The failure of vit. B_{12} to influence favorably the course of cyanide poisoning, despite its capability of binding 1 or 2 moles of cyanide in addition to the cyano group already present in its molecule(8) may be due in part to the lability of the resulting complex.

Summary and conclusions. 1. Vit. B_{12a} (hydroxo-cobalamin), but not vitamin B_{12} (cyano-cobalamin), has been found to be capable of preventing in mice the toxic symptoms and death due to cyanide administration. 2. When injected into mice exhibiting complete respiratory arrest and coma due to cyanide poisoning, vit. B_{12a} effected rapid recovery of most animals. 3. In mice injected with potassium cyanide followed by vit. B_{12a}, some of the cyanide appears in the urine as thiocyanate, but a greater percentage of the cyanide appears as vit. B_{12}, having formed this compound by reacting with the vit. B_{12a}.

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Received August 25, 1952. P.S.E.B.M., 1952, v81.

Crystalline Catalase, A Peroxidase. (19832)

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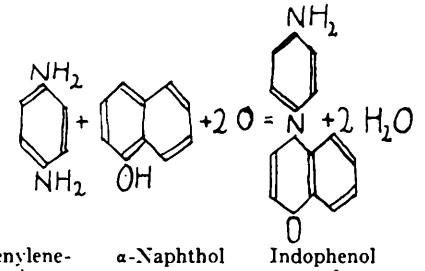
Catalase is generally classified as a non-oxidizing enzyme; its only function is said to be to destroy the toxic hydrogen peroxide. This classification was made because no other substrate has been found for this enzyme. Alkyl peroxides (which are not present in living organisms) are attacked only by concentrated catalase solutions(1). Keilin and Hartree(2) observed that a portion of the hydrogen peroxide, formed when certain oxidases aerobically oxidize their substrates, is utilized by catalase to oxidize ethyl alcohol to acetaldehyde. Oxidation by catalase and hydrogen peroxide was much slower. Theorell (3) and his school believe that catalases are a special group of peroxidases. Their deduction is based on studies concerning the mechanism of action of catalases and of peroxidases. No evidence is available, however, to indicate that catalase can oxidize any large or small molecule of biological importance, or any of the several types of the substances that are oxidized by peroxidases.

The experiments here described demonstrate a series of new functions of catalase. Since the reactions require the presence of hydrogen peroxide, they are peroxidatic in nature.

Experimental. Preparation of crystalline catalase. The crystalline cow liver catalase employed in these experiments was prepared according to the method of Tauber and Petit (4). The crystals which formed during dialysis were recrystallized by dissolving them in the least volume of 0.01 N sodium hydroxide. Without centrifuging, the solution was immediately adjusted to pH 5.8 by the addition of the calculated volume of 0.1 N acetic acid. A small quantity of insoluble matter was removed by centrifuging at room temperature. The clear supernatant was placed in a refrigerator at 4°. In about 3 hours the catalase began to crystallize. The crystals were dissolved in sodium chloride-phosphate buffer at pH 7.3(5). The activity of the crystals, as found by the method of von Euler and Joseph-

son(6). was 34,000 Kat. F. The stock catalase solution was kept in a refrigerator. All reagents and solutions of the compounds used in these experiments were prepared daily. α-Naphthol and p-phenylenediamine were decolorized with activated charcoal. For comparison commercial (Worthington) crystalline catalase was also employed.

A. The Nadi reaction with catalase and hydrogen peroxide. 1 ml of freshly prepared α-naphthol solution (0.05 M in 95% ethyl alcohol). was placed in a test tube. One ml of freshly prepared p-phenylenediamine solution (0.05 M in distilled water), 4 ml of 0.05 phosphate buffer at pH 7.3, and 0.1 ml of 0.3 M solution of hydrogen peroxide were added. After mixing the solution was divided into 2 parts. To one-half 0.1 ml (10 μg) of catalase solution was added. Both tubes were kept for 5 minutes at room temperature. The tube containing the catalase turned purple (indophenol purple). Then 2 ml of 95% ethyl alcohol was added to each tube. After mixing the purple color in the catalase-containing tube became more brilliant. The control had a pink color. The reaction occurs in the following manner:



p-Phenylene-diamine α-Naphthol Indophenol purple

If dimethyl p-phenylenediamine is employed instead of p-phenylenediamine, indophenol blue is formed. A similar oxidation reaction is known to be catalyzed by the cytochrome oxidase-cytochrome c enzyme system. The name of the reaction, "Nadi," was derived from the compounds α-naphthol and p-phenylenediamine.

B. Oxidation of other large molecules by catalase and hydrogen peroxide. To 1 ml of each of p-aminobenzoic acid, sulfathiazole, adrenalin, ephedrine, and tyrosine solution, 1 ml of catechol (0.05 M) in 0.05 M pH 7.0 phosphate buffer, 0.2 ml of a 0.3 M hydrogen peroxide solution and 0.1 ml (2 μg) of catalase solution in phosphate buffer of pH 7.3 were added. The tubes were kept at 37°. After 5 minutes the appearance of colors was noted (Table I). The control tubes without catalase were either slightly colored or colorless. In the tyrosine experiment 10 μg of catalase were employed.

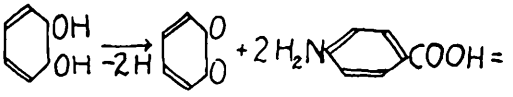
TABLE I. Peroxidation of Large Molecules by Catalase.

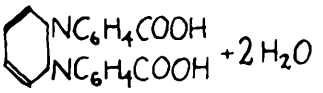
	Color after 5 min. at 37°	Changing gradually to
p-Aminobenzoic acid	Deep carmine	Red brown
Sulfathiazole	Rose red	" "
Adrenalin	Brick "	Deep red brown
Ephedrine sulfate	Pink	Lilac rose
L-Tyrosine	Rose red	Olive black

The compounds were dissolved in 0.05 M phosphate buffer of a final pH of 7.0. except the adrenalin which was in 0.05 M phosphate buffer of a final pH of 7.2, and the tyrosine solution which had a final pH of 8.3; the other substrates were adjusted to a final pH of 7.0 with sodium hydroxide. The concentrations were 0.005 M, except the ephedrine sulfate solution, which was 0.05 M.

Here, part of the oxidations probably occur in the following manner:

Catechol o-Quinone p-Aminobenzoic acid





Similar results were obtained with a commercial (Worthington) crystalline catalase preparation, and with crude horse radish peroxidase and hydrogen peroxide.

C. Peroxidation of phenols and amines by catalase and hydrogen peroxide. All 4 compounds of this group were dissolved in 0.1 M phosphate buffer of pH 7.3 and were of 0.05 molarity except the adrenalin solution which had a molarity of 0.005. Into 1 of each of 4 test tubes, 2 ml portions of each of the 5 compounds and 0.1 ml of 0.3 M hydrogen peroxide solution were added. The contents of each of the 5 tubes was divided into 2 parts.

To 1 part of each 0.1 ml catalase solution (10 micrograms) was added. Catechol gave an intense yellow color, pyrogallol a brown color, adrenalin a pink color, and p-phenylenediamine gave a light-reddish-purple color.

Discussion and summary. Our experiments show that dilute solutions of crystalline catalase, in the presence of hydrogen peroxide, can peroxidize a variety of compounds. α-Naphthol and p-phenylenediamine are oxidized to indophenol purple; p-aminobenzoic acid, sulfathiazole, adrenalin, ephedrine sulphate, and tyrosine, are coupled with catechol by oxidation to form colored compounds. Pyrogallol, catechol, adrenalin and p-phenylenediamine are oxidized by catalase and hydrogen peroxide. Related compounds (m-aminobenzoic acid, sulfadiazine) are also oxidized. A commercial crystalline catalase preparation, as well as crude horse radish peroxidase gave similar results. A catalase solution which had been boiled for 5 minutes did not give the described reactions.

The difference between catalases and peroxidases is: catalases can act as peroxidases but peroxidases can not act as catalases (decompose hydrogen peroxide without the presence of a second substrate). The present experiments are in full harmony with Theorell's theory which states that catalases are a special group of peroxidases.

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Received August 29, 1952. P.S.E.B.M., 1952, v81.

Combination Chemotherapy of Cancer with 8-Azaguanine and a Riboflavin Analog.* (19833)

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Quantitative biochemical differences between normal and tumor tissues have been reported by many investigators(1-5), and reveal, for the most part, lower metabolite concentrations in neoplastic tissue. Metabolite inhibition by an antimetabolite depends upon the concentration ratio of metabolite to antagonist. Thus, the function of a metabolite in low concentration in cancer cells may be blocked by an antagonist while only minimally depressing the metabolic activities of the higher metabolite level in normal cells. The possibility that the utilization of combinations of drugs against experimental neoplasms may result in simultaneous damage to different metabolic pathways, achieving greater tumor damage, has been reported by a number of investigators (6-9). For example, the vit. B_6 antagonist, desoxypyridoxine, was chosen for chemotherapeutic trial because it had been demonstrated that pyridoxine is in lower concentration in many solid tumors than in most normal tissues (1,3,4). Desoxypyridoxine was found to be a weak carcinostat against a mammary adenocarcinoma, and, in combination with a guanine antagonist (8-azaguanine), produced a carcinostatic effect not obtainable with either drug alone(6).

* This work was supported from funds supplied by the Ruth Cutting Auchincloss Memorial Fund, Mr. Robert Chambers (deceased), Mr. Charles Allen, Jr., and Dr. William Grosfeld. We are indebted to Dr. K. Folkers of Merck and Co., Rahway, N. J. for supplies of flavotin; to Dr. J. M. Ruegsegger of the Lederle Laboratories, Pearl River, N. Y., for the 8-azaguanine; and to Dr. E. Severinghaus of Hoffmann-LaRoche, Nutley, N. J., for the riboflavin-5-phosphate.

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