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Determination of Very Small Amounts of Hydrogen Peroxide

It has been found that radiation doses of a magnitude as low as 10 r. damage cells in culture and interfere with their multiplication[1,2]. Although the damage is to be ascribed primarily to short-lived radicals produced by the rays rather than to the hydrogen peroxide also made, it is desirable to study the biological effects of small concentrations of this substance.

In this connexion, a very sensitive method for the determination of hydrogen peroxide is needed. The efficiency of ionizing rays in producing chemical species is measured by the G value, which is defined as the number of molecules formed per 100 eV. of energy absorbed. Using γ-rays (cobalt-60), G values for hydrogen peroxide production of 1·05–1·31 were found at dose-rates of about 3×10^3 r./min.[3–8]. Therefore, a dose of 10 r. will give in 1 ml. water hydrogen peroxide of about 10^{-11} mole ($\sim 3{\cdot}5 \times 10^{-10}$ gm.).

To develop a suitable method, we started from the report by Andreae[9] that scopoletin (6-methoxy-7-hydroxy-1,2-benzopyrone) is oxidized stoichiometrically (1 : 1) by hydrogen peroxide with loss of its fluorescence, provided horse radish peroxidase is present. The fluorescence before and after interaction with peroxide was measured with a Beckman spectrophotometer model *DU*, adjusted for fluorimetry. 10^{-10} mole of hydrogen peroxide could be measured. In the present work the determination was made with a Galvanek-Morrison fluorimeter, mark *V* (Jarrell Ash Co.), as usually employed for the determination of uranium in solid sodium fluoride pills. In this way, the sensitivity of the method has been improved by about one magnitude for the concentration, and by two magnitudes for the quantity of hydrogen peroxide.

The method was tested with hydrogen peroxide solutions. The solvent water had to be very pure to avoid spontaneous decomposition of the solute. Twice-distilled conductivity water was distilled three more times. First, with alkaline potassium permanganate ; secondly, with silver nitrate ; and finally, without any addition in a quartz apparatus. Hydrogen peroxide ('Perhydrol *p.A.*', Merck) was added to this water to give a solution about 0·2 per cent, and the exact concentration determined by iodometry. This stock solution was stable when stored in silica in darkness. For use it was diluted in steps, and the concentration measured by the scopoletin method after each step.

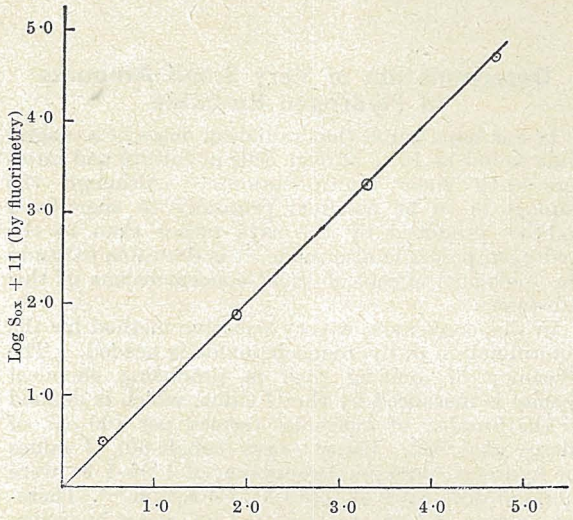

Fig. 1

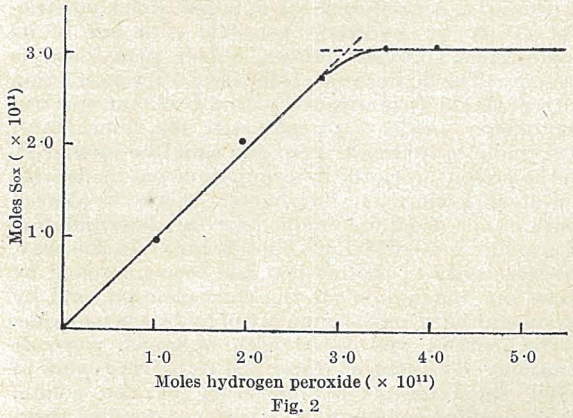

Fig. 2

For the purpose, 0·1 ml. of scopoletin solution, 0·1 ml. of horse-radish peroxidase (C. F. Boehringer) solution (concentration not critical), and 0·1 ml. of hydrogen peroxide solution were mixed. The amounts of scopoletin were chosen so that at most three-quarters were used up by reaction with the hydrogen peroxide. 15 min. after mixing, 0·3 ml. of 0·15 *M* borate buffer (*p*H 10·0) was added, exactly one-half of the mixture transferred to a platinum dish, and

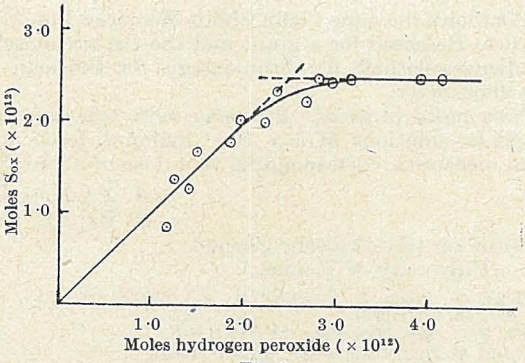

Fig. 3

the fluorescence measured. To obtain the fluorescence of the intact scopoletin, a blank mixture was made by including 0·1 ml. water instead of the same amount of hydrogen peroxide solution.

In Fig. 1, the logarithm of the number of moles of scopoletin oxidized (S_{ox}) is plotted against the logarithm of the number of moles of hydrogen peroxide as calculated on the basis of the iodometry with the stock solution. Clearly the method is applicable over a large range of concentrations. In Fig. 2, the destruction of the fluorescence in aliquots of one and the same scopoletin solution (containing initially $3{\cdot}12 \times 10^{-11}$ mole) by varying quantities of peroxide is shown, and Fig. 3 contains a similar plot for an initial concentration of $2{\cdot}5 \times 10^{-12}$ mole. It will be noted that the inclinations of the straight lines in the graphs are exactly 45°, as required by the stoichiometry 1 : 1.

It appears that in the range covered by Fig. 3 the limit of the method is reached. The scatter of the points in the lowest range is due to the fluctuations of the instrument when it works at its highest sensitivity. By proceeding statistically, that is, by measuring several identical samples in parallel and taking averages, the limit could be pushed down a little further.

In the method as used, the standard error is about 10 per cent in the measurement of $1{\cdot}5 \times 10^{-12}$ mole hydrogen peroxide, corresponding to a concentration of $1{\cdot}5 \times 10^{-11}$ mole/ml. in the initial solution, and to a radiation dose to this solution of about 15 r. Presumably even smaller concentrations and doses could be determined if the fluorimeter were adapted to take larger samples, or if the hydrogen peroxide could be diluted to a lesser extent by the reagents before measurement.

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Note added in proof. By using more concentrated reagents, solutions of 5×10^{-12} mole/ml. have now been measured, corresponding to a dose of about 5 r.

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