the sympathetic transmitter substance. This observation may explain the nature of the diarrhoea and hypotension seen in clinical practice.

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Gentisate and guinea-pig testis metabolism

SIR,—Gentisic acid (2,5-dihydroxybenzoic acid) is devoid of uncoupling activity in mitochondrial suspensions (Brody, 1956; Whitehouse, 1964) and does not produce toxic symptoms in large doses in man (Smith, 1952). Claims that gentisate is a therapeutically active antirheumatic drug have been challenged in print (cited in Whitehouse, 1964), but the absence of any well controlled clinical trial still leaves gentisate as one salicylate congener of potential therapeutic value. Recent studies on a liver succinate oxidase preparation (Hines, Bryant & Smith, 1963) and on testis mitochondria (Hines & Bryant, 1966a), both from the guinea-pig, have demonstrated several effects of gentisate on biochemical parameters often greater than those found for the parent molecule, salicylate. These experiments compare salicylate and gentisate effects on the metabolism of radioactive substrates by preparations of guinea-pig testis; and of gentisate on several isolated dehydrogenase enzymes. The tissue was isolated, the fractions prepared and the incubation techniques performed as described previously (Hines & Bryant, 1966b), using 1 μc of each carbon labelled substrate. The radioactively labelled intermediates were extracted with ethanol, separated by two-dimensional paper chromatography, visualised by radioautography and the ^{14}C measured by established techniques.

The results (Table 1) show that gentisate closely parallels salicylate in its effects on preparations of isolated guinea-pig testis. Both drugs decrease the utilisation of (2-^{14}C) - acetate by an homogenate preparation. The qualitative pattern of incorporation of the radiocarbon by each preparation was not altered by either drug. Quantitative relationships were altered, and these are shown as changes in the pool sizes of the amino-acids (alanine, aspartate and glutamate) acids of the tricarboxylic acid cycle (succinate, fumarate, malate and citrate), and those intermediates associated with glycolysis (phosphates and lactate). The inhibitory effect of salicylate on many isolated dehydrogenase enzymes is well established, and the mechanism of the inhibition involves competition with the appropriate coenzyme (Hines & Smith, 1964). The inhibitory action of sodium gentisate (5mM) on several dehydrogenases was also investigated. The inhibitions % (calculated from initial rates) for those dehydrogenases studied are: malate 46, isocitrate 31, lactate (NAD→NADH) 16, (NADH→NAD) 13, glyceraldehyde-3-phosphate 17, α-glycerophosphate 13, glucose-6-phosphate 21. It was possible to reduce the inhibition, in each instance, by the further addition of the respective coenzyme. The interference with transaminase enzyme activity is reflected in the reduced formation of radioactive amino-acids (Table 1) and conforms with established actions of salicylate on both glutamic - pyruvic and glutamic - oxaloacetic transaminase

TABLE 1. THE EFFECTS OF SODIUM SALICYLATE (5mM) AND SODIUM GENTISATE (5mM) ON THE METABOLISM OF RADIOACTIVE SUBSTRATES BY PREPARATIONS OF GUINEA-PIG TESTIS

(Results, the mean of four separate estimations, are expressed as the total % ^{14}C incorporated from the labelled substrates into the sum of all the separated soluble intermediates; the ^{14}C in each residual substrate being excluded)

Soluble intermediate	(2-^{14}C) Acetate homogenate			(U-^{14}C) Glucose soluble fraction			(1:4-$^{14}C_2$) Succinate mitochondria			(5-^{14}C)α-Ketoglutarate mitochondria		
	None	Salicylate	Gentisate	None	Salicylate	Gentisate	None	Salicylate	Gentisate	None	Salicylate	Gentisate
Alanine	0	0	0	8	7	6	0·7	0·2	0·5	0·3	0·6	0·4
Aspartic acid	3	2	2	20	24	24	30	18	14	4	3	3
Asparagine	1	0	0	0	0	0	2	1	0·8	0·7	0·4	0·6
Glutamic acid	64	59	56	0·5	0·3	0·2	4	2	2	12	10	11
Citric acid	7	11	10	0	0	0	12	16	15	50	42	39
Fumaric acid	2	1	2	0	0	0	15	19	22	6	8	6
Lactic acid	18	17	16	39	32	30	0·8	0·2	1	4	1	2
Malic acid	2	8	7	0·5	0·7	0·8	35	43	44	17	21	19
α-Ketoglutaric acid	0·5	1	2	0	0	0	0·5	0·6	0·7	—	—	—
Succinic acid	2	1	5	0	0	0	—	—	—	6	14	19
Phosphates	0·5	0	0	21	26	27	0	0	0	0	0	0
Oligosaccharides	0	0	0	11	10	12	0	0	0	0	0	0

activities (Smith, 1963). The diminished conversion of labelled glucose to lactate (probably a lactic dehydrogenase inhibition) may have been a causative factor in the unexpected increase in incorporation of radiocarbon into aspartate in the glucose experiments. There was no observable effect of either drug on the level of incorporation of radio-carbon from glucose in the oligosaccharide fraction, although γ-resorcylic acid (2,6-dihydroxybenzoic acid) is reported to double the incorporation of labelled glucose into an oligosaccharide fraction (Smith, 1963).

The demonstration of salicylate as an inhibitor of certain vital groups of cellular enzymes (those concerned with oxidative phosphorylation, the transaminases, and the dehydrogenases) can now, with the exception of uncoupling, be extended to its congener, gentisic acid.

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