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Papain Induced Chemical Changes in Adult Rabbit Cartilage Matrix.* (29726)

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Intravenous administration of papain to baby rabbits has been reported by Thomas (1) to produce marked softening of the ear cartilage which results in the collapse and drooping of the ears. These gross changes are accompanied microscopically by a loss of basophilia and metachromasia(2) of the cartilage matrix and chemically by a decrease in the chondromucoprotein (CMP) content of the matrix(3).

Previous reports(1,2) have indicated that animals weighing more than 1,200 g do not respond to this action of papain. Spicer and Bryant(2) noticed an occasional accumulation of metachromatic material in lymphatics of the ears following near lethal doses of papain. In a personal communication Thomas indicated findings similar to those of Spicer and Bryant.

Recent work from this laboratory has demonstrated that in spite of minimal or absent gross and histological changes, larger rabbits referred to as "adolescent," showed marked chemical changes in the matrix following papain(4). These animals weighed 3.1 $\pm$ 0.3 kg and demonstrated partially open epiphyseal plates by X-ray examination; hence, the term "adolescent."

This report deals with the action of papain on the cartilage of fully mature or "adult" rabbits. Maturity was determined on the basis of completely closed epiphyseal plates as demonstrated by X-ray of the upper end of the tibia. All animals in this group weighed between 3.6 and 4.1 kg.

Materials and methods. A total of 20 male and 22 female New Zealand white rabbits were used in these experiments. Of this group, 7 males and 9 females were used as controls; 13 males and 13 females were injected with papain. These animals were given by the ear vein an injection of 35 mg of papain per kg in NaCl solution. The papain was purified‡ but not crystalline. Controls received an equivalent amount of 0.15 M NaCl. The papain solution was prepared as follows: 2 g of powdered papain were mixed with 100 ml of 0.15 M NaCl. The mixture was placed on a magnetic stirrer and stirred for 30 minutes. The resulting turbid, yellowish solution was transferred to presterilized Boerner centrifugal filters, equipped with bacteriological filter pads, and centrifuged for 20 minutes at 800 $\times$ *g*. The filtrate was a clear, straw-colored solution. It was transferred under aseptic conditions into presterilized bottles and stop-

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‡ Papain was purified by the Sterling-Winthrop Research Laboratories, Rensselaer, N. Y. It was kindly provided free of charge.

TABLE I. Changes in Various Chemical Components of the Cartilage Matrix in Adult Female Rabbits Following Papain Injection Along with Their Statistical Evaluation.

Mature females	Control rabbits	After papain	% Change	$P_{(t)}$
No. of rabbits reported	9	13		
CMP (mg/g in wet cartilage)	40.21	33.55	17	$<.10$
Hexosamine (% in CMP)	7.46	6.76	9	$>.10$
Nitrogen (% in CMP)	8.69	9.45	9	$>.10$
Nitrogen/hexosamine (molar ratio in CMP)	15.29	18.27	19	$>.10$

pered with needle puncture rubber stopper. This papain solution was used within 6 hours after preparation to minimize possible oxidation of the enzyme.

Papain injected rabbits were sacrificed at various time intervals after papain by air embolism. Controls were sacrificed at similar times during the experiment.

The ears were removed promptly after death; they were stripped of skin and perichondrium as rapidly as possible. The cartilage was minced and the chondromucoprotein was extracted by the method of Malawista and Schubert(5). The hexosamine content of the extracted chondromucoprotein was determined by a modification of the Schloss(6) method. The nitrogen content of the extracted chondromucoprotein was determined by a micro-Kjeldahl method. A small segment of each ear was preserved in 10% aqueous formalin for histologic examination.

Results. The animals injected with papain demonstrated occasional curling and drooping of the tips of the ears. The upper ⅓ of the ear became soft and could not support its own weight, and curled over. This was much less dramatic than the gross changes seen in baby rabbits after injection of papain. Microscopically, sections stained with hematoxylin and eosin and with stains special for demonstrating changes in the matrix and connective tissue (toluidine blue and Masson's trichrome) failed to show any difference between controls and papain injected animals.

Pilot experiments, in which adult animals were sacrificed after papain at 24-hour intervals and up to 96 hours, indicated that the maximal depletion of the CMP from the cartilage matrix occurred 72 hours after papain injection. The results reported here, therefore, are from a group of animals killed at the time of maximal effect of papain on the cartilage matrix; *i.e.*, 72 hours after injection.

From the same experiments it was found that males and females responded differently to injection of papain. Consequently, the data presented here are divided into 2 subgroups according to sex of the animals. In this way, statistical evaluation of the data becomes more accurate.

The first two Tables show the average changes that occurred 72 hours after administration of papain in female and male animals.

Table I shows the average changes produced by injection of papain in the cartilage matrix of adult female rabbits. The amount of extractable CMP decreased by 17% but the statistical significance of these values is only borderline ($P_{(t)}<0.10$).

The hexosamine and nitrogen content of the extracted CMP changed but the values were below statistically significant levels ($P_{(t)}>0.10$). The same was true for the nitrogen to hexosamine molar ratio in CMP ($P_{(t)}>0.10$).

Table II shows the average results obtained from male animals receiving papain. Here the extractable CMP showed a surprising rise (28%) which was found to be statistically significant ($P_{(t)}<0.005$). The hexosamine and nitrogen content of the extracted CMP from these animals, as well as the nitrogen to hexosamine molar ratio, were found changed but not to any significant extent ($P_{(t)}>0.10$).

Since the changes in the chemistry of the cartilage in male animals were in a directly opposite direction from those in females (Tables I and II), and since in neither group were the average changes very great, it was decided to compare the changes between

TABLE II. Changes in Various Chemical Components of Cartilage Matrix in Adult Male Rabbits Following Papain Injection Along with Their Statistical Evaluation.

Mature males	Control animals	After papain	% Change	$P_{(t)}$
No. of rabbits reported	7	13		
CMP (mg/g in wet cartilage)	36.27	46.56	28	<.005
Hexosamine (% in CMP)	7.20	8.63	19	>.10
Nitrogen (% in CMP)	9.27	8.80	5	>.10
Nitrogen/hexosamine (molar ratio in CMP)	17.47	13.19	24	>.10

males and females and evaluate them statistically.

Table III shows the comparison between the average values for males and for females for the different chemical fractions in the control group and in those animals sacrificed at 48 hours and 72 hours after papain. The Wilcox Rank Sum Test was used for statistical evaluation of the data.

This Table shows that there is no statistically significant difference between male and female controls in any of the fractions studied. There is likewise no significant difference between males and females in any of the fractions studied 48 hours after administration of papain. However, 72 hours after papain there is a highly significant difference between the average amount of CMP extracted from the ears of females and males ($P_{(t)}<0.01$), between the hexosamine content of the extracted CMP from females and males ($P_{(t)}<0.01$), and consequently between the nitrogen to hexosamine molar ratio in the extracted CMP ($P_{(t)}<0.01$).

TABLE III. Comparison of Values Obtained from Various Chemical Fractions of Adult Male and Female Rabbits Receiving Papain Along with Their Statistical Evaluation.

	Hr after papain	Male	Female	$P_{(t)}$
CMP	Control	36.27	40.21	N.S.*
	48	34.68	34.51	N.S.
	72	46.56	33.55	<.01
Hexosamine	Control	7.20	7.46	N.S.
	48	7.16	6.93	N.S.
	72	8.63	6.76	<.01
Nitrogen	Control	9.27	8.69	N.S.
	48	8.82	8.98	N.S.
	72	8.80	9.45	N.S.
Nitrogen	Control	17.47	15.29	N.S.
	48	16.18	17.23	N.S.
Hexosamine	72	13.19	18.27	<.01

* Indicates statistically nonsignificant difference with $P_{(t)}>.10$.

Discussion. Intravenously administered purified papain appears to result in chemical changes in the ear cartilage of fully mature female rabbits similar to those previously reported in the cartilage of baby rabbits(3), and large but not fully mature "adolescent" rabbits(4). A comparison of the percentage changes in the various fractions with those previously reported in the younger animals shows that there appears to be a decreasing percentage change with increasing age. The percentage change in the extractable CMP in the babies was 42% compared to a 28% change in the adolescent group and to a 17% change in the present series of animals(3,4).

The percentage change in the nitrogen to hexosamine molar ratio in the baby rabbits was 55%, compared to 32% in the adolescent group, compared to 19% in the present group.

This seems to indicate a decreasing action of papain on the chondromucoprotein of the cartilage as the cartilage matures.

There appears to be a difference in response of the cartilage to intravenous papain between the sexes in the fully mature rabbits. The changes in the female were in the same direction as those in the younger groups, while the changes in the male in both the amount of CMP extracted and in the alteration of the chemistry of the chondromucoprotein molecule were in a directly opposite direction.

Another interesting observation was concerned with the time of maximal change among the 3 age groups. The baby rabbits showed maximal response to papain 24 hours after injection(3). In the "adolescent" group these changes occurred 48 hours after injection(4) and in the fully mature rabbits the

changes did not reach their maximum until 72 hours after injection of papain.

The delay in response to injection of papain could be explained on the basis of the recently published data by Stockwell and Barnett(7), who showed that aging reduces the permeability of cartilage. On the basis of these data, it could be concluded that papain diffuses with diminishing ease through the cartilage matrix as the animals age and, therefore, produces not only progressively smaller chemical changes in the matrix, but also requires a longer time to achieve these changes.

The difference reported here between adult male and female rabbits to injection of papain cannot now be explained.

Summary. Following intravenous administration of papain to fully mature female rabbits, there is a decrease of borderline significance in the amount of extractable chondromucoprotein (CMP) in the cartilage matrix of the ear. In the adult male animals there is a statistically significant increase in the extractable CMP. Highly significant differences were also found in the amount of CMP between male and female animals 72 hours after injection of papain. The overall effectiveness of papain in causing chemical changes in cartilage matrix appears to decrease as the animals mature. Similarly, the maximal response to papain, which in baby rabbits occurs 24 hours after the injection and in adolescent animals at 48 hours, is delayed further until 72 hours post papain in the adult. The reason for this delay is believed to be decreased permeability of the cartilage matrix with aging.

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Effects of Prolonged Residence in the Antarctica upon Some Organic Constituents of Human Parotid Saliva. (29727)

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The chemistry of human whole mixed saliva(1-8) as well as parotid saliva(6,9-11) has been investigated in both oral(1,2,4,5,11) and systemic disease(3,6-11) as an index of change in physiological status.

As part of the International Geophysical Year, specimens of parotid saliva were obtained from a group of naval personnel(12) before and during their assignment to duty at McMurdo Sound, Antarctica. It was presumed that, in addition to a change in climate, prolonged residence under adverse living conditions including isolation, extended night, and extreme cold, might cause physiological changes reflected in saliva chemistry. As far as is known, similar data cannot be found in the existing literature. Additional data from this same population for blood, urine, and saliva will be reported by others (13).

Methods. Under paraffin stimulation, parotid saliva was collected from 29 enlisted men and officers at 5 different periods and analyzed for rate of flow, protein, tyrosine, tryptophan, and uric acid as previously described(11,14,15). One collection was obtained prior to departure for duty in Antarctica (July-August). Four collections were obtained in the Antarctica, *i.e.*, in the fall (February-March), winter (April-May), deep winter (June) and spring (September). The samples were quick-frozen, and shipped to