on October 4 by the Chapter of Sigma Xi of the University of Cincinnati at which the following papers were given: "Radioactive Materials, the Geologic Source of Supply," Professor O. C. Von Schlichten, associate professor of geology; "Basic Principles of Atomic Energy," Dr. D. A. Wells, professor of physics; and "Radioactive Isotopes in Medical Research," Dr. G. M. Guest, associate professor of pediatrics.

THE annual meeting of the Society of Rheology will be held on October 26 and 27 at the Hotel Pennsylvania, New York City.

THE president and fellows of Harvard College have voted to establish a committee to develop a program in nuclear physics at the university. The corporation has further voted to allocate the sum of $400,000 to the committee to spend within a period of five years in developing the program.

THE School of Mathematics of the Institute for Advanced Study will allocate a small number of stipends to gifted young mathematicians and mathematical physicists to enable them to study and to do research work at Princeton during the academic year 1946–1947. Candidates must have given evidence of ability in research comparable at least with that expected for the degree of doctor of philosophy. Blanks for application may be obtained from the School of Mathematics, Institute for Advanced Study, Princeton, N. J., and are returnable by February 1, 1946.

THE *Journal* of the American Medical Association reports that the Governor of Wisconsin has signed a bill appropriating $25,000 annually "for study of and research into the causes, prevention and cure of cancer and for the purchase of necessary apparatus and supplies for the purpose of carrying on such study and research."

SPECIAL ARTICLES

THE ACTION OF HORSERADISH-PEROXIDASE ON ANGIOTONIN, PEPSITENSIN AND EPINEPHRINE[1]

FROM results obtained with a representative series of phenols, Elliott[2] concluded that all phenolic substances are oxidized with horseradish-peroxidase in the presence of hydrogen peroxide. Szent-Györgyi[3] showed that epinephrine was affected in a similar manner by this enzyme system. Bach and Chodat[4] found that peroxidase in a weak acetic acid medium catalyzed the oxidation of potassium iodide by hydrogen peroxide, releasing iodine. We wish to present data showing that the pressor peptides, angiotonin (hypertensin) and pepsitensin, as well as epinephrine, are oxidized by the action of hydrogen peroxide with horseradish-peroxidase and that this reaction is enhanced by the addition of a very small amount of potassium iodide.

The loss of the pressor response in a pithed cat was utilized as an index of the oxidative degradation of angiotonin, pepsitensin and epinephrine. In addition to this biological assay, the red color produced by the oxidation of epinephrine was measured photometrically.

The horseradish-peroxidase was prepared by the method of Elliott.[2] Angiotonin and pepsitensin solutions were standardized so that 0.5 cc produced a rise in arterial pressure of 50 to 70 mm Hg in a pithed cat. For the bio-assay 1: 250,000 epinephrine acidified with acetic acid was employed, and for the colorimetric test, 1: 10,000.

Each reaction mixture for bio-assay contained 5 cc of each pressor substance and, in different combinations, 1 cc of hydrogen peroxide solution (0.25 mg per cc), 1 cc of 0.001 N iodine in potassium iodide (KI_3), and 1 cc of peroxidase solution containing varying amounts of the dry horseradish preparation (0.5 to 2.0 mg). The final volume was adjusted to 10 cc with distilled water. The mixtures were incubated at room temperature (25° C) and the reaction was stopped at the desired time by immersion of an aliquot in boiling water for 10 minutes. Then a 1 cc sample was injected into the femoral vein of a pithed cat and the pressor response compared to that of the unmodified angiotonin, pepsitensin or epinephrine solution.

As a substrate for the colorimetric determination, 5 cc of the epinephrine solution was used; to this were added, in various combinations, 1 cc volumes of enzyme preparation (0.1 mg), hydrogen peroxide solution (0.25 mg per cc), and 0.0001 N iodine or potassium iodide. The change in color was measured in a Coleman spectrophotometer at a wave-length of 540 millimicrons.

RESULTS

Fig. 1 indicates the amount of angiotonin destroyed by hydrogen peroxide with peroxidase. In the presence of the same amount of hydrogen peroxide, increasing the quantity of peroxidase caused a greater destruction of angiotonin (compare Curve 2 and Curve 3). The marked, enhancing action of KI_3 is clearly evident (*cf.* Curve 1). The combination of

[1] We wish to express our appreciation to Mr. Robert Sanders for his technical assistance.

[2] K. A. C. Elliott, *Biochem. Jour.*, 26: 1281, 1932.

[3] A. Szent-Györgyi, *Biochem. Jour.*, 22: 1387, 1928.

[4] A. Bach and R. Chodat, *Ber.*, 37: 1342, 1904.

hydrogen peroxide and KI_3 had only a little destructive action on angiotonin (Curve 4); KI_3 alone or with peroxidase in the absence of hydrogen peroxide was inactive.[5] Although not shown in the figure (Fig. 1), oxidative destruction of pepsitensin paralleled

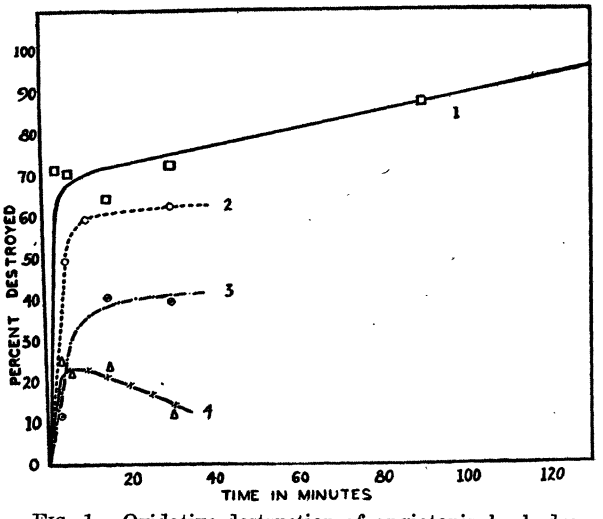

FIG. 1. Oxidative destruction of angiotonin by hydrogen peroxide + peroxidase. Curve 1. Angiotonin + hydrogen peroxide + KI_3 + 0.5 mg peroxidase preparation. Curve 2. Angiotonin + hydrogen peroxide + 1 mg peroxidase preparation. Curve 3. Angiotonin + hydrogen peroxide + 0.5 mg peroxidase preparation. Curve 4. Angiotonin + hydrogen peroxide + KI_3.

that of angiotonin very closely. Epinephrine was also readily oxidized by hydrogen peroxide with peroxidase.

The results of the colorimetric determination of the oxidation of epinephrine (Fig. 2) paralleled the destruction of angiotonin (Fig. 1) in a striking manner. In the colorimetric tests the iodine solution, compared with the potassium iodide solution of equal normality, had very little effect on the reaction. Without the addition of peroxidase neither iodine nor iodide in these concentrations had an appreciable effect even in the presence of hydrogen peroxide (Curves 4 and 5). The increased destruction of angiotonin and epinephrine with potassium iodide is probably due to the liberation of nascent iodine by the action of hydrogen peroxide with peroxidase.

DISCUSSION

The ability of peroxidase-peroxide systems to oxidize phenolic substances *in vitro* is well established, but their action *in vivo* is still open to question.[7, 8]

[5] Cruz Coke[6] showed that iodine inactivated angiotonin (hypertensin), but in the present experiments the small amounts of iodine used had relatively little effect.

[6] Eduardo Cruz Coke, N. Y. Acad. Sci., Section of Biology. Conference on Experimental Hypertension. Feb. 9 and 10, 1945.

[7] K. A. C. Elliott, *Biochem. Jour.*, 26: 10, 1932.

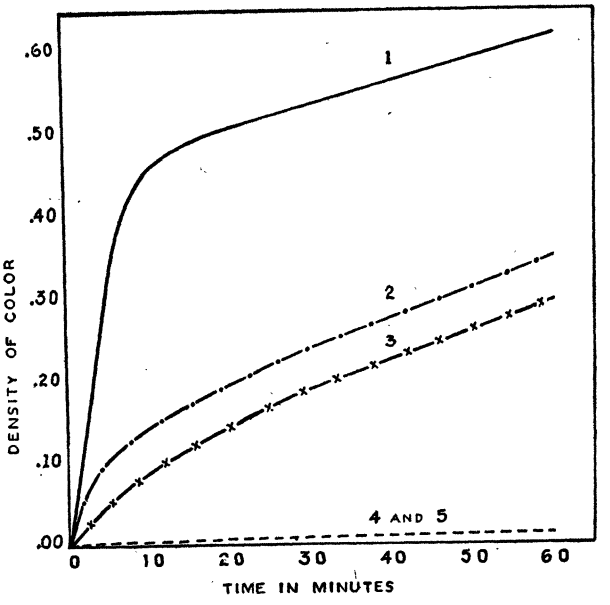

FIG. 2. Red color produced by oxidation of epinephrine. Curve 1. Epinephrine + hydrogen peroxide + potassium iodide + 0.1 mg peroxidase preparation. Curve 2. Epinephrine + hydrogen peroxide + iodine + 0.1 mg peroxidase preparation. Curve 3. Epinephrine + hydrogen peroxide + 0.1 mg peroxidase preparation. Curve 4. Epinephrine + hydrogen peroxide + potassium iodide. Curve 5. Epinephrine + hydrogen peroxide + iodine.

According to Bancroft and Elliott[9] "the enzyme shows its full activity in neutral solution and at concentrations of hydrogen peroxide which are possible within the organism." Dihydroxyphenyl-alanine[2] and tyramine[2] as well as angiotonin and epinephrine are oxidized by horseradish-peroxidase *in vitro*. These substances have all been considered as possible pressor agents in the "humoral" theory of hypertension. The marked effect of low concentrations of iodides in augmenting the action of horseradish-peroxidase suggests that such a combination may influence oxidative destruction of pressor substances *in vivo*. Studies are now in progress to determine the action of such a peroxidase system in hypertensive animals.

SUMMARY

(1) The pressor action of angiotonin, pepsitensin and epinephrine is rapidly destroyed by the horseradish-peroxidase-hydrogen peroxide system.

(2) This reaction is considerably enhanced by the addition of small amounts of potassium iodide.

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[8] J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," p. 179. New York: N. Y. Academic Press. 1943.

[9] G. Bancroft and K. A. C. Elliott, *Biochem. Jour.*, 28: 1911, 1934.