

EFFECT OF CERTAIN HYDROXYBENZOIC ACIDS ON THE OXYGEN CONSUMPTION OF WISTAR RATS

BY

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Despite the introduction of numerous other chemotherapeutic agents, salicylic acid and its derivatives retain an important place in the treatment of many rheumatic disorders; therefore any suggestion concerning their mode of action is of peculiar interest. Cochran (1952) has suggested that the increased tissue oxidation that he had observed after salicylate administration might be of fundamental importance in the therapeutic action of this drug. He gave 10 g. sodium salicylate intravenously to each of two normal subjects and to one patient with subacute rheumatism, and oral doses of 7.5 to 10 g. daily to three patients with subacute rheumatism; there was a marked increase in the oxygen consumption of about 60 to 70 per cent. after intravenous dosage and of 30 to 40 per cent. when the drug was given by mouth. This change in oxygen consumption was accompanied by a fall in the RQ, from which Cochran deduced that there was an increased utilization of protein or fat. Barbour and Devenis (1919) had previously studied the effect of oral doses of 1 to 1.25 g. acetylsalicylic acid on the metabolism of five normal men. They found that there was an average increase of 6.1 per cent. in oxygen consumption, whilst the carbon dioxide output increased concomitantly so that the RQ was unchanged. Denis and Means (1916) had

reported a similar experiment with variable results.

Present Investigations

Since not only salicylic acid derivatives have been used in the therapy of rheumatic fever, but also other hydroxybenzoic acids such as the 2 : 6, and 2 : 5 dihydroxy compounds it was decided to study their effect on the oxygen consumption of the Wistar rat to establish whether there was any correlation between the stimulation observed and the known therapeutic efficacy in man.

Method.—The oxygen consumption of pairs of 150-200 g. male Wistar rats was measured by the closed circuit method of Maclagan and Sheahan (1950).

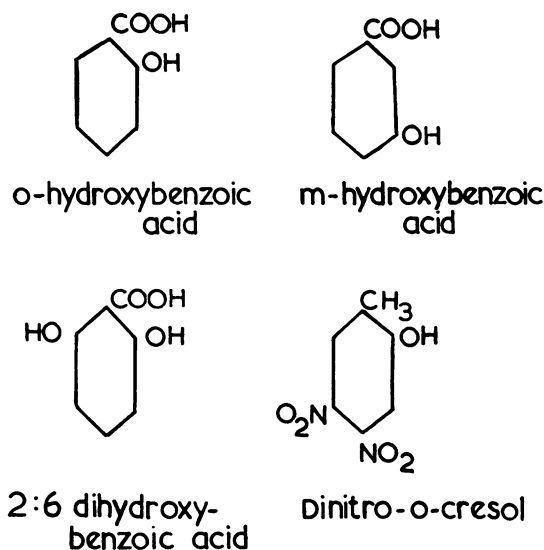
The drugs were given intraperitoneally in 1 ml. of either saline or 1 per cent. sodium carbonate at a dosage of 50 mg. per rat. A control group was injected with the vehicle only. The oxygen consumption of the treated group was measured over the succeeding 3-hr period and was expressed as a percentage of the mean oxygen consumption of the control group for that experiment.

Results.—The Table shows that salicylic acid was unique in increasing significantly the oxygen consumption. The only other positive finding was that *m*-hydroxybenzoic acid (see Figure, opposite) was notably depressant. The remaining compounds were without significant effect.

TABLE
EFFECT OF HYDROXYBENZOIC ACIDS ON OXYGEN CONSUMPTION OF RATS

Systematic Name	Trivial Name	No. of Observations	O ₂ Consumption compared with Control Group 100 per cent. (with standard deviation)	Probability (Student's <i>t</i> test)
Benzoic		18	102 ± 10.2	—
* <i>o</i> -hydroxybenzoic	Salicylic	12	183 ± 16.4	< 0.001
* <i>m</i> -hydroxybenzoic		24	65 ± 7.6	< 0.001
<i>p</i> -hydroxybenzoic		24	92 ± 15.2	—
2 : 3 dihydroxybenzoic		18	101 ± 9.6	—
3 : 4 dihydroxybenzoic	Protocatecheuic	18	102 ± 20.8	—
2 : 5 dihydroxybenzoic	Genticic	18	103 ± 19.4	—
*2 : 6 dihydroxybenzoic	γ resorcylic	18	106 ± 12.4	—
2 : 4 dihydroxybenzoic	α resorcylic	18	97 ± 13.8	—
cis-hexahydrosalicylic		18	103 ± 18.4	—

* See Figure for formulae.



Figure—Structural formulae of compounds discussed.

Discussion

Amongst the hydroxybenzoic acids, salicylic acid and its derivatives are most frequently used in the treatment of rheumatic fever.

Another compound, the sodium salt of 2:6 dihydroxybenzoic acid (Figure), has been reported by Reid, Watson, Cochran, and Sproull (1951) to be beneficial in doses lower than those of salicylate necessary to achieve comparable clinical results, but use of this compound was accompanied by many toxic manifestations.

Of questionable efficacy is 2:5 dihydroxybenzoic acid. This substance was reported as effective therapeutically both by Meyer and Ragan (1948), and by Camelin, Accoyer, Pellerat, Lafuma, and Coirault (1949), but subsequently Camelin, Accoyer, Vailhé, Roumagnac, and Rosset (1952) recorded an increase in the incidence of carditis in rheumatic disease during the use of this drug.

Amongst the remaining compounds tested, m- and p-hydroxybenzoic acids were described by Stockman (1920) as inactive in rheumatic fever.

Thus, of the two compounds, salicylate and 2:6 dihydroxybenzoic acid, reported to be useful in this disease, only the former had a stimulant effect on metabolism. Cochran's suggestion referred to above that the effect on metabolism is related to therapeutic value, therefore receives only partial support.

Baker (1936) pointed out that the dissociation constants of benzoic acid and of those mono-, di-, and trihydroxybenzoic acids, which do not possess

an hydroxyl group in the ortho position, are less than 1×10^{-4} . Those acids having such an ortho hydroxyl group have dissociation constants considerably higher than this; the highest is that of 2:6 dihydroxybenzoic acid which has a value of 5.0×10^{-2} . The increased acidity of the salicylic acid derivatives he ascribed to chelation of the anion. Reid and others (1951), in introducing 2:6 dihydroxybenzoic acid to clinical practice, suggested that this ability to chelate might be of significance in the therapy of rheumatic fever, an idea which was supported by the clinical efficacy of their compound. Thus there are two suggestions from the same department:

- that the therapeutic effect of salicylate is due to some, as yet undefined, stimulation of metabolism,
- that it is related to the ability of these compounds to form chelate rings.

It is apparent from the results given above that, in the rat at least, the increase in metabolism is unrelated to the dissociation constant of the hydroxybenzoic acids and hence to chelation, so that unification of these two concepts lacks experimental support.

The unique stimulatory action of salicylates amongst the hydroxybenzoic acids recalls the effect of the nitrophenols and related compounds. This similarity extends to observations on carbohydrate metabolism. Acute depletion of liver glycogen has been demonstrated to occur after the injection of salicylate into rats by Lutwak-Mann (1942), and by Smith, Meade, and Bornstein (1952). Bidstrup and Payne (1951) have reported that dinitro-ortho-cresol (Figure) experimentally causes an increase in oxygen consumption and the disappearance of glycogen from liver and muscles. Barker (1946) gave rats doses of 10 mg. DNOC per g. bodyweight, and found that oxygen consumption was increased. Moreover, Barnes (1953) has shown that the injection of DNOC into rats caused an initial fall in liver glycogen which was followed by a progressive rise, so that after 24 hours the level was higher than in control animals. Judah (1951) showed that under the influence of the dinitrophenols the uptake of inorganic phosphate by respiring mitochondrial preparations is decreased, but the consumption of oxygen increases. It seems possible that a similar uncoupling mechanism is the basis of the salicylate effect, though no direct evidence of this is yet to hand.

Only m-hydroxybenzoic acid (Figure) was found to exert an inhibitory effect. It is of interest to note that Rosenberg (1952) observed that the m-configuration confirmed inhibitory properties on phenols and amines when he measured both the ^{131}I

uptake by the thyroid of rats and milk peroxidase activity. The significance of this association is obscure, but it is remarkable that such a small change in molecular configuration leads to opposed experimental results.

Summary

(1) The effect of certain hydroxybenzoic acids on the oxygen consumption of Wistar rats is reported.

(2) Salicylic acid was found to stimulate the oxygen consumption whilst m-hydroxybenzoic acid depressed it. The remaining compounds were inactive.

(3) The salicylate results are discussed in the light of suggested modes of action of related compounds in rheumatic fever.

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Effets de certains acides hydroxybenzoïques sur la consommation d'oxygène par des rats de Wistar

RÉSUMÉ

(1) On présente un rapport sur l'effet de certains acides hydroxybenzoïques sur la consommation d'oxygène par des rats de Wistar.

(2) On trouva que l'acide salicylique stimule la consommation d'oxygène alors que l'acide m-hydroxybenzoïque l'abaisse. Les autres composés furent inactives.

(3) On discute ces résultats avec des salicylates à la lumière des modes d'action supposée des composés voisins sur le rhumatisme articulaire aigu.

Efectos de ciertos ácidos hidroxibenzoicos sobre la consunción de oxígeno por ratas de Wistar

SUMARIO

(1) Se informa sobre el efecto de ciertos ácidos hidroxibenzoicos sobre la consunción de oxígeno por ratas de Wistar.

(2) Se halló que el ácido salicílico estimula la consunción de oxígeno mientras que el ácido m-hidroxibenzoico la deprime. Los demás compuestos no fueron activos.

(3) Se discute los resultados con los salicilatos en la luz de los modos sugeridos de acción de los compuestos afines sobre el reumatismo poliarticular agudo.