ing values are shown in Table II. The steady residual activity observed after 24 h is mainly incorporated in the thyroid. For instance, after 16 days, out of 3.3% in the whole body 2.8% is in thyroid.

Discussion. The experimental finding that the RIAP is present only in the gastrointestinal tract after the first hour, indicates that this labelled compound is rapidly cleared out from the blood circulation and deiodinated immediately it reaches the liver (absence of RIAP in liver at 1 h). An interesting and unexpected result is the presence of RIAP in the gastrointestinal tract: 7% of the injected dose in the stomach and 9.8% in the intestine 1 h after the injection. Presumably, this RIAP is excreted by the gastric mucosa in the same way as the iodide. Later, this RIAP is also reabsorbed through the intestine giving its deiodination in the liver as well as any possible iodide reabsorption, the general rise in radioactivity being observed in the second peak at 6 h. It is significant that no RIAP is present in the gastrointestinal tract at this time (Table III). A similar two peaks excretion pattern has been observed[8] for other radioiodinated organic compounds.

These results corroborate the observations of STRAUB et al.[7] that RIAP is rapidly metabolized to iodide and also validate their suggestion of its possible use as a tagged agent for the hepatic function test because its deiodination process is performed almost exclusively by the liver.

Résumé. Nous avons étudié l'absorption et l'excrétion de l'antipyrine radio-iodée injectée par voie intraveineuse chez le Rat. L'activité incorporée par les différents organes présente deux sommets en fonction du temps. Cette courbe est spécialement significative dans le cas du tractus gastrointestinal. Les analyses chromatographiques des homogénats d'organes montrent une absence complète d'antipyrine radio-iodée au bout de 2 h. Nous n'en avons pas trouvé dans le foie, même après 1 h. Le foie semble responsable de la desiodation. La vie moyenne biologique pour le corps entier est de 4,5 h.

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Comisión Nacional de Energía Atómica, Buenos Aires (Argentine), August 10, 1965.

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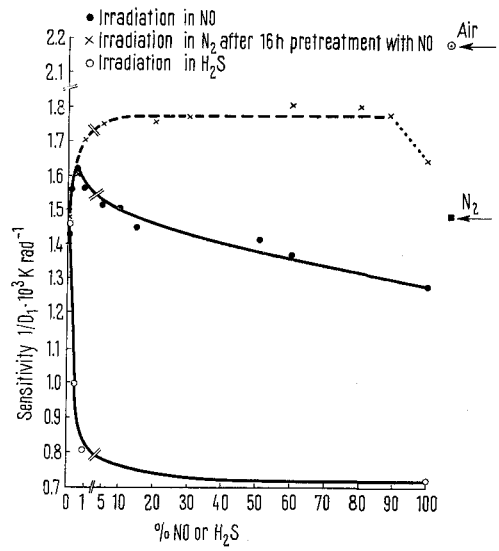
Effect of Nitric Oxide and Hydrogen Sulphide on Radiation Sensitivity of Spores of *Bacillus megaterium* in Suspension

The enhancement of radiation sensitivity by nitric oxide (NO) was first shown by HOWARD-FLANDERS[1]. It was later shown in this laboratory (DALE, DAVIES, and RUSSELL[2], and RUSSELL and DAVIES[3]) that the increase in sensitivity of *E. coli* and *S. flexneri* reached a maximum at 10% NO and then fell as the concentration of NO was raised further. Pre-treatment with NO before irradiation in N_2 also increased the sensitivity relative to that of untreated organisms.

In contrast to this, POWERS, WEBB, and KALETA[4] demonstrated that, relative to irradiation in N_2, NO protected dried spores of *B. megaterium*, both when NO was present during irradiation or was added afterwards, before exposure to oxygen. POWERS and KALETA[5] also showed that H_2S protected dried spores of *B. megaterium*, when present during irradiation or added afterwards.

It was therefore decided to examine the effect of NO and H_2S on the radiation sensitivity of wet spores in buffered suspension (the viability of a suspension was not affected by the gases themselves). The Figure shows the influence of different gases on radiation sensitivity, defined as the reciprocal of the dose (D_1) giving 1% survival. This is a measure of the change in both the shoulder and slope of the survival curve. The values in N_2 and NO (at 0.5% and 100% level) are based on six observations.

As can be seen from the Figure there is a slight sensitization by NO when present during irradiation at a concentration of 0.5% NO in N_2, whereas above that concentration the sensitivity of *B. megaterium* spores falls, being below that in N_2 when 100% NO is used. The difference between the values of $1/D_1$ in N_2 (1.485 $\pm$ 0.024) and



Radiation sensitivity of a suspension of spores of *B. megaterium* as a function of gas atmosphere. Abscissa: composition of gas atmosphere. Ordinate: radiation sensitivity, reciprocal of $D_{1\%}$.

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0.5% NO (1.616 ± 0.014) and between the values in N_2 and 100% NO (1.277 ± 0.038) are significant ($p < 0.001$, 't' test). Sensitivity is much lower when irradiation takes place in H_2S. The enhancement of sensitivity due to pre-treatment with NO required a minimum of 12 h contact with the gas. Four observations showed that the surviving fraction after irradiation in N_2 was unaltered by subsequent contact with 100% NO for 30 min.

Thus the effect of NO on wet spores is qualitatively similar to that found with a suspension of vegetative bacteria[3]. The far greater protection shown by H_2S may be a reflection of damage inflicted by NO upon the metabolism of the cell, making it more radiation sensitive. LYNCH and HOWARD-FLANDERS[6] have shown that NO reacts with free –SH groups in *S. flexneri*, and the present author[7] has found that treatment with NO causes an extended lag in subsequent growth of *E. coli*. Recent work by COTTON et al.[8] demonstrated that the presence of NO protected the non-metabolizing system, phage T_2r, as compared with irradiation in air or helium. Using phage T_2, HOWARD-FLANDERS et al.[9] found that NO did not increase radiosensitivity in similar conditions.

A low level of endogenous respiration has been found with spores of *B. megaterium* (RUSSELL, unpublished). The quantitative difference between the effect of NO on a suspension of spores and a suspension of vegetative cells might therefore be linked to differences in metabolic activity, and is in accord with the dual role of NO envisaged by DALE, DAVIES, and RUSSELL[2], whereby NO could exert both a protective and sensitizing influence[10].

Résumé. L'effet du NO sur la sensibilité à l'irradiation des spores de *B. megaterium* en suspension dans l'eau, est semblable à celui qui se produit dans les cellules d'*E. coli*. Les spores sont moins sensibles en présence de H_2S.

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On the Chemical Nature of the Heat-Stable Exotoxin of *Bacillus thuringiensis*

Bacillus thuringiensis Berliner produces several substances toxic for insects. One of these toxins is a heat-stable exotoxin found in the culture medium of *B. thuringiensis* var. *thuringiensis*[1,2]. Our attempts to isolate this toxin have not yet led to a pure preparation, but the results already permit us to characterize generally the nature of the toxin.

The toxicity of our preparations has been tested with larvae of *Drosophila melanogaster*. The tested fractions were added to the rearing medium. Fly maggots died as larvae when their food contained a sufficiently high quantity of the toxin.

Our starting material was a cell-free culture medium of *B. thuringiensis* var. *thuringiensis*, concentrated to a tenth of its original volume by boiling. This concentrate was mixed with an equal volume of ethanol. A bulky precipitate was formed which showed practically no toxicity. After 24 h the supernatant was decanted. The addition of more ethanol to an alcohol concentration of 60% of the volume resulted in further precipitation of non-toxic material. The supernatant was then mixed with twice the volume of ethanol (final concentration of alcohol = 90 volume%). After 24 h the supernatant was decanted and both supernatant and precipitate were tested. Only the latter proved to be toxic. The precipitate was dissolved in distilled water and will be called the pre-purified culture medium.

20 ml of the pre-purified medium were mixed with cellulose powder (Whatman No. 1) to a thick paste, and 80 ml of ethanol were then added. This mixture was introduced at the top of a cellulose column 20 cm high and 10 cm in diameter. The column was eluted with 80% ethanol until the eluate became colourless. All fractions were non-toxic for larvae of *Drosophila*. Then the column was eluted with 75% ethanol; 5 out of 15 200 ml fractions were found to be toxic. Since the most toxic fraction was also the least coloured one, we concluded that the toxin was probably colourless. All the toxic fractions had an absorption maximum at 258 to 260 nm and an absorption minimum at 230 to 234 nm, maximum and minimum being most pronounced in the most toxic fraction and least pronounced in the least toxic fraction. No maximum and minimum at these wave-lengths was found in the non-toxic fractions.

In a further experiment 50 ml of the pre-purified culture medium were run through a Dowex 2 anion exchange column on which the toxin was absorbed. The column was rinsed with distilled water and then eluted with 10% acetic acid until no more colour was eluted. These fractions were non-toxic. Elution with 5% KOH gave a non-toxic neutral fraction and a toxic alkaline fraction. However, 5% KOH alone also proved to be toxic in control tests. In order to remove the potassium, the alkaline fraction was neutralized with perchloric acid, two volumes of ethanol were added, and the precipitate was filtered off. After the alcohol had been evaporated, the filtrate proved to be highly toxic. The absorption spectrum of this preparation also showed a maximum at 260 nm and a minimum at 233 nm (Figure). This absorption spectrum corresponds closely to that of a nucleic acid. However, since the toxin is easily dialysable and soluble in ethanol

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