XL. NOTE ON ANAEROBIOSIS AND THE USE OF ALKALINE SOLUTIONS OF PYROGALLOL.

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EFFECTIVE precautions are usually taken to remove poisonous impurities from hydrogen used in securing anaerobiosis. It is difficult to find in bacteriological literature references to the need for recognising that a poisonous gas, carbon monoxide, is evolved when atmospheric oxygen is absorbed by alkaline solutions of pyrogallol. Wright [1928], following Burrows [1917, 1921] in his method of preparing pure nitrogen from commercial cylinder nitrogen, took precautions to ensure that no carbon monoxide, resulting from the small amount of oxygen present, should pass over into the anaerobic chamber.

The author, in conjunction with Drakeley [1925, 1929], has shown that all alkaline solutions of pyrogallol, of strengths likely to be used in practice, evolve carbon monoxide when oxygen of high purity is absorbed. With cylinder oxygen of about 98 % purity, amounts from 0.1 % up to practically 10 % of carbon monoxide may be formed, and absence of agitation of the absorbent solution favours the production of larger amounts of carbon monoxide than those formed when the solution is vigorously shaken. Oxygen of lower purity, such as atmospheric oxygen, liberated carbon monoxide in all cases investigated, though the percentage evolution was smaller.

These findings have a bacteriological import, since the conditions under which alkaline solutions of pyrogallol are used in bacteriology do not usually favour agitation of the solution during absorption of oxygen. A similar remark may be made with reference to some pomological investigations, as, for example, examination of the course of ripening of fruits in the absence of oxygen.

It was also shown that all alkaline solutions of pyrogallol absorb oxygen, though with varying degrees of efficiency. There appears consequently to be no warrant for the practice of some authors of specifying with exactness a particular concentration of alkali, while leaving vague the amount of pyrogallol.

The method of Rockwell, as quoted by Stitt [1927], is of much interest. Rockwell calls for the use of sodium bicarbonate solution with addition of sodium dihydrogen phosphate, according to the formula:

Sodium bicarbonate	...	...	...	...	50 g.
Sodium dihydrogen phosphate	...	...	...	...	0.75 g.
Water	...	...	...	...	500 cc.

"Keep tightly corked."

In use, "0.5 g. pyrogalllic acid and 1 cc. of the charged alkali solution" are mixed *in situ* (culture tube).

This might seem to be an attempt to avoid production of carbon monoxide, but it appears to be an omnibus method for producing carbon dioxide in replacement of the absorbed oxygen. As such, its chemistry is complicated. A qualitative study of a solution prepared according to Rockwell's direction was made. It was found that at room temperature a solution of pyrogallol in sodium bicarbonate solution did not appreciably absorb oxygen whether the acid phosphate were present or not. The amount of bicarbonate specified did not entirely dissolve. A roughly quantitative evolution of carbon dioxide took place when the acid phosphate was added to the bicarbonate solution. When the solution was warmed in an incubator at 38°, oxygen was slowly absorbed, owing to bicarbonate becoming partly transformed into normal carbonate; carbon dioxide was given off, partly as a product of that transformation, and partly from the interaction of acetic acid (an oxidation product of pyrogallol) with the carbonates.

A quantitative study was attempted, though, owing to the incomplete solubility of the components at room temperature, it was thought desirable to vary the quantities. 50 g. of pyrogallol were dissolved in 50 cc. of solution prepared according to the above formula, the solution being then made up to 200 cc. with access of air. After standing overnight at room temperature in a corked bottle, the solution was transferred to a Hempel pipette: thus free carbon dioxide was lost. The solution was treated with four successive amounts of commercial cylinder oxygen of about 98.5 % purity and it was allowed to stand in each case without agitation for periods of 24 or 72 hours in a thermostatically controlled water-bath at 38°. Results of analyses of the residual gases are given in the table.

No. of exp.	Volume of cylinder oxygen cc.	Hours in thermostat (approx.)	Percentage of unabsorbed oxygen	Percentage of carbon dioxide	Percentage of carbon monoxide
1	96.0	72	0.05	64	0.55
2	94.7	24	1.4	36	0.7
3	98.0	24	1.4	1.4	0.8
4	100.0	24	3.7	49	1.0

The percentage of carbon dioxide was variable, and depended largely upon the length of time which had elapsed between the moment the pipette was removed from the thermostat and the time analysis was begun. In Exp. 3 the pipette was allowed to stand 24 hours at room temperature before making analysis of the gases; the carbon dioxide was almost entirely absorbed during cooling.

When measured volumes of air were admitted into the pipette, which was then placed in the thermostat, it was found that the final volume of gas was always greater than the initial volume, owing to production of an amount of carbon dioxide greater than the volume of oxygen absorbed. A similar increase of volume was found when the pipette was filled with inert gases

(nitrogen and argon) left from a previous analysis of air. When the pipette was allowed to stand empty of gas, no gas was produced. It was evident that carbon dioxide was produced during the reversible thermal transformation of bicarbonate into carbonate, the acid phosphate taking no part in this production after the initial evolution, of which the effect is lost when the "charged solution" is mixed with pyrogallol before use.

No accurate figures can be given for the percentage of carbon monoxide evolved when this modification of Rockwell's solution was used as absorbent for atmospheric oxygen, owing to the fact that the gases were measured in a simple Hempel burette, and not in the Bone and Wheeler apparatus which was used in the earlier work. In four analyses of air, a small and just detectable amount of carbon monoxide was produced from about 100 cc. of air; since the limit of measurement of the burette readings was 0.05–0.1 cc., this indicated an evolution of carbon monoxide of the order of 0.3 %, calculated on the oxygen absorbed. The result was in agreement with analyses made, using solutions of pyrogallol in caustic potash.

It would seem desirable that the effect of small amounts of carbon monoxide upon the growth of micro-organisms should be investigated, since even such a weakly alkaline solution of pyrogallol as the modification of Rockwell's solution used in this work, is capable of evolving carbon monoxide when the solution is used as an absorbent of oxygen. In conclusion, it may be said that there is little evidence for the idea of combination between pyrogallol and alkalis in aqueous solution: the term "pyrogallate solution" should be abandoned in favour of "alkaline solution of pyrogallol".

REFERENCES.

- Burrows (1917). *Amer. J. Physiol.* **43**, 13.
— (1921). *Proc. Soc. Exp. Biol. Med.* **18**, 133.
Drakeley and Nicol (1925). *J. Soc. Chem. Ind.* **44**, 457 T.
— (1929). *J. Soc. Chem. Ind.* **48**, 62 T.
Stitt (1927). *Practical bacteriology*, 83.
Wright (1928). *J. Path. Bact.* **31**, 735.