THE EFFECTS OF ANTI-INFLAMMATORY DRUGS ON SOME ASPECTS OF INTERMEDIARY METABOLISM

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The effects of salicylate, 2,4-dinitrophenol, hydrocortisone, dexamethasone, phenylbutazone and chloroquine diphosphate on the incorporation of radioactivity from [^{14}C]glucose and [1,4-^{14}C]succinate into the soluble intermediates of rat liver preparations have been studied. It is concluded that the increased incorporation of radiocarbon into oligosaccharides, phosphates and malic and fumaric acids bears no relation to anti-inflammatory activity in the salicylate group of drugs.

THE anti-inflammatory compound, γ-resorcylic acid, produces several effects on intermediary metabolism such as an increased incorporation of radioactivity into the oligosaccharide, phosphate and organic acid fractions of rat liver preparations incubated with labelled substrates (Huggins, Bryant and Smith, 1961). The present work is concerned with the possible relation of these effects with anti-inflammatory activity. The effects of other anti-inflammatory drugs, salicylate, hydrocortisone, dexamethasone, phenylbutazone and chloroquine diphosphate, and of a related phenolic substance, 2,4-dinitrophenol, which is devoid of experimental anti-inflammatory properties, have therefore been studied in the same biochemical systems.

EXPERIMENTAL

The techniques used for the liver preparations and for the radioactive experiments were those described by Huggins, Bryant and Smith (1961). The concentrations of the drugs, after admixture with the tissue preparations and incubation media, were 5 mM for salicylate, 0·5 mM for 2,4-dinitrophenol, 10 μg./ml. for hydrocortisone, phenylbutazone and chloroquine diphosphate and 0·5 μg./ml. for dexamethasone (Moses and Smith, 1961).

TABLE I

METABOLISM OF [^{14}C]GLUCOSE BY RAT LIVER HOMOGENATE IN THE PRESENCE OR THE ABSENCE OF ANTI-INFLAMMATORY DRUGS

The ^{14}C present in each intermediate is expressed as a percentage of the total ^{14}C incorporated from the labelled substrate into the sum of all the soluble intermediates; ^{14}C in the residual substrate is excluded from all calculations

Soluble intermediate	None*	Resorcylate*	Salicylate	DNP	Hydrocortisone	Dexamethasone	Phenylbutazone	Chloroquine
Alanine	13	7	10	7	8	10	8	11
Lactic acid	14	0	0	3	20	16	24	20
Malic acid	0	0	0	0	2	1	2	2
Oligosaccharides	36	67	53	55	42	43	41	39
Phosphates	37	16	27	29	22	24	19	22
Unidentified compounds	0	10	10	6	6	6	6	6

* Data from Huggins, Bryant and Smith (1961).

RESULTS

The percentages of radiocarbon from [^{14}C]glucose which were incorporated into the soluble metabolic intermediates of a homogenate of rat liver, in the presence or the absence of the drugs, are given in Table I.

Salicylate and 2,4-dinitrophenol (DNP) resembled γ-resorcylate in causing substantial increases in the formation of radioactive oligosaccharides and a decreased incorporation of isotope into the phosphate fraction. The latter effect was also shown by the other anti-inflammatory drugs but these had much smaller actions on the oligosaccharide fraction. γ-Resorcylate, salicylate and DNP reduced the formation of radioactive lactic acid whereas the other drugs tended to produce the reverse effect.

TABLE II

METABOLISM OF [^{14}C]GLUCOSE BY A SOLUBLE FRACTION FROM RAT LIVER IN THE PRESENCE OR THE ABSENCE OF ANTI-INFLAMMATORY DRUGS

(Results expressed as in Table I)

Soluble intermediate	None*	Resorcylate*	Salicylate	DNP	Hydrocortisone	Dexamethasone	Phenylbutazone	Chloroquine
Alanine	16	10	19	19	18	23	24	29
Aspartic acid	16	13	20	16	18	21	24	26
Lactic acid	37	26	34	39	30	17	12	4
Malic acid	0·7	1·0	0·2	0·8	0·9	1·5	1·0	1·3
Oligosaccharides	1·6	2·1	1·2	1·3	3·7	5·3	4·0	5·4
Phosphates	24	48	23	21	24	28	29	31
Unidentified compounds	4·7	0	2·6	2·9	5·4	4·2	6·0	3·3

* Data from Huggins, Bryant and Smith (1961).

The results in Table II show that only γ-resorcylate caused an increase in the incorporation of ^{14}C into the phosphate fraction derived from [^{14}C]glucose in the soluble liver preparation. Hydrocortisone, dexamethasone, phenylbutazone and chloroquine enhanced the formation of the labelled oligosaccharide fraction whereas the other drugs were inactive. No consistent effects on the incorporation of isotope into the lactic acid were observed.

TABLE III

METABOLISM OF [1,4-^{14}C]SUCCINATE BY RAT LIVER MITOCHONDRIA IN THE PRESENCE OR THE ABSENCE OF ANTI-INFLAMMATORY DRUGS

(Results expressed as in Table I)

Soluble intermediate	None*	Resorcylate*	Salicylate	DNP	Hydrocortisone	Dexamethazone	Phenylbutasone	Chloroquine
Alanine	1	1	1	1	2	1	3	0
Asparagine	5	0	0	0	6	12	2	10
Aspartic acid	25	7	10	10	23	37	20	18
Glutamic acid	2	1	3	2	10	0	4	0
Glutamine	19	0	0	1	0	0	0	0
Citric acid	1	4	7	9	3	4	6	3
Fumaric acid	5	21	23	22	8	0	6	3
Malic acid	7	63	55	54	19	4	28	12
Lactic acid	16	1	3	1	11	9	14	12
Phosphates	12	0	0	0	14	19	13	23
Unidentified compounds	7	2	0	0	4	14	4	19

* Data from Huggins, Bryant and Smith (1961).

Table III shows the distribution of radiocarbon from [1,4-^{14}C]succinate among the soluble intermediates of mitochondrial suspensions from rat liver, in the presence or absence of the drugs. The most prominent action of γ-resorcylate, the accumulation of ^{14}C into the fumaric and malic acids, was shared by salicylate and DNP. However, this effect was much less pronounced with hydrocortisone and phenylbutazone and was absent in the dexamethasone and chloroquine experiments. Further differences between γ-resorcylate, salicylate and DNP on the one hand, and the steroids, phenylbutazone and chloroquine, on the other were concerned with the incorporation of isotope into the lactic acid, aspartic acid, asparagine and phosphate fractions. The first group caused a decreased formation of these labelled intermediates but the second group had little effect. All the drugs reduced the incorporation of ^{14}C into glutamine.

Discussion

Three separate effects of γ-resorcylic acid on intermediary metabolism were distinguished by Huggins, Bryant and Smith (1961). These were the increased incorporation of radioactivity from labelled glucose into an oligosaccharide fraction of a rat liver homogenate; into the phosphate compounds formed in a soluble preparation from rat liver and from labelled succinate into the fumaric and malic acids in rat liver mitochondria. The present results show that none of these biochemical effects appear to be connected with anti-inflammatory activity. The relevant data is summarised in Table IV where it is seen that although

TABLE IV

Effects of anti-inflammatory drugs and DNP on the incorporation of ^{14}C from [^{14}C]glucose or [1,4-^{14}C]succinate into some fractions of the soluble intermediates of rat liver preparations

The results are expressed as percentages of the corresponding control values

Drug	[^{14}C]Glucose		[1,4-^{14}C]Succinate
	Oligosaccharides in homogenates	Phosphates in soluble fraction	Malic plus fumaric acids in mitochondria
Resorcylate	186	200	700
Salicylate	152	96	650
Dinitrophenol	154	88	630
Hydrocortisone	117	100	225
Dexamethasone	119	117	33
Phenylbutazone	114	121	280
Chloroquine	104	129	125

salicylate resembles γ-resorcylate in causing at least a 50 per cent increase in the accumulation of ^{14}C in the oligosaccharides and into fumaric and malic acids, none of the other anti-inflammatory drugs shared these effects. More pertinently, DNP, which does not possess experimental anti-inflammatory activity (Marks, Smith and Cunliffe, 1961) also produced similar effects to γ-resorcylate in these systems. Of the drugs tested only γ-resorcylate caused a doubling of the incorporation of radiocarbon into the phosphate compounds of the soluble liver preparation. It is most unlikely that this action bears any relevance to the anti-inflammatory properties

of the dihydroxybenzoate since the closely related salicylate is without effect. The effect on the formation of radioactive phosphates in the soluble liver preparation appears to be restricted to γ-resorcylate whereas the other effects on oligosaccharides and the tricarboxylic acids are shared by compounds such as salicylate and DNP, which also possess phenolic hydroxyl groups. It must be concluded that none of the above effects can be related with anti-inflammatory activity in general.

The anti-inflammatory drugs used in the present work do not share common actions on intermediary metabolism. This is not surprising in view of their diverse chemical structures and it seems unlikely that they produce their beneficial effects in rheumatism by the same mechanism. The failure of the powerful uncoupling reagent, 2,4-dinitrophenol, to exhibit experimental anti-inflammatory properties (Adams and Cobb, 1958; Marks, Smith and Cunliffe, 1961) shows that this major action on cellular metabolism is not related to anti-inflammatory activity. However, both salicylate and γ-resorcylate inhibit glutamic-pyruvic transaminase activity (Steggle, Huggins and Smith, 1961) whereas DNP is inactive. The possible relation of this biochemical effect to anti-inflammatory activity in the salicylate group of drugs remains to be explored. In this laboratory it has been found that hydrocortisone, dexamethasone, phenylbutazone and chloroquine do not influence glutamic-pyruvic transaminase activity *in vitro* (Steggle and Smith unpublished data) but possess a common action on maltose formation from glucose in chopped liver preparations (Moses and Smith, 1961). These last four compounds also show consistent effects in increasing the incorporation of ^{14}C into oligosaccharide substances in the present experiments and it is possible that this action may bear some relevance to their anti-inflammatory properties.

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