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SHORT COMMUNICATIONS

Gentisate and human red cell metabolism

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THE ACTIONS of gentisate (5-hydroxysalicylate) have been compared to those of salicylate, both at the therapeutic and the biochemical level. Many of the biochemical actions are of a similar nature to those of salicylate (e.g. inhibition of rat serum glutamic-pyruvic transaminase,[1] inhibition of several dehydrogenase enzymes,[2] overall effects on guinea-pig testis metabolism[2]), but a number are very different (e.g. potent inhibition of cytochrome oxidase enzyme systems,[3] stimulation of pentose phosphate pathway metabolism[4]). The latter actions have been ascribed, in part, to the ease with which gentisate is oxidised to the para-quinone, 2-carboxy-1,4-benzoquinone, Quinones have been shown to be powerful inhibitors of cytochrome oxidase enzymes,[5] which would account for the inhibitory action of gentisate. It has been suggested[6] that hydroquinone-quinone systems may function as electron acceptors, and facilitate oxidation of $NADPH_2$ by molecular oxygen, thus increasing the proportion of glucose metabolised via the pentose phosphate pathway in the red cell.

Human red cells metabolise glucose by the glycolytic and pentose phosphate pathways. Glucose specifically labelled with ^{14}C in the 1-position enables the activity of the pentose phosphate pathway to be estimated by measuring the $^{14}CO_2$ produced. The experiments reported in this paper compared the effects of salicylate and gentisate, with those of a number of compounds capable of oxidation-reduction reactions, on glucose metabolism by haemolysed preparations of human red cells. The reactions were carried out in the presence of 1mM exogenous NADP, the concentration resulting in maximal pentose phosphate pathway activity.[7] The incorporation of radioactivity from [1—^{14}C] glucose into $^{14}CO_2$ in the haemolysates and the effects of the various added compounds were studied by the techniques described previously.[7]

TABLE 1. EFFECTS OF VARIOUS ADDITIONS ON THE METABOLISM OF [1—^{14}C] GLUCOSE TO $^{14}CO_2$ BY HUMAN RED CELL HAEMOLYSATES

Addition	Final conc. mM	[1—^{14}C] Glucose utilised dpm × 10^{-3}	$^{14}CO_2$ Produced dpm × 10^{-3}	% [1-^{14}C] Glucose utilised converted to $^{14}CO_2$
None	—	421±8	281±8	66·8±3·8
Salicylate	10	368±6	267±3	72·5±3·1
Gentisate	10	355±4	273±4	77·0±3·4
Quinol	10	369±5	320±7	86·7±4·3
Benzoquinone	10	378±5	312±4	82·5±2·9
Methylene blue	1	478±11	422±8	88·4±4·8
Reduced glutathione	10	288±6	269±7	93·4±5·2
Oxidised glutathione	10	297±8	250±5	84·3±4·7
Cysteine	10	298±8	222±7	74·2±4·8
Cystine	10	361±3	216±5	59·9±1·7
Ascorbic acid	10	327±5	280±4	85·8±2·1

* Each observation represents the mean of four separate experiments ± S.D. The radioactive measurements were performed at an efficiency of 70 per cent.

The results (Table 1) show that in the presence of a plentiful supply of NADP, the action of gentisate is similar to that of salicylate, causing decreased glucose utilisation and only slightly reduced $^{14}CO_2$ production. This contrasts sharply with the action of gentisate on intact human red cells in which the supply of NADP is limiting.[4] The results obtained in the presence of quinol closely resembled those

with benzoquinone, supporting the suggestion of a hydroquinone-quinone system operating. Methylene blue caused an increase in [1—^{14}C] glucose utilisation and in the proportion converted to $^{14}CO_2$, indicating that mere addition of NADP did not extend the capacity of the pentose phosphate pathway to its limit. The addition of certain compounds normally present in biological fluids, such as oxidised and reduced glutathione, cystine, cysteine and ascorbic acid, has been reported to increase the proportion of glucose metabolised via the pentose phosphate pathway in intact human erythrocytes.[8-10] The latter compounds all decreased [1—^{14}C] glucose utilisation in the haemolysates, and all but cystine increased the proportions of glucose converted to $^{14}CO_2$. The effects of glutathione, both oxidised and reduced forms, were consistent with its envisaged role in an oxidation-reduction cycle linking $NADPH_2$ oxidation with molecular oxygen.[11]

The results confirm that gentisate, although possessing certain modes of action resulting from the two hydroxyl groups being in the para-positions on the benzene ring, acts in a similar manner to salicylate as an inhibitor of certain vital groups of cellular enzymes.

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REFERENCES

1. R. A. Steggle, A. K. Huggins and M. J. H. Smith, *Biochem. Pharmac.* **7**, 151 (1961).
2. W. J. W. Hines, *J. Pharm. Pharmac.* **18**, 256 (1966).
3. W. J. Hines, C. Bryant and M. J. H. Smith, *Biochem. Pharmac.* **12**, 1109 (1963).
4. J. A. Sturman and M. J. H. Smith, *Biochem. Pharmac.* **16**, 220 (1967).
5. V. R. Potter and K. P. Dubois, *J. gen. Physiol.* **26**, 391 (1943).
6. L. F. Hewitt, *Oxidation-Reduction Potentials in Bacteriology and Biochemistry*, p. 21. London County Council (1937).
7. M. J. H. Smith and J. A. Sturman, *J. Pharm. Pharmac.* **19**, 108 (1967).
8. W. C. J. de Loecker and T. A. J. Prankerd, *Clin. chim. Acta*, **6**, 641 (1961).
9. A. Szeinberg and P. A. Marks, *J. clin. Invest.* **40**, 914 (1961).
10. G. Fornaini, P. Segni, G. Leoncini, A. Fantoni and O. Chieffi, *Ital. J. Biochem.* **12**, 374 (1963).
11. P. W. Spear and M. D. Sass, *Metabolism* **13**, 911 (1964).

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The depressor activity of trypsin-like enzymes purified from rat submandibular gland

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Earlier we have reported the purification and characterization of three alkaline trypsin-like proteases from the rat submandibular gland. They were trivially named salivain, glandulain and kallikrein-like peptidase.[1-4] All of the enzymes were capable of hydrolyzing synthetical ester or amide substrates typical for trypsin, e.g. *N*α-benzoyl-DL-arginine ethyl ester (BAEE) or *N*α-benzoyl-DL-arginine 2-naphthylamide. Salivain and glandulain, but not the kallikrein-like peptidase (all of the four isozymic forms), hydrolyzed readily also proteins. The high esterase activity of the enzymes resembles that of kallikrein, a depressor substance known to be present also in the submandibular gland. and