ULTRA-VIOLET IRRADIATED CARBOHYDRATES AND BACTERIAL GROWTH

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The following experiments were undertaken in an endeavour to confirm the work of Blank and Arnold.[1] These authors make the following assertions:

1. Exposure of any one of 20 different carbohydrates and 3 carbohydrate derivatives to U.V. light, (2537 Å) for periods varying from 1.0 to 5.0 hours, results in the growth inhibition of *Bacillus subtilis* spores, when such irradiated carbohydrate solutions are incorporated in the medium.
2. Similar irradiation of agar and agar-water gels so alters them that when used as a base for culture media, such media do not support the growth of *Bacillus subtilis* spores.
3. This results from the formation of a non-volatile, thermostable material, capable of diffusing through and from an agar gel, which prevents the growth of the organism.

Using as far as possible, the same technique as these authors and a mercury vapour lamp[2] giving 99 per cent of the emission in the line 2536 Å the results of our experiments were as follows:

Experiment 1. Irradiation of entire agar culture medium

An agar plate containing 10 ml. of sterile nutrient agar was irradiated for four hours at a distance of 5 cm. from the source of light. The pH of this medium was 7.2 before exposure. After irradiation the pH of the medium was found to be 4.6. Inoculation of this plate with spores of *Bacillus subtilis* and *Escherichia coli* was not followed by colony development within 72 hours. If, however, a plate is irradiated and

[1] Blank, I. H. and Arnold, W.: Jour. Bact., **30**, 507, 1935.

[2] Vi-Tan Ultra Violet Lamp, Manufactured by The Thermal Syndicate, Ltd.

before inoculation the pH is adjusted to 7.0, growth occurs with both types in 24 hours.

Experiment 2. Irradiation of agar gel

When 20 ml. of 2 per cent agar-water gel was irradiated for 3 hours, it was found that the pH of the gel dropped from 7.0 to about 4.0. This gel was used to prepare 20 ml. of nutrient agar, and the pH after sterilisation was 4.5 to 4.6. This medium did not permit growth of *Bacillus subtilis* spores or *Escherichia coli*. Adjustment of the pH of such medium to 7.0 resulted in colony formation by both types within 24 hours.

Experiment 3

Blank and Arnold state that if an irradiated agar gel is soaked in water, the gel removed and the soak water evaporated to dryness on a steam bath and the resulting solids incorporated in a culture medium, such medium does not support the growth of *Bacillus subtilis*.

We have found that a medium prepared in this fashion will have a final pH of about 4.6 to 4.8, and does not support the growth of *Bacillus subtilis*. If the medium is neutralised, however, growth occurs readily.

Experiments in which sucrose was irradiated have given similar results.

Experiment 4. Irradiation of 1 per cent sucrose

We have observed that irradiation of 1 per cent sucrose solution for 2 hours resulted in a drop in pH from 7.0 to 3.5–4.0. The use of such solutions as a base for nutrient media without subsequent pH adjustment was naturally followed by inhibition of the growth of bacteria.

If, however, the medium is neutralised before use, growth occurs readily.

Experiment 5. Irradiation of 1 per cent sucrose in the presence of excess of $CaCO_3$

If 1 per cent sucrose solution is irradiated in the presence of an excess of $CaCO_3$, there is little or no change in the pH of the solution and media prepared from it will readily support bacterial growth without any pH adjustment. This would seem to indicate that the sole inhibitive agent formed by the irradiation of carbohydrates is the acidity.

Our investigation has shown that approximately half the acidity formed by irradiation of a 1 per cent sucrose solution for 2 hours is due to formic acid. The remainder consists of non-volatile organic acids. Traces of formaldehyde were also present.

It should be mentioned that Blank and Arnold make no reference to the pH of their media before or after irradiation. They state that the question of the influence of H-ion concentration of the carbohydrate solution during irradiation still requires investigation (presumably in relation to the quantitative course of the reaction producing the inhibitory substance).

CONCLUSIONS

1. Confirmation has been obtained of the claim by Blank and Arnold that ultra-violet radiation (2357 Å) causes agar gels or sucrose solutions when subsequently included in culture media to inhibit the growth of *Bacillus subtilis* and *Escherichia coli*.
2. Such radiation of carbohydrates is accompanied by marked production of acid.
3. Approximately half this acid is formic acid.
4. Neutralisation of this acidity restores the ability of the culture media to support the growth of the bacteria.

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