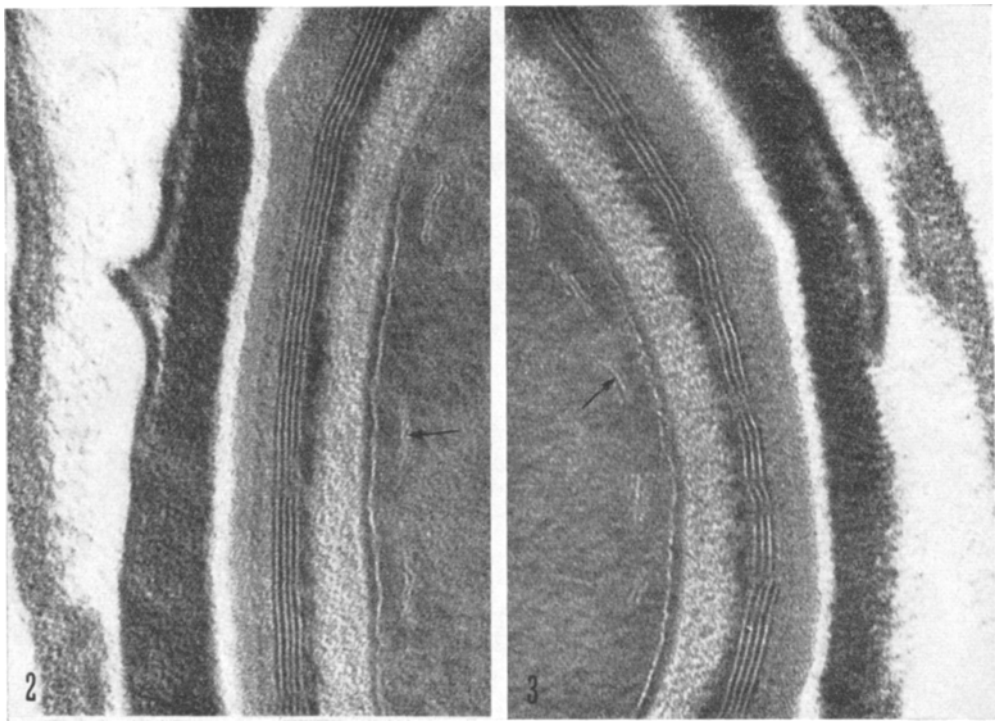

Fig. 2. Enlargement of a small section of the spore shown in Figure 1. Note the prominent segments of intracytoplasmic membrane. × 170,000.

Fig. 3. Section of another spore showing the intracytoplasmic membrane. × 187,500.

at 24°C for 3 h in 2% (w/v) $KMnO_4$ and 2% (w/v) OsO_4 in veronal acetate buffer, pH 6.1, bathed in 0.5% (w/v) uranyl acetate in veronal acetate buffer for 2 h, and embedded in Araldite (Cargille Laboratories Inc., New York). Sections were cut with a diamond knife on a Porter-Blum microtome and viewed in an RCA electron microscope (EMU 3G) with an accelerating voltage of 50 kV.

Figure 1 pictures a spore cut longitudinally. Inside the complex integument, consisting of the sporangial wall together with the various spore coats, and the cortical region, is the core, bounded by a 'unit' membrane[2] that lies just beneath the inner layer of the cortex. This spore core membrane encloses a cytoplasm that is packed with electron-dense particles corresponding in size to ribosomes. Centrally located is a large, ovoid body that probably contains the nucleoplasm. Just inside the spore core membrane, and coursing interruptedly but roughly parallel to it, is another membrane-like structure the dimensions of which are similar to those of the spore core membrane; but continuity of the two has not been observed. Details of this structure are shown in Figures 2 and 3.

The functional significance of this intracytoplasmic membrane is not known. These membranous wisps may be intrasporal mesosomes, the existence of which FITZ-JAMES[3] has shown in developing forespores of *Clostridium pectinovorum* but which, to our knowledge, have not been observed before in the core of mature spores of *Bacillus* species.

Zusammenfassung. Die Arbeit bringt den elektronenmikroskopischen Beweis für das Bestehen einer intracytoplasmatischen Membran im Cytoplasma ('core') der Sporen von *Bacillus popilliae.*

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Fate of Injected 4-Iodoantipyrine (^{131}I) in Rats

Radioiodinated 4-iodoantipyrine (RIAP) was used early in the study of the total body water compartment[1,2]. Recently an increasing use for it was found in the measurement of cerebral[3,4] and coronary blood flow[5,6]. STRAUB et al.[7] have pointed out that the RIAP is converted rapidly to a more diffusible compound: radioiodide, which invalidates the use of this compound in total body water studies.

In this experimental work, the distribution and elimination of the RIAP and its metabolic products have been studied on the wider basis of total body distribution and more time of observation.

Material and methods. 7 groups of 5 adult Wistar rats each were injected through the tail vein with 300 μC of RIAP (specific activity 480 μC/mg and radiochemical purity 99.0%). Then the animals were sacrificed at different intervals. The radioactivity of the different organs was measured with a scintillation counter and the values,

Table I. Radioactivity found in the different organs as % of the injected dose

	30 min	1 h	2 h	4 h	6 h	24 h	48 h
Brain	0.08 ± 0.06[a]	0.06 ± 0.02	0.02 ± 0.02	0.02 ± 0.03	0.04 ± 0.02	—	—
Heart	0.23 ± 0.05	0.18 ± 0.06	0.08 ± 0.06	0.17 ± 0.07	0.22 ± 0.06	0.03 ± 0.05	0.04 ± 0.03
Intestine	9.91 ± 5.16	13.89 ± 5.40	19.03 ± 3.82	10.64 ± 5.40	17.14 ± 0.26	4.93 ± 1.13	3.29 ± 0.95
Kidney	0.79 ± 0.36	0.71 ± 0.23	0.68 ± 0.19	0.52 ± 0.24	0.74 ± 0.15	0.22 ± 0.12	0.11 ± 0.04
Liver	4.07 ± 0.95	3.60 ± 0.26	3.06 ± 0.79	2.51 ± 0.96	3.60 ± 0.73	1.05 ± 0.52	0.73 ± 0.30
Lung	0.45 ± 0.23	0.44 ± 0.16	0.44 ± 0.24	0.34 ± 0.13	0.56 ± 0.10	0.12 ± 0.06	0.09 ± 0.07
Spleen	0.15 ± 0.08	0.91 ± 0.02	0.08 ± 0.04	0.11 ± 0.07	0.21 ± 0.13	—	0.02 ± 0.02
Stomach	6.22 ± 4.30	8.72 ± 3.20	17.77 ± 7.55	9.22 ± 3.14	15.62 ± 2.86	3.33 ± 2.30	3.21 ± 0.96
Thyroid	0.11 ± 0.07	0.17 ± 0.09	0.22 ± 0.59	0.43 ± 0.06	0.22 ± 0.05	2.59 ± 0.09	1.87 ± 0.77

[a] Standard deviation (group of five animals).

Table II. Whole body counting at different intervals

Time	c/p/m	% of injected dose
0	31,058[a] ± 4250[b]	100.0
1 h	21,977 ± 2910	70.7
3 h	20,325 ± 2520	65.4
48 h	2,937 ± 506	9.5
3 days	2,376 ± 475	8.5
6 days	885 ± 146	4.8
16 days	252 ± 71	3.3

[a] These values have been corrected by decay. [b] Standard deviation.

Table III. Chromatographic analysis of the different organ homogenates

	1 h	3 h	6 h	24 h
Brain	I RIAP	I	–	–
Heart	I RIAP	I	I	I
Intestine	I (30%) RIAP (70%)	I (32%) RIAP (26%) Y (42%)	I Y	I
Kidney	I	I	I	I
Liver	I	I	I	I
Lung	I RIAP	I	I	I
Spleen	I RIAP	–	–	–
Stomach	I (18%) RIAP (82%)	I (75%) RIAP (4%) X (21%)	I X	I
Thyroid[a]	I	I	I	I

I = iodide. RIAP = radioiodinated 4-iodoantipyrine. X = metabolite with Rf = 0.91. Y = metabolite with Rf = 0.57. [a] In all the chromatograms a part of the activity remains at the origin (thyronines synthesis).

expressed as % of the injected dose and corrected by decay, are shown in Table I. After this, the organs were homogenized in saline, filtered through filter paper and the filtrate chromatographed on Whatman paper 3 MM, using as solvent chloroform:ethanol:water (45:45:10). The ascending chromatography with this solvent gives the following Rf values: RIAP 1.0 and iodide 0.85. Posteriorly, the radioactivity was located on the chromatogram by autoradiography and identified by comparison with the Rf of standard activities chromatographied on the same paper.

Whole body counting was performed on a group of 5 animals throughout the whole experiment at different intervals. Table II gives the values of those determinations.

Results. Brain, heart, kidney, liver, lung, and spleen showed a similar pattern of uptake: the activity drops to a minimum between 2 and 4 h and then increases again to reach a maximum at 6 h. After this point it decreases with a lower rate. The chromatographic analysis of these organs indicates that the RIAP is only present during the first hour with the exception of kidney and liver in which no RIAP has been detected at the time assayed (1 h, 3 h, 6 h, and 24 h).

Intestine and stomach show a similar behaviour, but it differs from that of the other organs. In this case, the activity increases sharply to reach a first maximum after 2 h, followed by a considerable decrease and a new maximum after 6 h. The chromatograms of intestine and stomach present only RIAP and iodide after the first hour. At the third hour, iodide and RIAP and a new compound (one compound with Rf = 0.91 in the stomach and another compound with Rf = 0.57 in the intestine) have also been detected. These radioactive metabolites disappear after 24 h. Also, after 3 h no RIAP is present. Table III shows the percentage of the various compounds present in the stomach and intestine chromatograms after 1 h and after 3 h.

From the whole body counting, the biological half life has been graphically determined as 4.5 h. The correspond-

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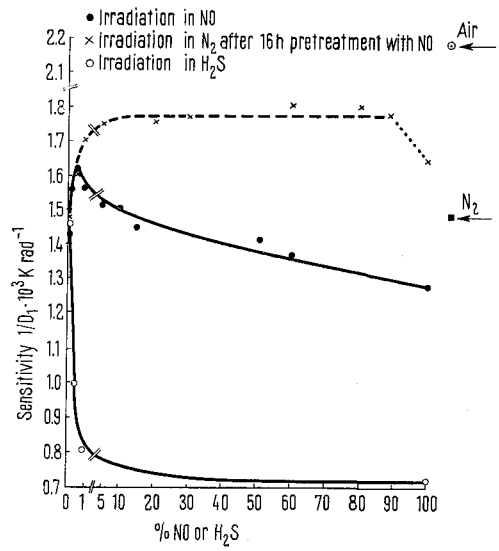
Effect of Nitric Oxide and Hydrogen Sulphide on Radiation Sensitivity of Spores of *Bacillus megaterium* in Suspension

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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