



LIBERATION OF LYSINE FROM INTERNAL ENVIRONMENT OF STREPTOCOCCI.

The rate of liberation of these amino-acids under the influence of tyrocidin can be demonstrated as in the accompanying graph. The internal concentration of lysine in a cell-suspension containing 45 mgm. dry weight *S. faecalis* cells/ml. was assayed as usual. In three further manometers the lysine-carbon dioxide output was followed with the intact cell suspension as substrate until this had ceased after 10-15 min.; amounts of tyrocidin corresponding to 1.0, 0.6 and 0.3 mgm. tyrocidin hydrochloride respectively were then added from a second side-bulb. An immediate evolution of carbon dioxide took place, showing the very rapid liberation of lysine from the cells after the addition of tyrocidin. 1.0 mgm. tyrocidin resulted in the liberation of all the internal lysine as assayed in disintegrated cells; the curve shows that the smaller quantities of tyrocidin resulted in the liberation of correspondingly smaller amounts of lysine. The curves suggest that the smaller quantities of tyrocidin liberate all the internal lysine from a proportion of the cells; this is confirmed by the addition of further amounts of tyrocidin after the evolution of carbon dioxide ceases, when the residual lysine is liberated. Addition of further tyrocidin has no effect in the manometer to which 1.0 mgm. has already been added.

The cytotoxicity produced by tyrocidin occurs very rapidly and there appears to be a quantitative relation between the amount of tyrocidin added and the number of cells lysed. The lytic effect is sufficient to explain the bactericidal action of tyrocidin, since in these experiments  $10^8$  cells are lysed by 1.0 mgm. and  $10^6$  cells by 0.1 mgm., while the amounts of tyrocidin necessary to sterilize cells in culture is found to correspond to 0.1 mgm./ml. for  $10^8$  cells/ml. or to 0.001 mgm./ml. for an inoculum of  $10^6$  cells/ml. Similar curves to those shown in the figure are obtained if the experiment is repeated in acetate buffer at pH 4.5 using the glutamic acid decarboxylase for the estimation of internal glutamic acid. It follows that neither the lysine nor the glutamic acid decarboxylase is inhibited by concentrations of tyrocidin up to 0.3 mgm./ml. under these conditions.

Repetition of these experiments with other antibiotics shows that similar results are obtained with approximately the same quantities of cetyl trimethyl ammonium bromide or Aerosol O.T. No such effect can be demonstrated with gramicidin, sulphathiazole, acriflavin or penicillin. A suspension of  $10^8$  cells/ml. requires 1.0 per cent phenol for sterilization in 30 min., and phenol in this concentration can be shown to release glutamic acid from the cells in the above manner. The lysine enzyme is inactivated by this concentration of phenol, but investigation of the lysine content of cells centrifuged out of 1.0 per cent phenol and washed prior to assay shows that the internal lysine has also been liberated. The bactericidal action of phenol would also appear to be due to disruption of the cell-membrane.

ERNEST F. GALE,  
E. SHIRLEY TAYLOR.

M.R.C. Unit for Chemical Microbiology,  
Biochemical Laboratory,  
Cambridge.  
Jan. 30.

<sup>1</sup> Gale, E. F., *Biochem. J.*, **39**, 46 (1945).

<sup>2</sup> Curren, H. R., and Evans, F. R., *J. Bact.*, **43**, 125 (1942).

<sup>3</sup> Gale, E. F., in preparation.

<sup>4</sup> Hotchkiss, R. D., "Advances in Enzymology", **4**, 153 (1944).

<sup>5</sup> Hotchkiss, R. D., and Dubos, R., *J. Biol. Chem.*, **141**, 155 (1941).

## Oxidation of Sulphur Dioxide During the Slow Combustion of Carbon Monoxide

IN many high-temperature oxidations, atomic oxygen has been postulated as playing a part in the development of reaction chains, and there is need of techniques which can give independent proof of their existence. Gaydon<sup>1</sup> has described a technique involving the introduction of nitric oxide to flames in which the presence of oxygen atoms in the flames is revealed by the yellowish-green emission observed due to the association reaction



Recently, it has been suggested<sup>2</sup> that the oxidation of sulphur dioxide added to carbon monoxide-air, hydrogen-air and methane-air flames respectively takes place through reaction with oxygen atoms formed in the combustion processes. The presence of a few parts per million of sulphur trioxide in combustion gases can be demonstrated qualitatively by observing the extent of sulphuric acid mist formation when the gases come into contact with water, and this technique has been extended to a study of the oxidation of sulphur dioxide during the slow combustion of carbon monoxide-oxygen mixtures.

A flow method was used in which a CO + 3O<sub>2</sub> mixture containing 1 per cent sulphur dioxide was passed at a rate of 400-500 c.c. a minute through a heated Pyrex tube, 3 cm. in diameter and 30 cm. in length. The gases were supplied direct from cylinders and were used undried, the water vapour content being about 0.09 per cent by volume. The temperature of the tube was raised at a steady rate of 5° C. a minute and the products of combustion were bubbled through water, any mist formed being observed in the bubbler.

No mist formation was observed until 600° C. was reached; thereafter it increased in intensity through the 'cool flame' region around 620° C. up to the ignition temperature; in the course of the first few runs this fluctuated slightly, but a steady value of 665° C. was finally obtained under these conditions. The growth in mist formation with increasing temperatures became so reproducible in successive runs that eventually it was possible to predict when ignition was about to take place.

In view of the well-known catalytic effect of water vapour on the oxidation of carbon monoxide near the upper limit<sup>3,4</sup>, some experiments were carried out on the influence of water vapour on the formation of acid mist. As the water vapour content was increased, mist formation set in at lower temperatures, but the ignition temperature was altered only slightly, as the accompanying table shows.

Water vapour (% by volume)	Temperature of mist formation (° C.)	Ignition temperature (° C.)
0.09	600	665
0.20	585	665
0.80	560	660
1.50	520	660

Subsidiary experiments showed that this increase in sulphur trioxide content in the water-catalysed oxidation was not due to the influence of water vapour on the heterogeneous oxidation of sulphur dioxide which was found to be negligible under these conditions.

The growth of active centres responsible for the oxidation of sulphur dioxide during the slow combustion of carbon monoxide, and the influence of water vapour on this, as shown by the above technique, have been confirmed quantitatively by measurement of the dewpoint of the gases; a detailed account of these results will be published shortly. The work forms part of a programme on the oxidation of sulphur dioxide in combustion sponsored by the Boiler Availability Committee.

G. WHITTINGHAM.

British Coal Utilisation Research Association,  
Coombe Springs Laboratories,  
Coombe Lane,  
Kingston-on-Thames.  
Feb. 1.

<sup>1</sup> Gaydon, A. G., *Proc. Roy. Soc., A*, **183**, 111 (1944).

<sup>2</sup> Dooley, A., and Whittingham, G., *Faraday Society General Discussion on Oxidation* held in September 1945.

<sup>3</sup> Hadman, G., Hinshelwood, C. N., and Thompson, H. W., *Proc. Roy. Soc., A*, **137**, 87 (1932).

<sup>4</sup> Topley, B., *Nature*, **125**, 560 (1930).

## New Iron Reagents for Alkaline Solutions

It has already been shown<sup>1</sup> that there are under suitable conditions distinct colour reactions between metallic iron and strongly alkaline solutions of special organic compounds, for example, dithio-oxamide, oximinocyanacetamide, nitrosoguanidine, oxalylhydrazide. Because this reaction may be of value in studying the passivity and corrosion of iron in alkaline solutions, it was desirable to find still more compounds acting as 'indicators' and especially to find reagents that are more stable than those mentioned. Unfortunately, conditions hitherto have not permitted a careful investigation of this matter, but preliminary experiments indicate that a large number of substances have the desired properties.

In seeking for new reagents for this purpose, it was found useful to try condensation products between hydrazides and 1,2-oximino-ketones. Among the former, oxalylhydrazide, malonylhydrazide, semicarbazide, thiosemicarbazide and nitroaminoguanidine have been used. The oximino-ketones tried were oximinacetone and ethyl oximinacetate. The resulting compounds are most probably the simple oxime-hydrazone, since the condensation was carried out under rather mild conditions. Also reaction products of salicylic aldehyde with hydrazides have been tried. They seem, however, to be not so suitable for the purposes proposed as some of the oxime-