Salicylate and Glutamate Metabolism

SIR,—Salicylate has been found to interfere with several important pathways involved in the metabolism of glutamate by animal tissues. The drug inhibits the synthesis of glutamine (Messer, 1958), glutamate-pyruvate transaminase activity (Steggle, Huggins and Smith, 1961), the enzymes responsible for the dehydrogenation and decarboxylation of the amino-acid (Gould, Huggins and Smith, 1963) and the incorporation of radioactive glutamate into muscle protein (Manchester, Randle and Smith, 1958). We have found that salicylate affects a further pathway of glutamate metabolism, its conversion to proline in preparations of rat costal cartilage. The drug also inhibited the incorporation of radioactive glutamate and proline into the cartilage protein but cortisol did not share these actions of salicylate.

Rat costal cartilage was freed from adherent muscle and cut into pieces, each of approximately 25 mg. Six pieces were incubated aerobically for 24 hr. at 37° in 1 ml. of the incubation medium of Salmon and Daughaday (1958) containing 50 μg. of Crystamycin (Glaxo) plus 2·5 μc of either [^{14}C]-glutamate or [^{14}C]-proline, in the presence or the absence of either 10 mM salicylate or 100 μg./ml. of cortisol. At the end of the incubation period the segments of cartilage were removed, washed for 10 sec. with fresh incubation medium, immersed in 0·5 ml. of boiling per cent (v/v) ethanol and allowed to extract for 24 hr. at room temperature. This process was repeated until no further radioactivity was extracted and all the aqueous ethanolic extracts were combined. The pieces of cartilage were then added to 2 ml. of 6N hydrochloric acid and heated in sealed glass tubes at 100° for 24 hr.; the hydrolysate was evaporated to dryness and the residue dissolved in 1 ml. of water. The radioactive substances present in the supernatants from the original incubation mixtures, in the aqueous ethanolic extracts of the cartilage and in the hydrolysates were separated by two dimensional chromatography and visualised by radioautography (Smith and Moses 1960). Radioactive glutamate and proline were presumptively identified by their chromatographic positions and this was confirmed by eluting the spots and separating the amino-acids by high-voltage paper electrophoresis, in the presence of authentic non-radioactive material, according to the directions of Atfield and Morris (1961). The ^{14}C in each spot was measured directly on the chromatograms by means of a Scott-type Geiger-Muller tube (Fuller, 1956).

Most of the carbon-14 from the labelled amino-acids which was incorporated into the soluble intermediates, appeared in substances such as malate, fumarate and aspartate. However, radioactive proline was detected in the supernatant, in the aqueous alcoholic extract and in the hydrolysate of the rat costal cartilage incubated with the ^{14}C-labelled glutamate. Only approximately 0·5 per cent of the glutamate carbons which had been metabolised by the tissue, appeared as proline and this conversion is therefore not a quantitatively important pathway for the metabolism of glutamate. The finding that glutamate carbons are incorporated into proline in rat costal cartilage is of interest since the overall conversion of glutamate does not seem to have been established *in vitro* with animal tissue although the intermediate reactions have been defined in microorganisms (Vogel, 1955) and nutritional and isotopic carbon studies (Sallach, Koeppe and Rose, 1951) suggest that glutamate is the precursor of proline in the intact rat.

The presence of cortisol did not reduce the extent of the conversion of the labelled glutamate to labelled proline but salicylate caused an approximately 50 per cent inhibition of the transfer of carbon-14 from the glutamate to proline. Strecker (1960) has suggested that the interconversion of glutamate and proline

in animal tissues may involve dehydrogenase enzymes requiring pyridine nucleotides as coenzymes. It has been shown (Bryant, Smith and Hines, 1963) that salicylate inhibits malate and isocitrate dehydrogenase activities by competing with either nicotinamide adenine dinucleotide or its phosphate and the drug may interfere with the biosynthesis of proline from glutamate by a similar mechanism. Trace amounts of radioactive glutamate were detected in the experiments in which the cartilage was incubated with the labelled proline; cortisol had no effect but in the presence of salicylate no radioactive glutamate was found.

TABLE I

EFFECTS OF SALICYLATE AND CORTISOL ON THE *IN VITRO* INCORPORATION OF RADIOACTIVE GLUTAMATE AND PROLINE INTO THE PROTEIN OF RAT COSTAL CARTILAGE

Labelled substrate	Amount of radioactive substrate (expressed as counts per min. × 10^{-3}) incorporated into the protein of 1 g. wet wt. of cartilage		
	Control	Salicylate	Cortisol
[^{14}C]-glutamate	177	89	192
[^{14}C]-proline	240	66	220

The results in Table I show that salicylate, but not cortisol, inhibited the incorporation of both the radioactive glutamate and the radioactive proline into the cartilage protein. Similar findings have been reported with isolated rat diaphragm in which salicylate reduced the incorporation of radioactivity from isotopically labelled amino-acids into the proteins (Manchester and others, 1958) whereas cortisol had no effect under similar experimental conditions (Manchester and Young, 1959).

We wish to thank Mr. D. Haylett for expert technical assistance and the Empire Rheumatism Council for a grant towards the cost of the work.

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June 12, 1963

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