Incorporation of Glucuronolactone-6-C^{14} into Connective Tissue Polysaccharides.* (24619)

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In view of recent findings that D-glucuronolactone serves as a precursor for synthesis of L-gulonic acid(1), L-ascorbic acid(1-5) and L-xylulose(6,7), it was considered of importance to investigate whether D-glucuronolactone could be incorporated into acid polysaccharides (APS) of connective tissues. For this reason large doses of D-glucuronolactone-6-C^{14} were administered over a period of 2½ days to actively growing rats. Experiments were also carried out in which this labeled compound was administered to guinea pigs after subcutaneous injection of carrageenin to stimulate formation of large amounts of connective tissue(8,9). The APS fraction was isolated from samples of connective tissue obtained from these animals and their C^{14} content determined. Results showed no significant incorporation of glucuronolactone into APS fraction of connective tissues.

Materials and methods. Albino rats of the Wistar strain, 25-30 days old, weighing 50-70 g were used. Thirty mg of D-glucuronolactone-6-C^{14} (1 μc/mg) was injected intraperitoneally in divided doses of 5 μc twice a day for 3 days and animals sacrificed 6 hours after last dose. The skins were shaved, removed, freed of fat and muscle, minced, defatted and dehydrated with acetone and ether and then dried. A pooled sample of tail tendon, rib cartilage, trachea and nasal septum was also obtained from these rats and treated the same as the skins. Guinea pigs weighing 400-500 g were injected subcutaneously with 5 ml of a solution containing 50 mg carrageenin. On fifth and sixth days after receiving carrageenin, D-glucuronolactone-6-C^{14} (20 μc) was injected intraperitoneally in doses of 5 μc twice daily for 2 days. Six hours after last dose, animals were sacrificed and newly formed connective tissue was removed from abdominal wall, freed of extraneous material, homogenized in acetone, and a homogeneous powder was obtained after removal of acetone. Two different methods were used for extraction and isolation of APS from connective tissues. (a) *Chemical.* Dried tissues were extracted with 1 N NaOH (15 ml/g) at 70° for 30 minutes, and the resulting solution was neutralized with acetic acid and clarified by centrifugation(10). From the supernatant, APS were precipitated with cetyltrimethylammonium bromide (cetavlon)(11), or benzidine dihydrochloride(12). (b) *Enzymatic.* Weighed samples of dried connective tissues were digested by trypsin, dialyzed and the APS were precipitated with acetone(13). The precipitate was washed with alcohol and ether-dried, and analyzed for radioactivity. The isolated APS were dissolved in water, aliquots were plated and radioactivity determined by a windowless gas-flow counter. The methods for C^{14} assay in tissues reported by Burns *et al.*(14) were used for determination of radioactivity in skin and granuloma tissue.

Results. Results in Table I show per cent incorporation of C^{14} from labeled D-glucuronolactone into the APS fraction of connective tissues. The small amounts of C^{14} found in these tissues could come from fixation of $C^{14}O_2$ formed in metabolism of D-glucuronolactone. Considerable radioactivity was found in the supernatant fraction obtained from cetavlon precipitation and in the dialysate of enzymatic digestion. These findings suggest that D-glucuronolactone was utilized for formation of labeled compounds of small molecular weight. Similar results were obtained in the APS fraction of the carrageenin induced connective tissue of guinea pigs (Table I). In this newly formed, highly metabolizing tissue, about 1% of administered dose of radioactive D-glucuronolactone was present, but no significant incorporation into the APS fraction was found as determined by both enzymatic and chemical methods. Two

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TABLE I. C^{14} in APS Fraction after Administration of Glucuronolactone-6-C^{14}.

Exp.	Tissue	Dry wt tissue, g	Method of isolation	Total C^{14}, cpm	% of dose
1*	Skin	3.54	Cetavlon	5660	.011
			Enzymatic	9200	.02
	Pooled tissue	.28	Cetavlon	5200	.01
2†	Skin	1.3	"	1580	.01
			Enzymatic	2400	.015
	Pooled tissue	.075	Cetavlon	1650	.01
3†	Granuloma	1.5	"	1500	.01
			Enzymatic	3000	.02
4†	"	1.8	"	4000	.025

* Tissues from 2 rats representing 60 μc total dose.

† One animal/exp. receiving 5 μc twice a day for 2 days.

control experiments carried out under the same conditions with glucose-6-C^{14}, showed a .1% incorporation of C^{14} into APS fraction of granuloma tissue and skin.

Discussion. The C-6 labeled glucuronolactone was used in these experiments instead of the uniformly labeled compound, to eliminate non-specific incorporation from carbons 1-5; studies have shown that the C-6 of glucuronolactone forms CO_2 but carbons 1-5 go to glucose(15).

Newly formed connective tissue in the actively growing rat and the carrageenin induced granuloma rich in acid polysaccharides has provided a good method of studying whether D-glucuronolactone is utilized for formation of APS. Although D-glucuronolactone is utilized for formation of L-gulonic and L-ascorbic acids to a greater extent than glucose(1-5), the .01% conversion of this compound, or 1/10 that of glucose, into APS found in our studies appears to be insignificant. These results and reports of other investigators that D-glucuronolactone does not serve as a precursor of menthol and borneol glucuronides(16,17) suggest that there may be a different pathway of formation of D-glucuronic acid for synthesis of glucuronides and tissue APS, than that for L-gulonic acid and L-ascorbic acid.

Summary. D-glucuronolactone-6-C^{14} was administered to actively growing rats to study its incorporation into connective tissue acid polysaccharides. The same compound was administered to guinea pigs after injection of carrageenin which stimulates formation of new connective tissue. No significant incorporation of glucuronolactone into the APS fraction of connective tissues was found.

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