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 pretreatment 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A low level of endogenous respiration has been found with spores of *B. megaterium* (RUSSELL, unpublished).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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[6] J. P. LYNCH and P. HOWARD-FLANDERS, Nature, Lond. *194*, 1247 (1962).
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[8] I. M. COTTON, R. R. RINEHART, R. PETRUSEK, and W. S. STONE, Rad. Res. *21*, 481 (1964).
[9] P. HOWARD-FLANDERS, J. LEVIN, and L. THERIOT, Rad. Res. *18*, 593 (1963).
[10] Acknowledgments: The author wishes to thank Mrs. J. HOPKINS for technical assistance.
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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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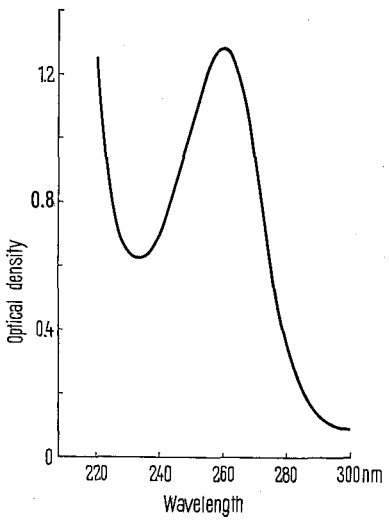
[2] A. BURGERJON and H. DE BARJAC, C. r. Acad. Sci. *T251*, 911 (1960).

up to 75% it must be a much smaller molecule than that of an ordinary nucleic acid.

Purified preparations gave a positive sugar reaction after Molisch, negative reactions after Fehling and Feulgen, but a red colour with HCl and phloroglucine and a blue-violet colour with HCl and diphenylamine (aldopentoses). No positive pentose reaction was obtained with non-toxic fractions. The toxic fractions therefore contain at least one aldopentose (possibly ribose).

From the absorption spectra and the chemical reactions of the toxic fractions, we infer that the toxin is likely to be a nucleoside or a nucleotide of adenine or of uracile. The chemical analysis will follow as soon as the preparations are completely purified.



Absorption spectrum of a purified preparation of the heat-stable exotoxin of *Bacillus thuringiensis*.

We may suppose that a toxin of this constitution acts as an antimetabolite in the nucleic acid metabolism. We would therefore expect it to act in the same way in all animals. However, this substance seems to be toxic for insects only. Preliminary experiments have shown that the toxin is practically non-toxic for fish, *Tubifex*, and aquarium plants. Fish will live unharmed for at least one month in water containing toxin in a concentration that kills larvae of *Drosophila* within 24 h. In addition it has been demonstrated by KRIEG and HERFS[3] that the toxic culture filtrate of *B. thuringiensis* var. *thuringiensis* had no adverse effects on young and adult mice when injected intraperitoneally or administered with the drinking water. Thus the metabolic step with which the toxin interferes must be specific for insects. This knowledge could incite the development of a new class of specific chemical insecticides which might be entirely harmless for vertebrates and most animals other than insects.

Zusammenfassung. Das hitzestabile Exotoxin von *Bacillusthuringiensis* – auf Insekten spezifisch toxisch wirkend – wurde aus dem Kulturmedium angereichert und stark gereinigt. Sämtliche toxischen Fraktionen zeigen ein Absorptionsmaximum bei 258–260 nm, ein Absorptionsminimum bei 232–234 nm und eine positive Aldopentosereaktion. Bei der toxischen Substanz scheint es sich um ein Nucleosid oder Nucleotid des Adenins oder Uracils zu handeln.

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[3] A. KRIEG und W. HERFS, Z. PflKrankh. *70*, 11 (1963).

Effet du pH sur la radioprotection des grains d'orge par les sels minéraux

Le traitement des grains d'orge par des solutions de chlorures alcalin et alcalino-terreux avant l'irradiation, détermine une radioprotection de la croissance des plantules. Cet effet n'est pas spécifique d'un cation déterminé. Il augmente avec la concentration saline jusqu'à un potentiel osmotique de -14 joules/cm^3 environ[1]. Mais il ne se manifeste que si, pendant la durée du traitement, la germination des grains n'a pas dépassé la phase précoce d'activation enzymatique[2]. Il semble que cette propriété des solutions salines soit due pour une large part à leur action physico-chimique sur la structure de certains gels polyélectrolytes de la graine. En vue d'étayer cette hypothèse, nous nous sommes demandé si le pH des solutions testées pouvait avoir une influence sur cette radioprotection.

Matériel et méthodes. Les essais ont porté sur des grains d'orge (*Hordeum sativum* Jess. var. Piroline; InstitutAgronomique de Gembloux), trempés avant l'irradiation, pendant 12 h, dans des solutions tampons 1,5 *M* de phosphates ($KH_2PO_4 - K_2HPO_4$) et de carbonates ($K_2CO_3 - KHCO_3$). Les grains ont reçu 4, 8, 12 kR provenant d'un générateur de rayons X, travaillant avec filtre de 2 mm d'aluminium sous une tension de 140 kV et une intensité de 20 mA. Le débit est d'environ 1000 R/min.

Après l'irradiation, les grains sont repiqués sur de l'ouate imbibée d'eau distillée et cultivés pendant 6 jours en conditions normalisées. Après ce laps de temps, les hauteurs du coléoptile et de la première feuille sont mesurées et exprimées en % des témoins non irradiés mais trempés dans les mêmes solutions. Pour chaque essai nous avons testé 3 lots de 50 grains.

Résultats. Quel que soit le pH des solutions de phosphates, le contenu en eau des grains est de 20–22% du poids frais. Il ne varie donc pas au cours des différents essais et correspond approximativement à celui des grains trempés dans une solution 1,5 *M* de $CaCl_2$.

Les hauteurs du coléoptile et de la première feuille issus de grains irradiés après avoir été traités par les différentes solutions tampons sont plus grandes que celles des plantules provenant des grains simplement trempés dans l'eau distillée. Il suffit de comparer les résultats des Figures 1

[1] C. GILLET, Nature, *207*, 99 (1965).
[2] C. GILLET, Bull. Classe Sci. Acad. Roy. Belg., 1069 (1965).