Antipyretic Action and Catalase Activity

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Antipyretics are well known as stabilizers of commercial hydrogen peroxide solutions (Kausch, 1938; Slater, 1945), and it has been suggested by Bailey (1937) that 'antipyretic substances are in general aromatic, many of them phenolic, and quite possibly lower the temperature of the body by reducing the rate of oxidation', conceivably by interfering with H_2O_2 metabolism. In tissue metabolism, H_2O_2 may be produced by reactions involving, for example, D-amino-acid oxidase, xanthine oxidase, uricase (Sumner & Somers, 1943), and the chemical identification of H_2O_2 as a metabolic product has recently been demonstrated by Tosic & Walton (1946). The H_2O_2 produced is decomposed by the widely distributed cellular enzyme catalase, but simple protective decomposition of H_2O_2 into O_2 and water is now considered by Keilin & Hartree (1945) to be less likely than catalysis of coupled oxidation of alcohols, and possibly of other biologically important compounds.

The possibility that antipyretic action may not be wholly central in origin is suggested by the hyperthermic action of dinitro- α -naphthol in a dog deprived of its central thermoregulatory mechanism (Heymans, 1928), and by the antipyretic action of acetanilide, quinine and pyramidone in decerebrate rabbits exhibiting pyrexia induced by β -tetrahydro-naphthylamine (Skowronski, 1929). The antipyretic substances, sodium salicylate, quinine and pyramidone, have a depressant action on isolated-tissue respiration (Nitzescu & Cosima, 1923), and the reduction of methylene blue by liver tissue is inhibited by antipyretics such as aspirin, antipyrine, phenacetin, quinine and sodium salicylate, as well as by a number of typical antioxidants (Boutaric & Jacquinet, 1931).

Antipyretic activity is broadly associated with particular chemical structures, and several representative antipyretic substances were tested for inhibitory activity in the isolated catalase- H_2O_2 system, in an attempt to provide evidence for the hypothetical mechanism suggested above for possible peripheral action of antipyretics. Of the four main types of antipyretic substances, acetanilide and phenacetin represent typical aniline derivatives, salicylic acid and acetylsalicylic acid represent typical phenol derivatives, antipyrine is a representative pyrazolone derivative, and quinine repre-

sents a number of quinoline derivatives. *p*-Hydroxybenzoic acid is a phenol derivative not ordinarily used as an antipyretic.

EXPERIMENTAL

A horse liver catalase extract, prepared by the method of Sumner, Dounce & Frampton (1941), had a 'Katalase *f*' value (determined by the method of von Euler & Josephson, 1927) of 2560. The catalase content was calculated by colorimetric estimation of the Fe content, by the method of Delory (1943), assuming 0.062% Fe in the dry enzyme (von Euler, Zeile & Hellström, 1930).

The test substances used were A.R. grade, further purified as follows: acetanilide, phenacetin, aspirin, salicylic acid and *p*-hydroxybenzoic acid (recrystallized from conductivity water), quinine (recrystallized from pure benzene), antipyrine (recrystallized from pure toluene). The pH of the Sorensen buffer was checked electrometrically. All solutions were prepared in glass-distilled water, and dust was excluded as far as possible.

Reaction rates were determined at 37° using the Barcroft manometer. The right-hand manometer flask contained 2 ml. of 0.32M- H_2O_2 (M.A.R.), and 2 ml. of 0.0067M-phosphate buffer, pH 6.8; the left-hand flask, 2 ml. of 0.32M- H_2O_2 , 1 ml. of 0.0067M-buffer, 1 ml. of a solution of the test substance, and a Keilin pipette holding 0.16 ml. of undiluted catalase extract. For the calculation of percentage inhibitions, reaction rates were compared with that of the control, in which the amount of O_2 evolved over a period of 5 min. was taken as a measure of initial reaction velocity.

RESULTS

The percentage inhibitions obtained with the substances tested are given in Table 1. The observed inhibitions with the hydroxybenzoic acid derivatives can be due only in negligible degree to the slight lowering of pH, and probably result from the presence of phenolic OH groupings. The latter may interrupt the chain reaction, postulated by Sumner (1941), which involves the formation of the free radicals OOH and OH. A number of workers (e.g. Richter, 1934; Alyea & Pace, 1933; Yakushi, 1937; Collier, 1940) have shown, in fact, that catalase is inhibited by phenolic compounds. Collier has shown that, in general, catalase is inhibited by phenols, oximes and amino derivatives which may give rise to HO.NH groupings on oxidation, and that these compounds produce methaemoglobin from haemoglobin, reduce Folin's phenol reagent,

Table 1. *Inhibition by test substances of the decomposition of 0.16M-H₂O₂ by catalase*

Conc. of antipyretic (M $\times 10^{-3}$)	Percentage inhibitions						
	Acetanilide	Phenacetin	Salicylic acid	Acetyl-salicylic acid	p-Hydroxybenzoic acid	Antipyrine	Quinine
0.4	—	—	—	—	—	—	0
1.1	—	0	44	17	—	0	—
1.8	0	—	—	—	47	—	—
2.3	—	—	76	42	—	—	—
2.6	—	—	—	—	—	0	—
3.5	0	—	—	—	68	—	—
4.5	—	—	89	70	—	—	—
5.3	—	—	—	—	—	0	—
7.0	0	—	—	—	—	—	—
10.5	—	—	—	—	—	0	—
14.0	28	—	—	—	—	—	—

give colour reactions with FeCl₃ and resemble anti-oxidants.

Experiments with concentrations higher than those recorded for phenacetin and quinine were excluded by the low solubility of these compounds. With antipyrine there is obviously no correlation with antipyretic activity, and inhibition with acetanilide was only slight at relatively high concentrations. It is thus not possible to associate antipyretic activity in every case with the *in vitro* decomposition of H₂O₂ by catalase.

SUMMARY

1. Since many antipyretics can be used to stabilize H₂O₂ solutions, it has been suggested that they may also conceivably interfere with H₂O₂ metabolism. To test this theory, a number of representative antipyretics have been tested for inhibitory activity in the isolated enzyme system, catalase-H₂O₂.

2. Inhibition was observed with some, but not all, of the substances tested, i.e. there was no parallelism with antipyretic activity.

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