

and one half minutes after watering, and made the periodicity much more pronounced.

The plant never ceased growing. The periodicity was evident before and after the changes in the environmental conditions. The average value of the growth-rate was calculated for each hour of the three cycles. From the values obtained, the general average rate of growth was obtained and found to be 1.092 mm. per hour. Employing this value the calculated final height was 6.8 cm. By actual measurement it was found to be 6.4 cm. The evidence justifies the conclusion that the method is accurate and practical.

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#### Flavin-Adenine-Dinucleotide in Tissues of Rats on Diet Deficient in Flavin

Ochoa and Peters<sup>1</sup> found cocarboxylase decreased in tissues of pigeons fed on a diet deficient in vitamin B<sub>1</sub>. Axelrod and Elvehjem<sup>2</sup> reported a diminution of cozymase in tissues of animals on a nicotinic acid free diet. We now find a similar diminution in the flavin-adenine-dinucleotide content of boiled extracts of tissues from rats fed on a diet deficient in flavin. The rats (average weight 35 gm.) were fed a synthetic diet of rice starch, casein (alcohol extracted), salt mixture, supplemented by cod liver oil, crystalline vitamin B<sub>1</sub> hydrochloride, a 50 per cent alcohol yeast concentrate<sup>3</sup>, and a liver filtrate from a previous fuller's earth and franconite adsorption. These supplements were flavin-free.

Mean flavin-adenine-dinucleotide content of rat tissues (expressed in  $\mu$  gm./gm. fresh tissue).

Group	Diet	No. of animals	Mean weight increase (gm. $\pm 2 \times$ S.E. mean)	Tissue	Mean nucleotide ( $\pm 2 \times$ S.E. mean)
A	Flavin deficient	6	14.9 $\pm$ 4.0	Brain Heart Kidney Liver	10.5 $\pm$ 0.6 38.0 $\pm$ 5.0 72.4 $\pm$ 11.0 40.7 $\pm$ 10.0
B	Flavin deficient plus 50 $\mu$ gm. flavin per day	6	79.8 $\pm$ 14.0	Brain Heart Kidney Liver	13.0 $\pm$ 2.2 93.2 $\pm$ 17.9 78.6 $\pm$ 12.5 87.3 $\pm$ 22.2

The nucleotide was determined after the method of Warburg and Christian<sup>4</sup>, and solutions standardized from a pure specimen kindly sent by Prof. O. Warburg. Group A, fed the above diet for 5 weeks, increased in weight but slightly (see table), while Group B, receiving in addition 50  $\mu$  gm. flavin per day, had a normal growth rate. Some rats in Group A showed a dermatitis, especially around the eyes, and others (results not recorded in table) died before the conclusion of the experiment. It is seen that the mean flavin-adenine-dinucleotide values of Group A are all lower than those of Group B. This is especially so in the case of heart and liver tissue. Application of Fisher's *t* test to the difference of means of the heart and liver figures gives *t* = 6.1 and 3.81 respectively. For *P* = 0.01, *t* = 3.17. These figures are thus clearly significant, and afford direct evidence of a further instance of a deficiency in the vitamin B complex affecting biological oxidation systems.

These results will be reported in detail elsewhere. We are indebted to the Nuffield Trustees and the Hildebrand Harmsworth Trustees (Merton College) for financial support.

Note added in proof. In a letter which has just appeared (NATURE, 144, 670; 1939) Axelrod, Sober and Elvehjem report a decrease of *d*-amino-acid oxidase in kidney and liver of rats fed on a flavin-deficient diet, suggesting a deficiency of its coenzyme. Our own independent results furnish the direct proof of this.

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<sup>1</sup> Ochoa and Peters, *Biochem. J.*, **32**, 1501 (1938).

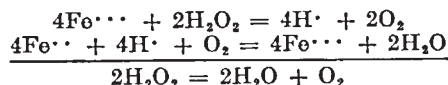
<sup>2</sup> Axelrod and Elvehjem, *NATURE*, **143**, 231 (1939).

<sup>3</sup> Kinnersley, O'Brien, Peters and Reader, *Biochem. J.*, **27**, 225 (1933).

<sup>4</sup> Warburg and Christian, *Biochem. Z.*, **293**, 150 (1938).

#### Mechanism of Decomposition of Hydrogen Peroxide by Catalase

WE have recently suggested<sup>1</sup> that the decomposition of hydrogen peroxide by catalase is brought about by the successive reduction of the catalase iron by the peroxide and its re-oxidation by molecular oxygen; the reaction proceeding according to the following equations:



The evidence supporting this suggestion can be summarized as follows:

(1) It can be shown spectroscopically that the trivalent iron of azide- or hydroxylamino-catalase is rapidly reduced by hydrogen peroxide, although a powerful reducing agent such as sodium hyposulphite reduces neither the free catalase nor its azide or hydroxylamine derivatives.

(2) The reduced azido- or hydroxylamino-catalase is readily re-oxidized by molecular oxygen but not by hydrogen peroxide or potassium ferricyanide<sup>2</sup>.

(3) It has been demonstrated manometrically that the complete removal of oxygen inhibits the catalytic activity of the enzyme by at least 88 per cent in acid phosphate solution and only by 40-70 per cent in a more alkaline phosphate buffer solution. The remaining activity of the enzyme in these experiments is due to a slow non-enzymic decomposition of hydrogen peroxide, which increases with the *pH*, and which liberates a sufficient amount of oxygen to promote the catalytic activity of the enzyme<sup>1</sup>.

In our oxygen-free manometric experiments, strong or even complete inhibition of catalase was obtained only when the non-enzymic decomposition of hydrogen peroxide was almost completely abolished. The formation of oxygen during the experiments from non-catalytic decomposition must be avoided because, according to the cyclic mechanism of decomposition proposed by us, the slightest trace of oxygen can, by initiating a series of oxidations and reductions, bring about an extensive decomposition of hydrogen peroxide limited only by the stability of the enzyme preparation. The experiments, therefore, had to be carried out in absence of impurities containing other catalytic metals or h ematin compounds, at a low *pH*, in thoroughly cleaned flasks and using purified