# THE EFFECT OF HYDROGEN PEROXIDE ON THE PERMEABILITY OF THE CELL

By DAVID LANDSBOROUGH THOMSON
(1851 Exhibition Senior Student).

From the Institute of Biochemistry, University of Cambridge.

(*Received 12th December* 1927.)

Szücs (1913) reported that hydrogen peroxide increased the permeability of the living cell. Following up a previous observation [Bokorny, 1886], he found that if threads of *Spirogyra* were treated first with hydrogen peroxide and then with ferrous sulphate solutions, a dark precipitate appeared within the cells. He concluded that the hydrogen peroxide had so increased the permeability of the cell that the ferrous ions were able to enter and form a dark iron-tannin compound. It was also found that hydrogen peroxide increased the permeability of the cell to various dyes. In view of the ever-increasing importance of the rôle assigned to hydrogen peroxide in cell metabolism it seemed worth while to re-open this question. As Szücs gives no details of his experiments, an exact repetition was impossible.

## PREPARATION AND PROPERTIES OF HYDROGEN PEROXIDE.

It seemed desirable to prepare a solution of hydrogen peroxide which should be as free as possible from organic impurities, which might exert a narcotic action, and be as nearly as possible neutral in reaction. For this purpose perhydrol (Merck) was distilled in oxygen at reduced pressure over crystalline barium peroxide. The peroxide content of the crystals was found to be 99 per cent. of the theoretical value, but it is possible that the inclusion of some hydrogen peroxide of crystallisation makes this value too high.

The perhydrol, to which a few crystals of barium peroxide were added, was allowed to fall drop by drop [Maass and Hatcher, 1920] into a distilling flask containing barium peroxide. The flask was maintained at a temperature of 55°, and the receiver was cooled with ice and salt. A slow stream of oxygen, bubbled through 10 per cent. NaOH, was drawn through the apparatus, and the pressure was brought down to 12 mm. Hg with a Geryk pump. A wash-bottle containing a strong solution of permanganate prevented any hydrogen peroxide passing through to the pump.

In this way a 9·25 per cent. solution of hydrogen peroxide was obtained, the yield being about 20 per cent. The solution left no visible residue on evaporation, and various impurities were tested for and found to be absent. The hydrogen-ion concentration was determined by adding drops of various indicator solutions to

the solution and noting the colour changes. Potentiometric measurements are not possible, since hydrogen peroxide reacts with all available electrode materials [Jones, Barnes and Hyde, 1902]; still less is it possible to use colorimetric methods. The solution was alkaline to congo red, methyl orange, and brom-phenol-blue, and was therefore of *p*H 4·5–5. Perhydrol diluted to the same strength had a *p*H of 3·5–4.

It appears to be established that hydrogen peroxide in aqueous solution dissociates as an acid, probably as a binary electrolyte [Carrara and Bringhenti, 1903], since the acidity of such solutions disappears when the hydrogen peroxide is destroyed with platinum [Merck]. Direct measurements of this dissociation have not yet been obtained. Maass and Herzberg (1920) found the freezing-point of a 4·9 per cent. solution to be $-3{\cdot}4°$, indicating an apparent molecular weight of 28; the writer calculates that on the assumption that this anomalous result depends on the dissociation of the peroxide as a binary electrolyte liberating hydrogen cations, such a solution should be of *p*H 0·5 roughly—an incredible value. It is conceivable that the hydrogen peroxide, by its high dielectric constant, increases the dissociation of the solvent.

The published description of the latest method of preparing hydrogen peroxide [Kilpatrick, Reiff and Rice, 1926] reached this country too late to be of service to the writer.

## EFFECT OF HYDROGEN PEROXIDE ON PLASMOLYSIS.

The epidermal cells of the leaf of *Tradescantia discolor* were used for the study of the effect of previous treatment with $H_2O_2$ on plasmolysis. A small strip of epidermis was immersed for ten minutes in a 1 per cent. solution of $H_2O_2$, washed for one minute, and then brought into a hypertonic solution in a hollowed slide, and plasmolysis followed under the microscope. A cover-slip was used to retard evaporation. In order to minimise the action of light, a feeble illuminant was employed and switched off between observations. Controls were either placed directly in the hypertonic solution, or previously treated with a phthalic buffer solution of *p*H 5·0 for ten minutes. It was found that mere counting of the number of cells plasmolysed did not give regular results, and it was not possible to demonstrate a shift of the limiting plasmolytic concentration after treatment with $H_2O_2$. Accordingly a group of cells was selected in each experiment, and sketched on squared paper with the aid of a camera lucida and a squared micrometer ocular, and the area of the cytoplasm at suitable intervals was similarly drawn. These areas were then computed by counting the squares covered on the paper, calculated as volumes by bringing them to the power $\frac{3}{2}$, and expressed as percentages of the initial volume.

### Series A. Hypertonic solution, 8 per cent. cane sugar solution.

A considerable number of the cells failed to plasmolyse (it was remarked that in general solutions stronger than usual for this material were required to produce plasmolysis): 38 per cent. of those treated with $H_2O_2$, 33 per cent. of those treated

with buffer solution, 12 per cent. of those untreated. It is not justifiable to regard these figures as indicative of increased permeability, since failure to plasmolyse may be ascribed to various other causes [Szücs, 1913]. The following table gives the amount of shrinkage in those cells which did plasmolyse and which survived throughout the observations:

| Previous treatment | Number of cells observed | Volume at time (minutes) | | | |
|---|---|---|---|---|---|
| | | 0 | 15 | 30 | 45 |
| 1 % $H_2O_2$ | 132 | 100 | 47 | 51 | 52 |
| Buffer *p*H 5·0 | 120 | 100 | 51 | 49 | 52 |
| None | 138 | 100 | 53 | 47 | 46 |

SERIES B. HYPERTONIC SOLUTION, 1·15 PER CENT. POTASSIUM NITRATE.

In this series of experiments attention was given to the question of recovery from $H_2O_2$; some of the specimens, after treatment, were washed for one hour in an isotonic solution of NaCl, KCl and $CaCl_2$ (ratio Na : K : Ca = 25 : 1 : 1), and controls were also treated with this solution. It was found that a large number of the cells treated with $H_2O_2$ disintegrated in this interval, or in the hypertonic solution.

| Previous treatment | Number of cells observed | Volume at time (minutes) | | | |
|---|---|---|---|---|---|
| | | 0 | 15 | 30 | 45 |
| 1 % $H_2O_2$ | 151 | 100 | 56 | 56 | 61 |
| 1 % $H_2O_2$ and 1 hour isotonic salt solution | 118 | 100 | 59 | 58 | 60 |
| Buffer and 1 hour isotonic salt solution | 133 | 100 | 61 | 57 | 58 |
| 1 hour isotonic salt solution | 146 | 100 | 62 | 55 | 56 |

## SERIES C. HYPERTONIC SOLUTION, BALANCED SALT SOLUTION ($NaCl$, $KCl$, $CaCl_2$).

Osmotic pressure at 0° = 5·25 atmospheres.

| Previous treatment | Number of cells observed | Volume at time (minutes) | | | |
|---|---|---|---|---|---|
| | | 0 | 15 | 30 | 45 |
| 1 % $H_2O_2$ | 65 | 100 | 46 | 47 | 49 |
| 1 % $H_2O_2$ and 1 hour isotonic salt solution | 63 | 100 | 49 | 49 | 50 |
| Buffer | 65 | 100 | 49 | 48 | 49 |
| Buffer and 1 hour isotonic salt solution | 60 | 100 | 48 | 49 | 49 |
| None | 62 | 100 | 51 | 47 | 48 |
| 1 hour isotonic salt solution | 64 | 100 | 52 | 47 | 46 |

Finally, further series of experiments were made in which the treatment with $H_2O_2$ was prolonged, or in which higher concentrations of $H_2O_2$ were employed; but so many of the cells were injured by this treatment that the measurements made on the survivors seem to be untrustworthy. They did not differ greatly from the results tabled above.

## EFFECT OF HYDROGEN PEROXIDE ON THE PERMEATION OF VARIOUS SUBSTANCES.

### Series D. Permeation of sodium hydroxide.

As the red pigment of the epidermal cells of the leaf of *Tradescantia discolor* is an indicator, changing to blue in alkaline media, the permeation of alkalies into these cells is readily followed under the microscope. Strips of the tissue, with or without previous treatment with $H_2O_2$, are immersed in isotonic NaCl containing 0·01*N* NaOH; the time at which the colour within the cells matches a standard prepared by adding 0·005*N* NaOH to an aqueous extract of the pigment is noted.

| Previous treatment | Number of observations | Time in minutes |
|---|---|---|
| 1 % $H_2O_2$ (10 mins.) ... ... ... ... ... | 5 | 73 |
| 2 % $H_2O_2$ (10 mins.) ... ... ... ... ... | 5 | 68 |
| 3 % $H_2O_2$ (10 mins.) ... ... ... ... ... | 5 | 43 |
| 1 % $H_2O_2$ and 1 hour neutral isotonic salt solution ... | 5 | 70 |
| 2 % $H_2O_2$ and 1 hour neutral isotonic salt solution ... | 5 | 69 |
| Buffer *p*H 5·0 (10 mins.)... ... ... ... ... | 5 | 61 |
| Buffer *p*H 5·0 and 1 hour neutral isotonic salt solution | 5 | 74 |
| None ... ... ... ... ... ... ... | 10 | 87 |
| 1 hour isotonic salt solution ... ... ... ... | 5 | 80 |

### Series E. Permeation of ferrous sulphate.

The permeation of ferrous sulphate into the cells of *Spirogyra* sp. is detected by the appearance of a dark precipitate within the cells. Solutions of $FeSO_4$ of various strengths were brought to isotonicity with sodium sulphate, and the presence or absence of a precipitate within the cells after 30 minutes' immersion noted.

| Previous treatment | Concentration of ferrous sulphate | | | |
|---|---|---|---|---|
| | 2 % | 3 % | 4 % | 5 % |
| 1 % $H_2O_2$ (10 mins.) | 0 | 0 | Trace | Precipitate |
| 1 % $H_2O_2$ (10 mins.) and 1 hour isotonic salt solution | 0 | 0 | . 0 | ,, |
| Buffer *p*H 5·0 (10 mins.) | 0 | 0 | Precipitate | ,, |
| Buffer (10 mins.) and 1 hour isotonic salt solution | 0 | 0 | Trace | ,, |
| None | 0 | 0 | 0 | ,, |

### SERIES F. EXOSMOSIS OF PIGMENT.

The exosmosis of the red pigment from thin slices of beetroot in 1·8 per cent. NaCl solution, with or without previous treatment with 1 per cent. $H_2O_2$, was studied. In the case of the slices treated with $H_2O_2$ the individual experiments gave such divergent results that no significant average values could be obtained. The controls, however, gave regular results. It is possible that decolorisation of the pigment by the peroxide is an interfering factor, though the specimens were thoroughly washed after treatment.

## EFFECT OF HYDROGEN PEROXIDE ON HAEMOLYSIS.

### SERIES G. HAEMOLYSIS IN HYPOTONIC SOLUTIONS.

The effect of hydrogen peroxide on the haemolysis of ox blood corpuscles in hypotonic NaCl solutions was studied. The corpuscles were centrifuged and washed and suspended in NaCl solutions of various concentrations. In this way a set of standards was obtained, by comparing the colour at a given time (5 minutes) with that in a parallel experiment in which 1 per cent. $H_2O_2$ was present in the salt solution. Since the cell-membrane is permeable to $H_2O_2$ [Overton], the osmotic activity of the added peroxide may be neglected. The method is nevertheless objectionable, since hydrogen peroxide has a considerable if not fully investigated power of forming additive compounds with inorganic salts [Tanatar, 1901]; moreover, the peroxide reacts with the haemoglobin and disturbs the tint. Specimen results may be cited:

| | | | | |
|---|---|---|---|---|
| Concentration of NaCl, used as standard | 0·65 | 0·64 | 0·63 | 0·62 |
| Concentration of NaCl in suspensions giving the same tint at equal time, in presence of $H_2O_2$ | 0·67 | 0·66 | 0·64 | 0·62 |

These experiments were completed before the author had access to the paper by Rigoni (1926), who obtains rather different results. The divergences can, however, be ascribed with confidence to the very different experimental conditions.

## DISCUSSION.

The plasmometric experiments show that while the amount of shrinkage of the protoplast in hypertonic solutions is not affected by previous treatment with $H_2O_2$, the time-relations are changed. Plasmolysis is rapid, and deplasmolysis sets in relatively early. This is intelligible if it be supposed that the peroxide initiates an increase in the permeability of the plasma membrane, which in hypertonic media continues progressively; so that in the early stages of the experiment there is increased permeability to water, with rapid exosmosis, and in later stages a permeability to dissolved substances with accompanying deplasmolysis. When the cells, after treatment with $H_2O_2$, are allowed to rest for an hour in isotonic medium, they subsequently show a similar but less marked alteration in the time-relations of plasmolysis; suggesting that in the isotonic solution the disintegrative

changes are arrested and partially reversed. The action of the slightly acid buffer solution is similar, but less marked; it follows that the action of the peroxide cannot be ascribed to its acidity alone.

On the other hand, the experiments on the permeation of NaOH show that the buffer solution was rather more active than 1 per cent. $H_2O_2$ in increasing permeability; and there is no evidence of recovery from $H_2O_2$ in one hour in isotonic salt solution. The ferrous sulphate experiments in general confirm these findings.

The experiments on haemolysis do not show any increased permeability to NaCl in the presence of $H_2O_2$; on the contrary, the amount of haemolysis is increased. It seems improbable that the degree of dissociation of the electrolyte, at such concentrations, is so significantly increased by the presence of $H_2O_2$ that the experimental results can be ascribed to this effect; the view that the elasticity of the plasma membrane is impaired by the $H_2O_2$ is preferred.

It is concluded that hydrogen peroxide increases the permeability of the plasma membrane by damaging or disintegrating it, and impairs its resistance to osmotic forces. $H_2O_2$ is known to have a disintegrating effect on cellular structure [Karczag and Steinberg, 1922; Rigoni, 1926] and to affect the stability of protein sols [Fernau, 1923]. This action of $H_2O_2$ is apparently not wholly due to its acidity, though mineral acids have an analogous effect [Brenner, 1918] as the experiments with the acid buffer solution indicate. In favourable circumstances there may be some recovery from the effects of $H_2O_2$, but this is regarded as a process of active repair rather than as a true reversal effect. Höber (1926) is incorrect in ascribing to Szücs a claim that the action of $H_2O_2$ is reversible. In the absence of experimental details no criticism of the work of Szücs can be offered.

## SUMMARY.

Experiments on plasmolysis, permeation of alkali, and haemolysis do not confirm the view that hydrogen peroxide plays a specific part in the physiological control of permeability.

## REFERENCES.

Bokorny (1886). *Jahrb. f. wiss. Bot.* **17**, 347.
Brenner (1918). *Öfversigt af Finska Vetensk. Soc. Förhandl.* **60**, No. 4.
Carrara and Bringhenti (1903). *Gazz. Chim. Ital.* **33**, 362.
Fernau (1923). *Kolloid-Zeits.* **33**, 89.
Höber (1926). *Physikalische Chemie der Zelle*, Leipzig (6th ed.), p. 569.
Jones, Barnes and Hyde (1902). *Journ. Am. Chem. Soc.* **27**, 22.
Karczag and Steinberg (1922). *Biochem. Zeits.* **132**, 279.
Kilpatrick, Reiff and Rice (1926). *Journ. Am. Chem. Soc.* **48**, 3019.
Maass and Hatcher (1920). *Journ. Am. Chem. Soc.* **42**, 2548.
Maass and Herzberg (1920). *Journ. Am. Chem. Soc.* **42**, 2569.
Rigoni (1926). *Arch. di Fisiol., Suppl.* **24**, 664.
Szücs (1913). *Jahrb. f. wiss. Bot.* **52**, 269.
Tanatar (1901). *Zeits. anorg. Chem.* **28**, 255.