Effect of Hydrocortisone on C^{14}OOH—P-Aminosalicylic Acid Distribution in Cartilage of the Rat.* (23259)

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The current practice of using salicylates to treat numerous rheumatic disorders is widespread and includes the use of hydrocortisone and similar steroids in combination with salicylates. Thus we became interested in studying the distribution of salicylates, specifically p-aminosalicylic (C^{14}OOH-PAS) in the rat and the effect of hydrocortisone (HC) on concentration of C^{14} in the cartilage, following intraperitoneal injection of C^{14}OOH-PAS. Previous workers(1) have reported that PAS is widely distributed in soft tissues of the normal rat following intraperitoneal injection of C^{14}OOH-PAS. Studies on the fate of C^{14} carboxyl labeled PAS have shown that this drug is not significantly decarboxylated when administered interperitoneally(2), indicating that *in vivo* the carboxyl label is quite stable.

Methods. Litter-mate young male rats,‡ normal and hypophysectomized, served as test animals. Normal animals, weighing about 100 g, were given intraperitoneally 5 million counts of C^{14}OOH-PAS§ in saline/100 g body weight. They were etherized at 30, 60 and 240 minute intervals. Tissues were removed, cleaned, weighed and homogenized in ground glass grinder. The tissue homogenate was plated on disks, dried, weighed and radioactivity determined in a windowless gas flow counter (efficiency 49%) to 3 to 5% probable error. These values were then corrected for self absorption. The tibial cap was removed uniformly at the metaphyseal-epiphyseal junction and represented mainly articular cartilage with a little bone. The costal cartilage was easily obtained as pure cartilage. Two animals were used in each group.

Results. The data are presented in Table I. In the hormone work, rats hypophysectomized at about the age of 25 days were used experimentally on tenth day postoperatively. They received intraperitoneally 100 μg of hydrocortisone acetate in saline or equal volumes of isotonic saline, daily for 4 days. One group of rats received both hydrocortisone and growth hormone (GH). On the fourth day, 4 hours after the last dose of hormone solution, these rats received intraperitoneally C^{14}OOH-PAS, 5 million counts/100 g body weight. Then the animals, 2 in each group, were etherized at 15, 30, 60 and 120 minute intervals. Tissues were then prepared as previously outlined. Experiments were repeated twice with results that were correspondingly similar. Average values of 2 animals are presented in Table II.

In the normal rat C^{14} was found in appreciable concentration 30 minutes after injection of C^{14}OOH-PAS, especially in the costal cartilage, tibial cap and heart tissue (Table I). After 4 hours all tissues had insignificant levels, approximately 4 counts/minute/mg (dry weight). The costal cartilage retained

TABLE I. C^{14}OOH-PAS in Tissues of Normal Rats.

Tissue	+ 30 min.	+ 60 min.
	Counts/min./mg wet wt	
Skeletal muscle	18-16.5	15.3-13.6
Heart "	39-38.6	21.9-23
Tibial cap	112-90.7	67.5-84
Costal cartilage	86-86.0	66.0-68
	Counts/min./mg dry wt	
Skeletal muscle	72- 66	61- 68
Heart "	170-168	95-100
Tibial cap	178-144	107-133
Costal cartilage	176-176	134-138

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‡ Obtained from Hormone Assay Laboratory, Chicago, Ill.

§ Obtained from Abbott Laboratories, N. Chicago, Ill.

TABLE II. Effect of Hydrocortisone on C^{14}OOH-PAS in Costal Cartilage.

	Control	H + HC	H + HC + GH
	Counts/min./mg wet wt		
+ 15 min.	157	57	151
+ 30	92	76	72
+ 60	68	50	44
+120	40	12.2	7.4
	Counts/min./mg dry wt		
+ 15 min.	320	117	309
+ 30	188	155	147
+ 60	139	102	89
+120	82	25	15

H = hypophysectomized; HC = hydrocortisone, 100 μg; GH = growth hormone, 100 μg.

higher levels at one hour than did the heart tissue. These values for skeletal muscle are in good agreement with those previously reported(2).

When levels of C^{14} activity are considered on a dry weight basis, costal cartilage, tibia cap and cardiac muscle are almost identical. However, when C^{14} activity is expressed on a wet weight basis there appears to be a localization of C^{14} labeled drug in the cartilage, a finding which is related to the fact that the relative moisture content of these tissues is quite different. It should also be pointed out that none of the tissues used was perfused, so that levels of activity given include radioactivity in blood present in the tissues. However, the cartilage, an avascular tissue, contains no blood, thus drugs probably reach it in an ultrafiltrate from the plasma. It has been shown that a large portion of blood salicylate (50-60%) is bound to plasma proteins(3), and is therefore not readily accessible to the avascular cartilage tissue. The ratio of free salicylate in the cartilage to the free salicylate in the heart is probably much higher than indicated by the figures for C^{14} activity because the heart contains protein-bound salicylate in the blood while cartilage, devoid of blood, presumably contains chiefly unbound salicylate.

Hypophysectomized rats attained high concentrations of C^{14} in the costal cartilage within 15 minutes after receiving C^{14}OOH-PAS (Table II). These levels gradually decreased during the 2 hour observation period following the high initial values. In the hydrocortisone-treated animals, the initially observed C^{14} deposition at 15 minutes was appreciably below that of control animals. At no time in the 2-hour study did the C^{14} contained in the costal cartilage of the steroid-treated rat reach values observed in the control rats. In animals given both hydrocortisone and growth hormone, the C^{14} salicylate content of the costal cartilage at 15 minutes approximated that noted in the control animal. However, none of the subsequent determinations presented such results. Since growth hormone has been noted to counteract many of the inhibitory actions of hydrocortisone it may be possible, by using the proper balance of hormone levels to correct this inhibitory activity of hydrocortisone on C^{14} salicylate concentration in cartilage. These studies are being continued using C^{14}OOH-acetylsalicylic acid and C^{14}OOH-sodium salicylate.

Summary. C^{14} as PAS and/or its metabolites is deposited in appreciable amounts in the heart, costal cartilage and tibial cap of the normal rat following the intraperitoneal injection of C^{14}OOH-PAS. Localization in the avascular cartilage is significant in that this salicylate is mainly protein-free and thus available for cellular metabolism. In hypophysectomized rats hydrocortisone inhibited noticeably the concentration of C^{14}OOH-PAS in the costal cartilage of steroid-treated rats when compared to the untreated control. Growth hormone tends to counteract this inhibitory action of hydrocortisone in the deposition of C^{14} noted 15 minutes, but not at longer intervals, after intraperitoneal injection of C^{14}OOH-PAS. This investigation indicates that salicylate-C^{14} can localize in cartilage, both articular and costal, and that this concentration in the costal cartilage is decreased markedly by hydrocortisone treatment.

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