylation *in vivo* and so diminish the supply of ATP required for the biosynthesis of ester sulphates *in vivo.* Salicylates are known to diminish the

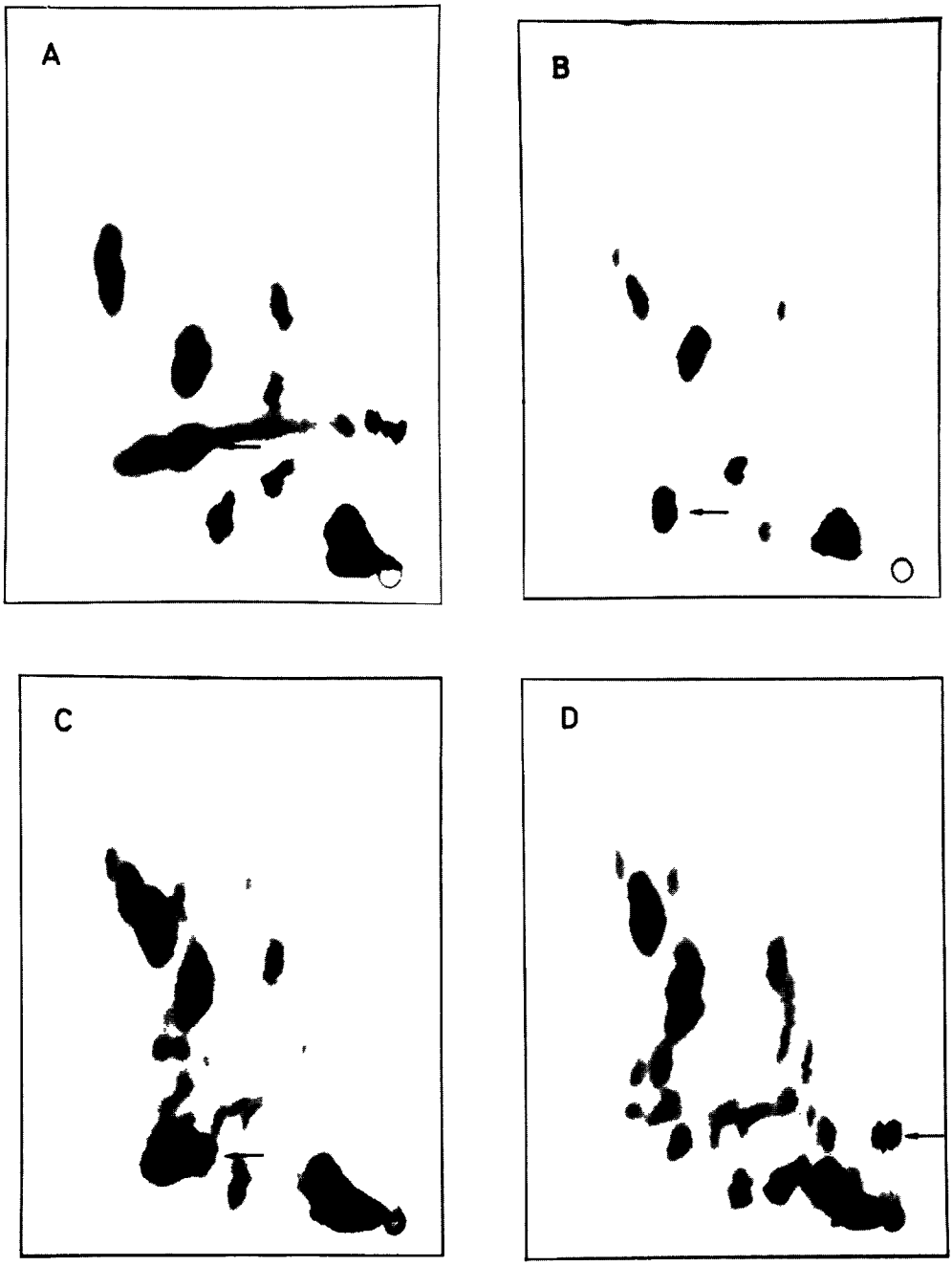

FIG. 1. Autoradiographs of chromatograms of urinary ester sulphates-^{35}S excreted by male Wistar rats after administration of the following drugs (doses as in Table 1) and sodium sulphate-^{35}S. (Radioactive zones not present on chromatograms of urine from control animals = "extra-normal" or "unphysiological" ester sulphates, are indicated by arrows.)

A: Sodium salicylate
B: p-OH-benzoic acid
C: 2,5-Dihydroxybenzoate
D: 2,6-Dihydroxybenzoic acid

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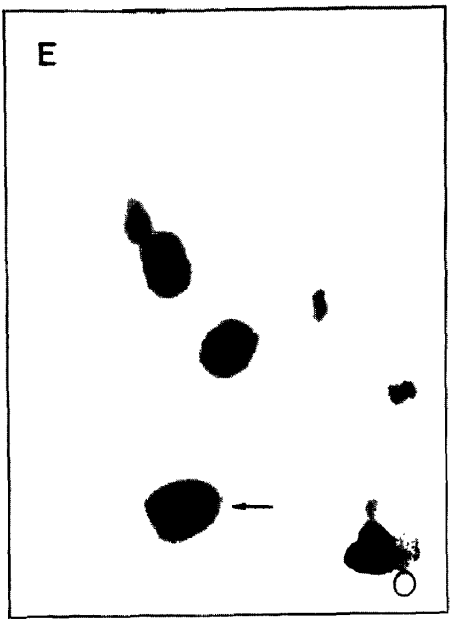
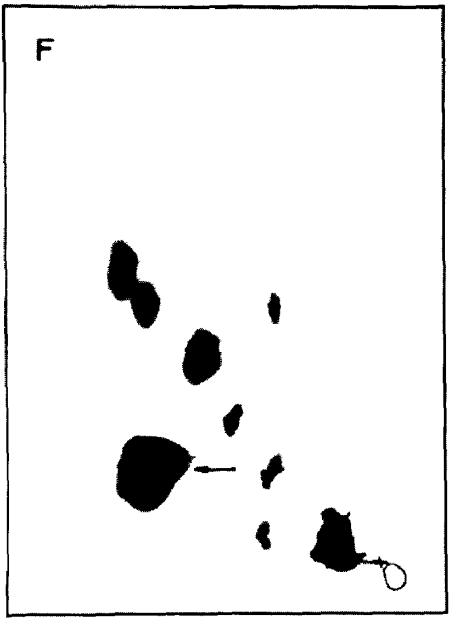
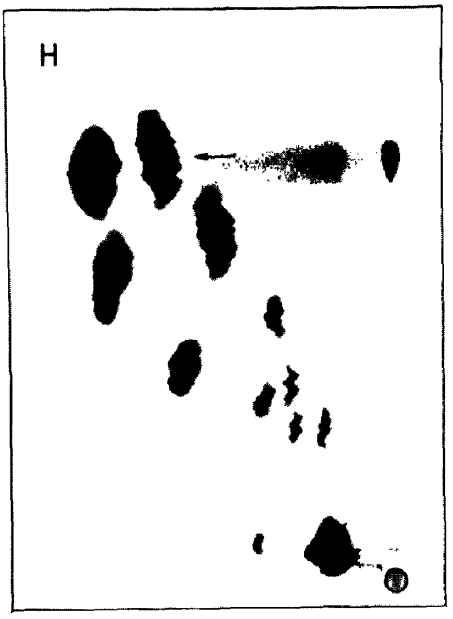
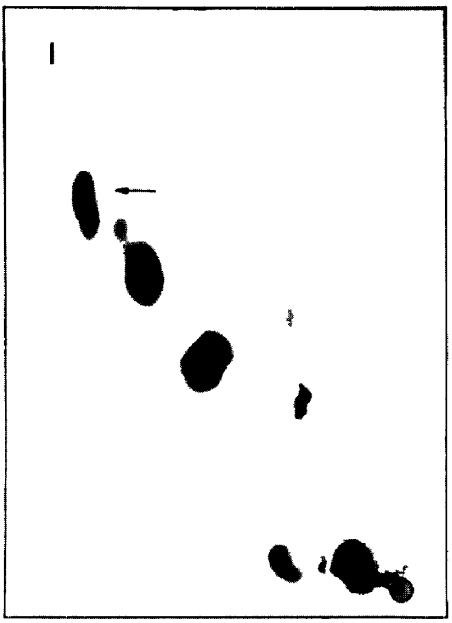


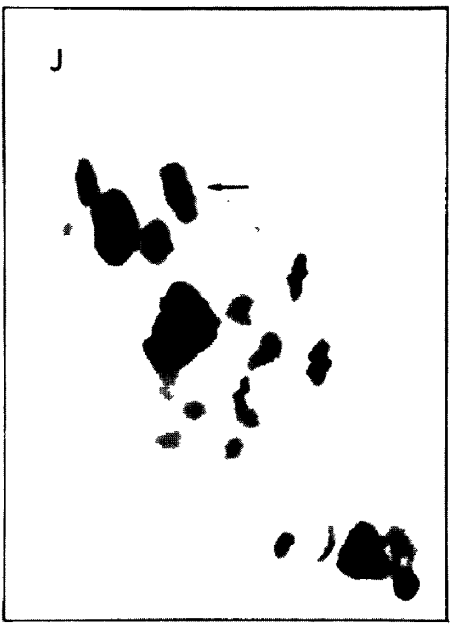


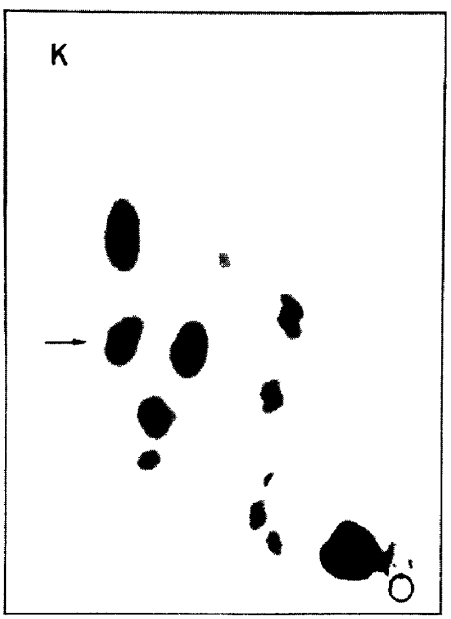


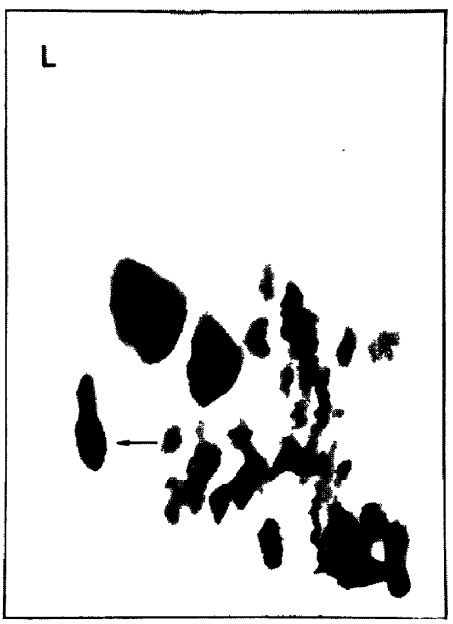
E: 1-Hydroxy-2-naphthoic acid
G: Phenylbutazone

F: 2-Hydroxy-6-naphthoic acid
H: Antipyrine



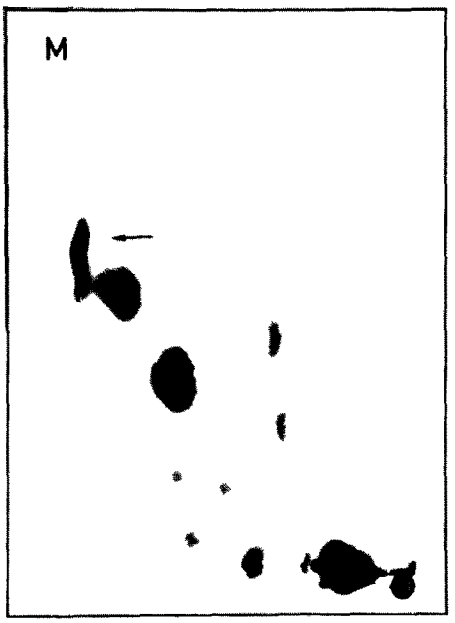


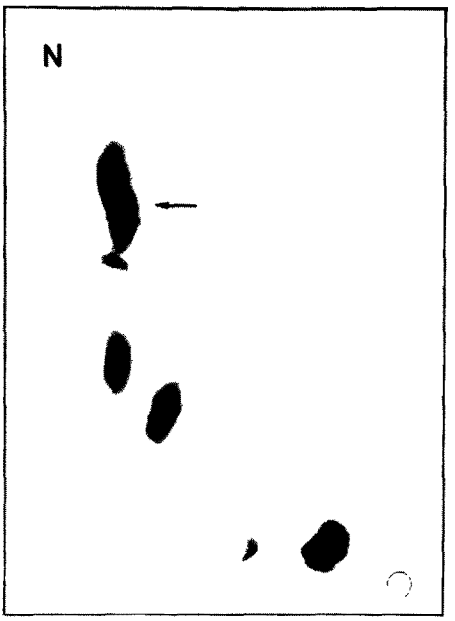


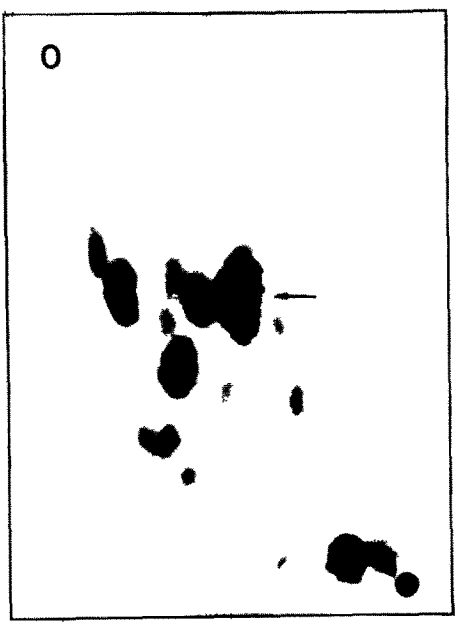


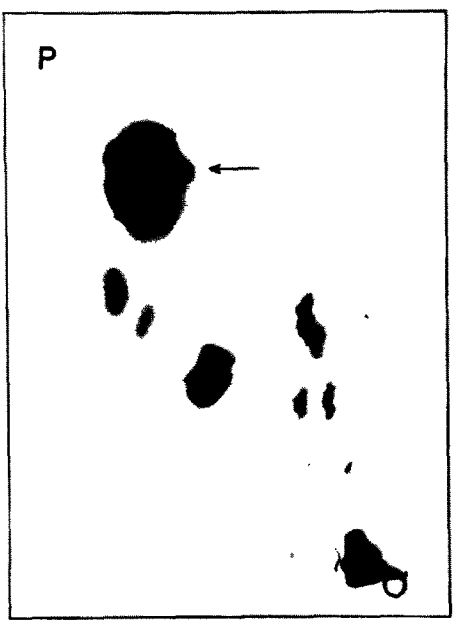
I: 1,3-Diphenylpyrazolone
K: Dicoumarol

J: 2,5-Dinitrophenol
L: Flufenamic acid









M: Chloroquine phosphate
O: 2-OH-quinoline

N: Oxine
P: Hydrocortisone (female Wistar rats)

excretion of urinary steroid conjugates, without affecting the excretion of non-conjugated steroids, by human subjects[23] which suggests that this action of salicylate in uncoupling oxidative phosphorylation is not restricted to the rat alone.

By contrast, hydrocortisone and ACTH did not alter the urinary ester sulphate excretion which affords evidence that these drugs do not affect renal or hepatic oxidative phosphorylation *in vivo* at dose levels which influence cartilage metabolism. Only when administered at high dose levels (100 mg/kg) was any evidence obtained that hydrocortisone was metabolised in rats and excreted in the urine as an "extra-normal" or unphysiological ester sulphate.

Sex and strain differences in excretion of sulphated metabolites

Only male Wistar rats excreted extra-normal urinary sulphates after phenylbutazone administration. The urines of female Wistar rats and of both male and female Sprague-Dawley rats did not contain these extra-normal radioactive sulphates, following the administration of both sodium sulphate-^{35}S and phenylbutazone to these animals.

After high doses of hydrocortisone (100 mg/kg) female Wistar and Sprague-Dawley rats each excreted an extra sulphate ester in the urine (Fig. 1P). Sprague-Dawley females excreted less of this compound than the Wistar female rats. Male rats of both strains did not excrete any significant quantity of this extra urinary sulphate ester, following hydrocortisone administration.

GENERAL DISCUSSION

These findings lend further support to the concept that anti-inflammatory drugs owe their property of supressing the inflammatory response, in part at least, to an ability to deprive the irritated tissue of its normal supply of energy in the form of ATP derived from cellular metabolism. They do not preclude other (additional) drug actions such as discussed by Bollet.[24] Drugs established as anti-inflammatory agents by pharmacological assays in experimental animals were potent inhibitors of metabolism in one experimentally amenable connective tissue, cartilage, both *in vivo* and *in vitro*. Some of their chemical analogues which had been found to be potent drugs *in vitro* such as the *o*-hydroxynaphthoates or diphenylpyrazolone,[4] were relatively ineffective *in vivo*, emphasising that the uncertainties of drug distribution *in vivo* will always preclude pharmacological exploration by *in-vitro* assays alone. However the fact that other analogues such as 4-hydroxybenzoate or antipyrine were without activity *in vitro* and *in vivo* indicates that preliminary screening for potential antirheumatic drugs might well be carried out with *in-vitro* studies of their action on the metabolism of isolated connective tissues.

Other workers have observed similar effects of salicylate, phenylbutazone and oxyphenbutazone upon the incorporation of ^{35}S into the polysaccharide sulphates of experimental granulomae in rats.[25–27] In contrast to our findings with costal cartilage, some of these workers failed to observe any effect of phenylbutazone upon ^{35}S incorporation into (a) the polyuronides of the cartilaginous xiphoid process[26] or (b) the total sulphate(s) of rat subcutaneous connective tissue.[25]

It has been claimed that the anti-inflammatory activity of salicylates is not associated with their ability to uncouple oxidative phosphorylation because 2,4-dinitrophenol, unlike salicylate, had no effect on the increased capillary permeability in

guinea pigs elicited by anaphylaxis.[28] It is not known though whether 2,4-dinitrophenol is distributed throughout the whole animal in the same manner as salicylate. The present experiments show that 2,4-initrophenol does have a salicylate-like action on cartilage *in vivo*, apparently associated with its uncoupling action (contrast 2,5-dinitrophenol). 2,4-Dinitrophenol does manifest anti-inflammatory activity in experimental animals under certain conditions[29]

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