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THE INFLUENCE OF SODIUM SALICYLATE ON THE METABOLISM OF COLLAGEN

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Abstract—The effect of 300 mg/kg of sodium salicylate on the metabolism of collagen was studied by injecting ^{14}C-proline into rats and determining the specific and total activities of ^{14}C-hydroxyproline in skin collagen fractions and in urine. The administration of sodium salicylate began 28 days before the isotope injection and was continued for a further 28 days. During the first 24 hr after isotope administration specific and total activity of ^{14}C-hydroxyproline in urine was elevated in sodium salicylate treated rats. The total activity of ^{14}C-hydroxyproline in skin and partial activities of acid extractable and insoluble collagen were higher in sodium salicylate treated rats between the first and seventh day after the injection of isotope. Between the 7th and 28th day the total skin activity and partial activity of insoluble collagen was reduced in comparison with control group. These results suggest that sodium salicylate administered in a chronic experiment accelerates the metabolic turnover of collagen.

ALTHOUGH salicylates belong to the common drugs for treating rheumatic diseases, still little is known of the mechanism of their antirheumatic effect. Since pathologic lesions of rheumatic and so called collagen diseases occur in connective tissue, a number of studies has been devoted to studying the influence of salicylates on the metabolism of the connective tissue. It has been found, that salicylates inhibit mucopolysaccharide biosynthesis in heart valve, cornea and cartilage *in vitro* in a concentration of 2 mM and higher (as measured by the incorporation of radioactivity from acetate-^{14}C, glucose-^{14}C and inorganic sulphate-^{35}S into the tissue polysaccharide sulphates)[1–4]. In rats the above drugs also inhibit the incorporation of radioactive inorganic sulphate into the polysaccharides of the costal cartilage *in vivo*.[5] This effect is explained by the capacity of salicylates to uncouple oxidative phosphorylation.[6] Reports on the influence of salicylate on the collagen component of connective tissue are encountered in isolated cases only. *In vitro* inhibition of the incorporation of labelled proline in bone collagen has been observed in the presence of acetylsalicylic acid in the medium (in a concentration of 10^{-3} M).[7] Results of investigations of the influence of salicylates on non-collagenous tissue protein are contradictory. Manchester *et al.*[8] have found that in a concentration of 5 mM salicylate decreases the incorporation of radioactive glycine, lysine and glutamate into proteins of an isolated rat diaphragm. On the other hand, Weiss *et al.*[9] have observed stimulation of the incorporation of labelled leucine into proteins of cell-free preparations of rat liver after exposure to a concentration of 0·6–6 mM. Smith and collaborators prove that low as well as high concentrations of

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salicylates inhibit incorporation of labelled leucine into organ proteins of rats and mice *in vitro* as well as *in vivo*.[10, 11]

This report describes the influence of administering sodium salicylate on the metabolism of collagen in rats in a chronic experiment.

MATERIAL AND METHODS

Forty male albinos rats, of 80 g initial weight, were fed Altromine—commercial diet in pellets. Twenty rats were given daily doses of 300 mg sodium salicylate/kg body wt. The daily dose was divided into two portions so as to keep the salicylate level in the body constant as far as possible. A control group for 20 rats was administered physiological solution in the same manner. On the 28th day of the experiment, 10μc of uniformly labelled ^{14}C-proline/100 g body wt. was administered to the rats i.p. The administration of sodium salicylate continued, the whole experiment continuing for 56 days. Groups of 4 treated and control rats were sacrificed in intervals of 0·5; 1; 3; 7 and 24 days after administration of the isotope. Twenty four hour urine was collected each time from 4 rats of the treated and 4 of the control group on the 1st, 7th and 28th day after administration of the ^{14}C-proline dose.

After sacrificing the rats their skin was shaved, fat was removed mechanically and a part was homogenised. The homogenate was extracted with the 10-fold amount of 0·45 M NaCl (pH 7·4) at 4°, 24 hr, in a rotating agitator. The samples were centrifuged at 25,000 *g* for 1 hr. Extraction was repeated three times. The joined supernatants—*neutral-salt extractable collagen* (*NSC*)—were dialysed against flowing water at 4° for 2 days. The fibriles formed were briefly centrifuged and hydrolysed with 6 N HCl for 20 hr at 105°. The rest of the skin homogenate after extraction with 0·45 M NaCl was then extracted three times for 24 hr with 0·066 M sodium citrate buffer pH 3·7. The combined supernatants—*acid-extractable collagen* (*ASC*)—were treated in the same way as NSC. The rest of the skin homogenate after extraction with sodium citrate buffer—*insoluble collagen* (*ISC*)—was hydrolysed with 6 N HCl for 20 hr at 105°. All hydrolysates were evaporated to dryness and in each sample, the hydroxyproline content was determined according to Stegemann[12] and the sp. act. of hydroxyproline-^{14}C was determined by the method of to Juva and Prockop[13] modified by Nordwig, Kobrle and Pfab.[14] The counting efficiency for the liquid scintillation counter (Packard Tri-Carb Scintillation Spectrometer) employed was 69 per cent. All reported values for ^{14}C-hydroxyproline are based on observed counts per minute, which were at least 10 times the background of the counting system.

The 24-hr urine sample from each rat was hydrolysed after addition of the same volume of concentrated hydrochloric acid for 20 hr at 105°. The hydrolysates were evaporated to dryness and in each sample, the hydroxyproline content was determined according to Prockop and Udenfriend[15] and sp. act. of ^{14}C-hydroxyproline was determined by means of the Juva and Prockop method modified by Nordwig, Kobrle and Pfab.

RESULTS

The administration of sodium-salicylate in this experiment was a long-term process —a total of 56 days. The rate of growth of the treated rats was approximately the same in the course of the experiment as that of the control group, (Table 1).

TABLE 1. INCREASE IN WEIGHT OF CONTROL RATS AND OF RATS TREATED WITH SODIUM SALICYLATE

Days of the experiment	Days after introduction of ^{14}C-proline	Number of the rats	Wt. of rats (g)		Increase in wt. (g)	
			Control	Sodium salicylate	Control	Sodium salicylate
0		25	80	80		
7		25	111	105	31	25
18		25	151	145	71	65
28	0·5	4	175	161	95	81
29	1	4	192	180	112	100
31	3	4	196	188	116	108
35	7	4	200	210	120	130
56	28	4	180	186	100	106

Yields of extractable and insoluble collagen in skin and hydroxyproline in urine control and sodium salicylate treated animals.

The results shown in Table 2 indicate that the administration of sodium salicylate does not alter the collagen content of the individual fractions extracted from the rat skin. The values given are mean values (± M.E.M.) obtained by measuring at the five individual intervals after administration of the isotope.

TABLE 2. YIELDS OF COLLAGEN FROM NEUTRAL SALT EXTRACTABLE, ACID EXTRACTABLE FRACTIONS AND INSOLUBLE COLLAGEN mg/100 mg TOTAL COLLAGEN

	NSC	ASC	ISC
Control*	3·6 ± 1·1	5·8 ± 2·7	80·1 ± 4·9
Sodium salicylate*	4·0 ± 1·5	4·5 ± 1·8	76·5 ± 6·3

*The mean from all rats in the group (20). Results are presented as means together with confidence limits of the 5 per cent level of probability.

Administration of the sodium salicylate, however, has a significant influence on the excretion of hydroxyproline in urine—during the entire experiment, hydroxyproline excretion of the treated group was elevated—Table 5, first column.

The incorporation of ^{14}C-proline into the collagen fractions of the skin

(a) *Specific activities.* Table 3 shows the course of sp. act. s of ^{14}C-hydroxy-proline in the individual collagen fractions after administration of ^{14}C-proline. The sp. act. of NSC in animals treated with sodium salicylate is increased on the first two days after administration of the isotope, in the latter course of the experiment it does not differ any more from the control group. The sp. act. of ASC is lower 12 hr after administration of the isotope than in controls, but it rises rapidly above the control value between the 1st and 7th day. On the 7th day it does not differ from the control value, and on the 28th day after administration of the isotope the value of specific ASC

activity in the treated group is somewhat lower than in the control group. The sp. act. of ^{14}C-hydroxyproline in insoluble collagen is increased between the first and seventh day in sodium salicylate treated rats, it then decreases slowly and on the 28th day it is again a little lower than in the control group.

TABLE 3. SPECIFIC ACTIVITIES OF THE THREE COLLAGEN FRACTIONS cpm/μg HYDROXYPROLINE

Days after introduction ^{14}C-proline	NSC		ASC		ISC	
	Control	Sodium salicylate	Control	Sodium salicylate	Control	Sodium salicylate
0·5	8·89 (8·0–10·5)	13·00 (10·7–16·5)	0·51 (0·30–0·60)	0·22 (0·17–0·24)	0·08 (0·07–0·10)	0·04 (0·01–0·08)
1	6·61 (6·45–6·88)	10·10 (9·45–10·60)	0·51 (0·39–0·69)	1·18 (1·00–1·40)	0·13 (0·10–0·15)	0·25 (0·22–0·28)
3	3·82 (3·00–4·20)	3·27 (3·00–3·70)	1·27 (0·90–1·50)	1·45 (1·10–1·80)	0·23 (0·20–0·26)	0·28 (0·24–0·32)
7	1·65 (1·40–1·70)	1·21 (1·10–1·20)	0·90 (0·80–1·20)	1·00 (0·90–1·05)	0·19 (0·17–0·20)	0·35 (0·34–0·35
28	0·11 (0·09–0·11)	0·05 (0·05–0·06)	0·57 (0·53–0·63)	0·42 (0·39–0·45)	0·23 (0·17–0·26)	0·17 (0·16–0·18)

(b) *Total and partial activities.* Quantitative values for the distribution of the overall amount of activity of ^{14}C-hydroxyproline in the individual collagen fractions of the skin are given in Table 4. The partial activity of the individual fractions shows a part of the total activity found in the fraction given. The total activity is the sum of all three partial activities—NSC, ASC and ISC partial activities. All the values were calculated using 100 mg overall collagen as basis. The values of ^{14}C-hydroxyproline total activity in the skin of rats treated with sodium salicylate is somewhat lower 12 hr after administration of ^{14}C-proline than in the controls, but it rises very rapidly over the control values afterwards. The total activity curve of treated animals continues to be high throughout the experiment, decreasing only toward the end. On the 28th day the total activity is lower than in controls. Partial NSC activity is higher only during the first 24 hr after administration of the isotope, later its course does not differ from the controls. The course of the ASC and ISC partial activity curve in the treated group is similar, 12 hr after administration of the isotope it is lower, rising very rapidly and keeping above the normal values practically throughout the experiment. Only in the last phase of the experiment does the curve decrease below the control value.

Specific and total activities of urine hydroxyproline

As already mentioned, hydroxyproline excretion in the group of sodium salicylate treated rats is always higher than in the control group (Table 5). In the course of the first 24 hr after administration of ^{14}C-proline, specific and total ^{14}C-hydroxyproline activity is also elevated in the treated group. On the 7th and 28th day, however, the activity values do not differ from the control values.

TABLE 4. TOTAL AND PARTIAL ACTIVITIES cpm/100 mg COLLAGEN

Days after introduction ^{14}C-proline	Total activity		NSC		ASC		ISC	
	Control	Sodium salicylate	Control	Sodium salicylate	Control	Sodium salicylate	Control	Sodium salicylate
0·5	7157 (6240–8860)	6650 (5390–7940)	5263 (4570–5800)	5833 (4590–6900)	417 (255–567)	230 (166–326)	1001 (700–1240)	578 (430–705)
1	7060 (5500–8880)	8793 (7900–10100)	4280 (3770–4790)	5175 (4210–5900)	881 (565–905)	1358 (927–1580)	1615 (1095–1710)	2780 (2780–2780)
	5596 (4700–6880)	6546 (5750–7670)	2002 (1805–2330)	2226 (1850–2650)	751 (496–974)	1051 (982–1120)	2780 (2520–3170)	3423 (2920–4040)
7	3126 (2170–3700)	5365 (50000–5730)	554 (447–685)	591 (470–748)	450 (225–604)	735 (705–765)	2250 (2130–2580)	3980 (3740–4220)
28	3247 (2630–3790)	2405 (2320–2480)	61 (49–83)	49 (42–53)	486 (392–565)	266 (241–295)	2543 (2120–2990)	1961 (1835–2160)

DISCUSSION

The results described above give a partial picture about the influence of the chronic administration of sodium salicylate in a dosis of 300 mg/kg body wt. on the metabolism of collagen.

TABLE 5. EFFECT OF SODIUM SALICYLATE ON THE URINARY EXCRETION OF HYDROXYPROLINE ON THE SP. ACT. AND TOTAL ACTIVITY OF ^{14}C-HYDROXYPROLINE AFTER THE ADMINISTRATION OF ^{14}C-PROLINE

Collection period after ^{14}C-proline	Group	Urinary hydroxyproline		
		μg During collection	Sp. act. (cpm/μg)	Total activity (cpm)
0–24 hr	Control	364 (342–402)	3·56 (3·34–3·70)	1297 (1142–1447)
	Sodium salicylate	559 (448–655)	4·48 (3·55–5·65)	2218·9 (1590–2776)
6–7 day (24 hr)	Control	297 (238–388)	0·86 (0·47–1·38)	249·6 (143–306)
	Sodium salicylate	388 (346–464)	0·46 (0·36–0·59)	176·1 (124–209)
27–28 day (24 hr)	Control	243 (218–278)	0·50 (0·33–0·57)	120·6 (93–140)
	Sodium salicylate	323 (312–332)	0·31 (0·18–0·53)	99·8 (59–165)

One of the important effects of sodium salicylate is the enhancement of the total activity of ^{14}C-hydroxyproline in the skin. However, the activity of NSC in the course of the first 24 hr after isotope administration was not increased too significantly. From an overall view on the further fate of labelled molecules in the organism it follows that there are several reasons explaining why the enhancement of the total activity of ^{14}C-hydroxyproline in skin of sodium salicylate treated rats there is a relatively small increase of NSC activity. The first of these reasons is to be found in the higher values of partial ASC and ISC activities between the 1st and 7th day after the administration of the isotope, indicating a higher transformation rate of the NSC fraction into the ASC and especially ISC. The administration of sodium salicylate causes a higher maturation rate of collagen. The second reason are the findings in the urine, where an elevated specific and total activity of ^{14}C-hydroxyproline was found during the first 24 hr. This could be explained by increased degradation of newly synthetised forms of collagen, i.e. the fraction NSC.[16, 17] During the further course of the experiment the activity in the urine does not differ from the control values any more. However, the quantitative excretion of hydroxyproline during the whole experiment is higher in sodium salicylate treated rats. It is clear that this is a consequence of the enhanced degradation of newly synthetised collagen. The increased transformation rate of NSC to the less soluble and insoluble fractions as well as the higher NSC degradation which can be proved in the urine, is the reason why no

quantitative alterations were found considering the amount of collagen in the NSC fraction as compared with the control group.

From the course of the curve of total skin activity and partial activity of insoluble collagen between the 7th and 28th day after the isotope administration it follows that in the sodium salicylate treated group an appreciable reduction of activity can be established while in the controls the activity values are almost constant. Sodium salicylate thus causes a higher degradation rate of mature collagen, too. The collagen half-time in salicylate treated rats is clearly shorter. Therefore no alterations in the quantity of collagen present in both fractions ASC as well as ISC could be found in these experimental conditions. We assume therefore that the influence of sodium salicylate on collagen metabolism is manifested by an overall acceleration of the metabolic collagen turnover.

One of the difficulties in comparing the metabolic effects of salicylates in various experiments is the difference of doses used by individual authors and the duration of administration of the drugs. Morever the majority of studies of the metabolic effect of salicylates has been carried out *in vitro*. The dose of 300 mg/kg of sodium salicylate used in the present study corresponds approximately to a plasma level of 3–4 mM and a dose of 21 g/70 kg body wt. in humans. This dose is higher than the maximum dose applied, e.g. in the treatment of rheumatic fever. In relation to the experiments performed with other types of antirheumatics in animals it is, however, not too high considering that for the proof of antiinflammatory activity of antirheumatic much higher doses are used than those administered in clinical practice.

In a study of the biosynthesis of non-collagenous proteins *in vitro*, salicylates inhibited the incorporation of aminoacids in a concentration of 5–10 mM. Dawkins, Gould and Smith[10] have described the inhibition of incorporation of ^{14}C-leucine in a wide salicylate concentration range of 0·1–20 mM. Weiss, Campbell, Deibler and Sokoloff[9] believe, however that salicylates in cell-free preparations of rat liver, in a concentration of up to 5 mM, stimulate, in higher concentrations they suppress aminoacid incorporation. The importance of the dependence on the administered dose of salicylates also follows from Wilhelmi's experiments,[18] who found that regeneration of *Amblyostoma mexicanum* was reduced by higher doses of salicylates, while lower doses supported regeneration. Similar observations have been made by the same author in a study of the influence of salicylates on healing of skin defects in rats.

A significant metabolic effect of salicylates is the increased oxygen uptake in man, experimental animals and tissue preparations.[19] This effect is distinct in concentrations of 5 mM and probably depends on uncoupling of oxydative phosphorylation. In higher concentrations (which inhibit the system of dehydrogenases bound to NAD and NADP directly *in vitro*) oxygen uptake is suppressed. Increased oxygen uptake is accompanied by a roughly 20 per cent increase of saturation of venal blood. This property of salicylates may be a significant factor in the metabolism of collagen. In experiments *in vitro* on tissue slices of chicken embryos, Chvapil, Hurych and Ehrlichová found that an increased oxygen content of the medium causes a more rapid synthesis of collagen hydroxyproline and also more rapid aggregation of collagen molecules.[20]

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