Technical Papers

Mast Cells in Human Atherosclerosis

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The observation that in some mammals there is an inverse correlation between tissue mast cell content and susceptibility to alimentary lipemia, or atherosclerosis (*1*), prompted a quantitative study of mast cells in human hearts (*2*).

Table 1. Heart mast cell counts of mixed male-female groups.

No. of individuals per group	Group	No. of mast cells/cm² myocardium ± ε	p
46	I. Young adults	297 ± 18.2	I vs. II: < 0.001
48	II. Senile atherosclerotic	198 ± 20.8	I vs. III: 0.5 – 0.4
48	III. Senile nonatherosclerotic	273 ± 23.9	II vs. III: 0.02 – 0.01

Table 2. Heart mast cell counts of male and female groups.

No. of individuals per group	Group	No. of mast cells/cm² myocardium ± ε	p
23	I. Female young adults	351 ± 30.1	I vs. II: 0.02 – 0.01
23	II. Male young adults	239 ± 33.1	I vs. III: < 0.001
24	III. Female senile atherosclerotic	193 ± 28.5	I vs. V: 0.2 – 0.1
24	IV. Male senile atherosclerotic	201 ± 30.7	
24	V. Female senile nonatherosclerotic	276 ± 33.5	
24	VI. Male senile nonatherosclerotic	270 ± 34.9	

Mast cells were counted in toluidine blue-stained 7 μ paraffin sections from the formalin-fixed myocardium of 46 young adults (20 to 35 yr old) without any evidence of atherosclerosis, 48 senile adults (60 to 90 yr old) with marked generalized atherosclerosis, and 48 senile adults (60 to 90 yr old) without gross evidence of atherosclerosis. The three groups were evenly divided in males and females.

The counts were made without knowledge of the identity of the samples. Two hundred high-power fields, covering an actual myocardium area of 8 mm², were counted in every sample. It should be noted that the occasional infarcts or scars were excluded from the counting and that mast cells were counted only in pure cardiac muscle tissue. The results, expressed as average number of mast cells (± standard error ε) per square centimeter myocardium, are presented in Tables 1 and 2.

Table 1 shows that (i) atherosclerotic seniles had a lower count than nonatherosclerotic young adults ($p = < 0.001$); (ii) there was no significant difference between the counts of nonatherosclerotic seniles and nonatherosclerotic young adults ($p = 0.5 - 0.4$); (iii) senile atherosclerotics had a lower count than senile nonatherosclerotics ($p = 0.02 - 0.01$).

Table 2 reveals that (i) among the nonatherosclerotic young adults, the males had a lower count than the females ($p = 0.02 - 0.01$); (ii) among the females, atherosclerotic seniles had a lower count than nonatherosclerotic young adults ($p = < 0.001$); (iii) among the females, there was no significant difference between the counts of nonatherosclerotic seniles and nonatherosclerotic young adults ($p = 0.2 - 0.1$); (iv) although the male counts followed the female pattern, there were no significant differences between them.

The high statistical significance of the difference between the counts of nonatherosclerotic young adults and atherosclerotic seniles (mixed or female) is remarkable if it is taken into consideration that the groups under comparison were very heterogeneous. These groups consisted of individuals who died from a great variety of diseases of diverse duration and severity (acute and chronic infections, tumors, endocrine-metabolic disorders, poisons, and so forth). The only common features of each group were the presence or absence of atherosclerosis, the sex, and, within very broad limits, the age. Since there are indications that tissue mast cell concentrations can be influenced by adrenal and other hormones (*3–5*), it is possible that variable conditions of endocrine imbalance prevailing in each individual accounted for part

of the count variation within each group. More homogeneous groups could thus be expected to present smaller standard errors and greater intergroup differences.

The interpretation of these findings will have to await further morphological and biochemical studies.

References and Notes

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2. This study was supported by the National Research Council of Canada. The cooperation of the pathology department of the University of British Columbia in providing human tissues is gratefully acknowledged.
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Sulfide Inhibition of Catalase

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Current investigations on the mechanism of inhibition of catalase by anions (*1*) have revealed several phenomena peculiar to sodium sulfide inhibition of catalase. In view of the recent report by Boeri and Bonnischen (*2*) of the oxidation of thiol groups by catalase, we are presenting these preliminary findings in advance of the general work on anion inhibition (*3*). Sulfide exhibits two distinct kinds of inhibition. The first, typical of monovalent anions such as acetate and chloride, appears to occur only in the presence of the substrate; the second occurs in its absence and is actually reversed by the substrate.

In these studies, the rate of destruction of hydrogen peroxide (Merck), initial concentration approximately $10^{-2}M$, by crystallized beef liver catalase (Worthington) was followed spectrophotometrically by the method previously described (*4*). The reaction mixture was maintained at *p*H 7.0 with 0.01*M* phosphate buffer at 25°C. The concentration of the uninhibited enzyme at any time t was determined graphically from the tangents of the semi-log slope of the optical density of H_2O_2 versus time curve (*5*).

If sodium sulfide is added simultaneously with the substrate to the catalase solution, the initial inhibition is slight or absent, but during catalysis the enzyme is rapidly inhibited in a manner identical to that found with acetate and chloride (Fig. 1, upper curve). In contrast, if sodium sulfide is added to the catalase solution before the substrate, the initial inhibition is appreciable, and its magnitude increases slowly with the incubation time (5 to 15 min) allowed before the substrate is added. This inhibition appears to occur at a much slower rate than that observed when peroxide is present and is proportional to the hydrogen ion

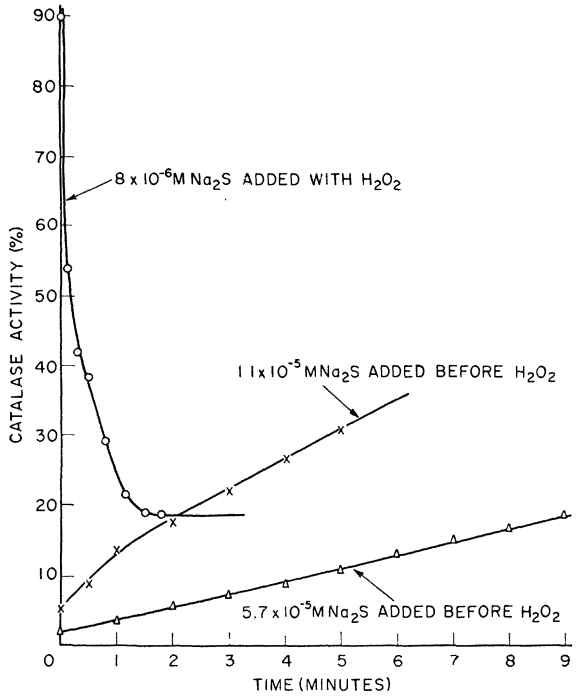

Fig. 1. Change in activity of catalase during the catalysis of hydrogen peroxide (initial concentration $10^{-2}M$). (Upper curve) Sulfide and peroxide added simultaneously to the catalase solution. (Lower curves) Sulfide and catalase incubated together for 10 min before the addition of peroxide.

and sulfide concentrations. However, this inhibition is partially reversed during the catalysis of hydrogen peroxide (Fig. 1, middle and lower curves). It is also reversed to a small degree by bubbling nitrogen and to a greater degree by bubbling oxygen through the catalase and sodium sulfide solution. This represents a true reactivation of the enzyme and not a destruction of the substrate through a stoichiometric reaction with the sulfide, because (i) the initial rate, when the sulfide concentration is the highest, is the slowest rate, and (ii) the concentration of the hydrogen peroxide is approximately 10^4 times greater than that of the sulfide (*6*). By choosing the proper concentration of sulfide and time of contact with the enzyme before adding the substrate, it is possible to obtain inhibition curves intermediate between the extremes shown in the figure.

During the course of these inhibition studies, we have observed that the oxidation of sodium sulfide to colloidal sulfur (*p*H 7.0) is accelerated by catalase in the presence or absence of peroxide. This oxidation is manifest by the appearance at the surface of the reaction mixture of a cloudy area of colloidal sulfur, which rapidly extends downward as oxygen mixes with the system. Since, in all probability, the enzyme is refractory to hydrogen peroxide under these conditions, it is very probable that the mechanism for oxidation of sulfide is a nonspecific phenomenon asso-

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