# SURVIVAL OF MICRO-ORGANISMS IN SPACE

## *Comparison of Survival Data Obtained in Fifteen Balloon-, Rocket-, and Satellite-Borne Exposure Experiments with Incident Solar Photons*

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**Abstract.** Survival of coliphage $T_1$, tobacco mosaic virus (TMV) and spores of *Penicillium roqueforti* Thom after direct exposure to space on board 6 balloons, 6 sounding rockets and 3 satellites is related to the numbers of solar UV photons incident during exposure. The survival followed exponential curves leading to complete inactivation. Solar ultraviolet radiation of wavelengths 2000 Å to 3000 Å appears to be the main cause of inactivation of broth suspended phage and, probably, TMV. $T_1$ phage and *Penicillium* spores prepared in saline were also affected by radiation shorter than 2000 Å, while TMV seems to be resistant to radiation of these wavelengths. The biological effectiveness of the solar spectrum in the area between 3000 Å to 50000 Å was not significant. Sterilization of interplanetary spacecraft appears necessary since micro-organisms can easily be shielded against lethal radiation.

## 1. Introduction

Previous experiments showed that solar ultraviolet radiation (UV) was the main cause of inactivation of dried preparations of micro-organisms directly exposed to space (Lorenz *et al.*, 1968a, c; Orlob and Lorenz, 1968). Exposure of filter-shielded micro-organisms to space on board a sounding rocket permitted an estimate of the biological effectiveness of solar UV (Lorenz *et al.*, 1968b). Knowledge of the biological effectiveness of solar UV is important in relation to planetary quarantine and spacecraft sterilization (Reynolds and Nicks, 1965; Hall, 1965).

The present report compares the survival of micro-organisms after direct exposure to space on board balloons, sounding rockets and satellites with incident solar photons. Due to its higher stability during drying and transportation (Lorenz *et al.*, 1968a, c), broth-suspended $T_1$ phage was flown more frequently than other microbiological preparations. It was hoped that a comparison of the survival of space-exposed micro-organisms with the various ranges of the solar spectrum at the balloon and rocket/satellite altitudes would indicate the spectral range with the highest biological effectiveness. Results previously published as individual flight experiments (Lorenz *et al.*, 1968a, b, c; Hotchin *et al.*, 1965; Orlob and Lorenz, 1968) are included in this report.

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## 2. Methods

### A. MICRO-ORGANISMS

Coliphage $T_1$ broth concentrate, broth lysate and synthetic medium lysate, purified tobacco mosaic virus (TMV) suspended in phosphate buffer or saline, and *Penicillium roqueforti* Thom spores suspended in saline were used. The approximate titers of the suspensions were $10^{11}$ PFU (plaque forming units) per ml ($T_1$ phage broth concentrate), $5 \times 10^9$ PFU/ml ($T_1$ phage broth lysate), $10^{10}$ PFU/ml ($T_1$ phage synthetic medium lysate), 10–34 mg/ml (TMV), and $3 \times 10^7$ colony forming units (*Penicillium*). Detection thresholds (lowest possible survival fractions) were calculated from the experiments with the highest numbers of incident photons (see Section 2B). The threshold values were $3 \times 10^{-7}$ ($T_1$ phage broth concentrate), $9 \times 10^{-7}$ ($T_1$ phage broth lysate), $2 \times 10^{-4}$ ($T_1$ phage synthetic medium lysate), $7 \times 10^{-4}$ (TMV), and $8 \times 10^{-7}$ (*Penicillium*). Droplets of 0.01 ml or 0.02 ml of the microbial suspensions were seeded and dried on autoclaved, plastic-coated aluminum plates. Details of preparation,

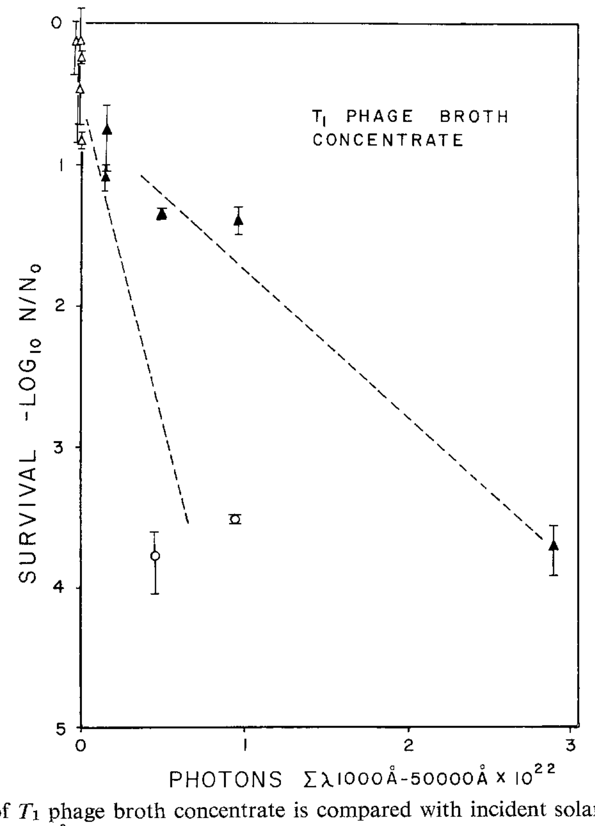


Fig. 1. Survival of $T_1$ phage broth concentrate is compared with incident solar photons of wavelengths 1000 Å to 50000 Å. Open triangles represent average survival obtained in rocket experiments, black triangles represent average survival obtained in balloon experiments, and circles represent average survival obtained in Gemini-satellite experiments. Vertical *T* bars indicate the average faults of sample means.

assay and exposure techniques, and elution after flight are described elsewhere (LORENZ *et al.*, 1968a, c; ORLOB and LORENZ, 1968). Survival is defined as the ratio of survival of directly exposed micro-organisms to survival of corresponding 'flight controls' (see LORENZ *et al.*, 1968b).

B. FLIGHT CONDITIONS

Micro-organisms were exposed to space on board 6 sounding rockets at altitudes between 70 km to 150 km, on board 6 balloons at an altitude of approximately 34 km, and on board the Gemini satellites IX-A and XII and the Gemini-VIII Agena space

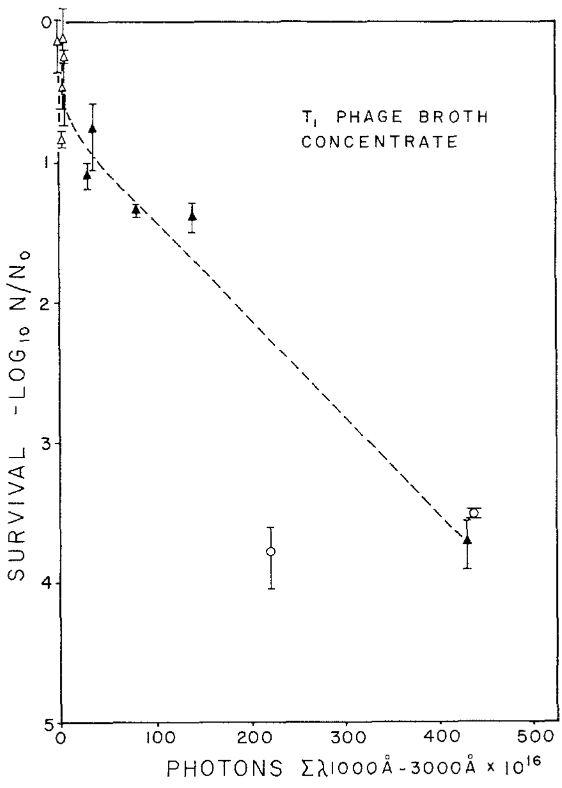


Fig. 2. Survival of $T_1$ phage broth concentrate is compared with incident solar photons of wavelengths 1000 Å to 3000 Å. See legend of Figure 1 for further explanations.

rocket. To permit an estimate of solar photons incident under these conditions (see Table I), the payload orientation towards the sun was determined. Angles of incidence of solar radiation were estimated for some of the balloon- and rocket-borne experiments (LORENZ *et al.*, 1968a, b; ORLOB and LORENZ, 1968). Solar flux data (DETWILER *et al.*, 1961; JOHNSON, 1954) were corrected for oxygen and ozone absorption at various altitudes. Data on the atmospheric density as a function of altitude and absorption coefficients were obtained from the literature (ELTERMAN, 1964; LADENBURG and VAN VOORHIS, 1933; VIGROUX, 1953). To estimate incident

TABLE I

Numbers of Solar Photons Incident on Micro-organisms

| Flight Experiment | Sum of Incident Photons[a] | |
|---|---|---|
| | 1000 Å–50000 Å | 1000 Å–3000 Å |
| Pandora rocket 1[b] | $4.2 \times 10^{19}$ | $2.0 \times 10^{16}$ |
| Luster rocket 2[c] | $4.7 \times 10^{19}$ | $2.2 \times 10^{16}$ |
| Luster rocket 3[b] | $9.7 \times 10^{19}$ | $4.8 \times 10^{16}$ |
| Luster rocket 4[d] | $1.3 \times 10^{20}$ | $6.3 \times 10^{16}$ |
| Luster rocket 5[e] | $1.4 \times 10^{20}$ | $6.5 \times 10^{16}$ |
| Balloon 1[b] | $1.4 \times 10^{21}$ | $2.1 \times 10^{17}$ |
| Balloon 2[b] | $1.6 \times 10^{21}$ | $2.4 \times 10^{17}$ |
| Gemini-XII[f] | $4.6 \times 10^{21}$ | $2.2 \times 10^{18}$ |
| Balloon 3[g] | $5.0 \times 10^{21}$ | $7.4 \times 10^{17}$ |
| Balloon 4[e] | $8.0 \times 10^{21}$ | $1.2 \times 10^{18}$ |
| Gemini-IX-A[f] | $9.5 \times 10^{21}$ | $4.4 \times 10^{18}$ |
| Balloon 5[h] | $9.7 \times 10^{21}$ | $1.4 \times 10^{18}$ |
| Balloon 6[i] | $2.9 \times 10^{22}$ | $4.3 \times 10^{18}$ |
| Gemini-VIII Agena[j] | $1.6 \times 10^{24}$ | $7.1 \times 10^{20}$ |

[a] Data published elsewhere (Detwiler *et al.*, 1961; Johnson, 1954) were corrected for absorption by oxygen and ozone, angle of incidence of solar radiation, and exposure time. See text for further explanation. The irradiated area unit was 0.16 $cm^2$ in most experiments, and the fluxes/area of Rocket 5 and Balloon 4 (Hotchin *et al.*, 1965) were corrected for this area. It was assumed that the micro-organisms were randomly distributed.**

[b] See Lorenz *et al.* (1968a) for flight conditions.

[c] See Lorenz *et al.* (1968b) for flight conditions.

[d] The rocket ('Luster D') was launched on June 6, 1967 from White Sands Missile Range at 09:00 MST. The exposure lasted 205 sec. at altitudes of 79 km to 142 km. During this period, sunlight was incident on the micro-organisms at an average angle of incidence of $48.5° \pm 6°$.

[e] See Hotchin *et al.* (1965). Rocket 6 was flown during the night when solar photons were absent (Orlob and Lorenz, 1968).

[f] See Hemenway *et al.* (1968) for flight conditions.

[g] The balloon was launched on August 14, 1966 from Palestine, Texas, at 20:15 CST. It traveled towards the West coast and was brought down near Jal, New Mexico. During 13 hr 10 min of exposure at an altitude of approximately 34 km, the payload was exposed to sunlight for 5 hr 55 min incident at angles between 86°–50° from normal.

[h] The balloon was launched on November 16, 1966 from Palestine, Texas, at 02:23 CST. It traveled towards the East coast and was brought down 40 miles West of Columbia, South Carolina. The payload was exposed to sunlight for 5 hr 33 min incident at angles between 90°–52° from normal.

[i] The balloon was launched on June 9, 1967 from Palestine, Texas, at 20:37 CST. It traveled towards the west coast and was brought down after 20 hr 40 min of total exposure near Snyder, Texas. The payload was exposed to sunlight for 10 hr 50 min incident at angles between 90°–11.5° from normal.

[j] Photon estimates were obtained by extrapolation of Gemini-IX-A data; see Hemenway *et al.* (1968) and Lorenz *et al.* (1968c) for flight conditions.

** Survival of micro-organisms after drying and storage varied between 100% and 0.01% (Lorenz *et al.*, 1968c). It is at this time not possible to determine the accurate cause of this loss. No attempt was therefore made to estimate the quantum yield of the inactivating radiation. This is also justified because solar radiation is not monochromatic.

solar radiation during the Gemini IX-A and XII satellite experiments, flight data (HEMENWAY *et al.*, 1968) were corrected for spacecraft orientation as recorded by the flight test data of the Manned Spacecraft Center, Houston, Texas.* During the Gemini-XII experiment, the payload orientation relative to flight direction was fixed in a $-16°$ pitch angle and a $33°$ roll angle. Corrections for random tumbling during the Gemini-IX-A exposure were made according to equation

$$I = \frac{I_0}{2} \int_0^{\pi/2} \cos\alpha \sin\alpha \, d\alpha = \frac{I_0}{4}$$

where $I_0$ = number of photons during the exposure period assuming normal incidence, $I$ = actual number of incident photons, and $\alpha$ = angle of incidence of solar radiation. Flux intensities during 4 months of direct space exposure of micro-organisms on board the Gemini-VIII Agena spacecraft were estimated by extrapolation from the numbers of photons incident during the Gemini IX-A exposure.

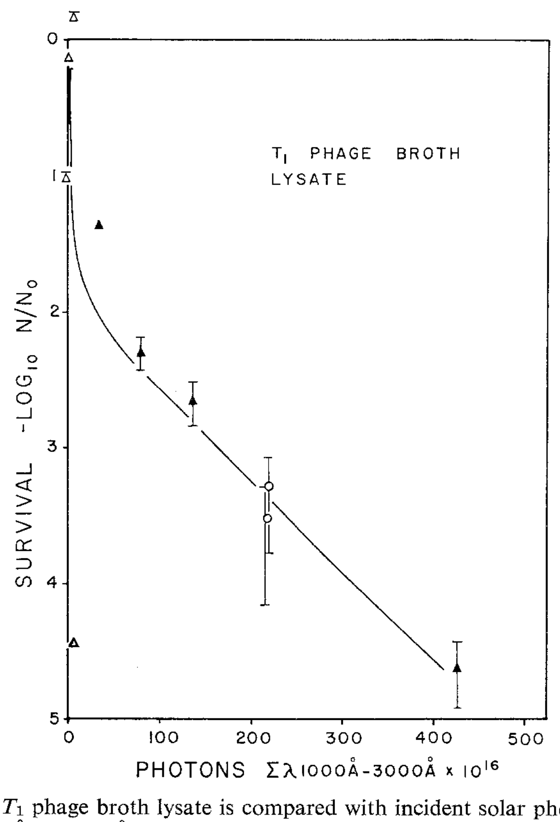


Fig. 3. Survival of $T_1$ phage broth lysate is compared with incident solar photons of wavelengths 1000 Å to 3000 Å. See legend of Figure 1 for further explanations.

* Gemini-IX and Gemini-XII spacecraft flight test data, CO3E Trajectory, Manned Spacecraft Center, Houston, Texas, June 3 and November 11, 1966.

## 3. Results

Figure 1 shows the survival of $T_1$ phage broth concentrate as a function of solar photons of wavelengths 1000 Å to 50000 Å. It can be seen that the survival depended on the number of photons incident on the micro-organisms. After approximately 5-fold inactivation in the rocket experiments, survival data can be arranged in 2

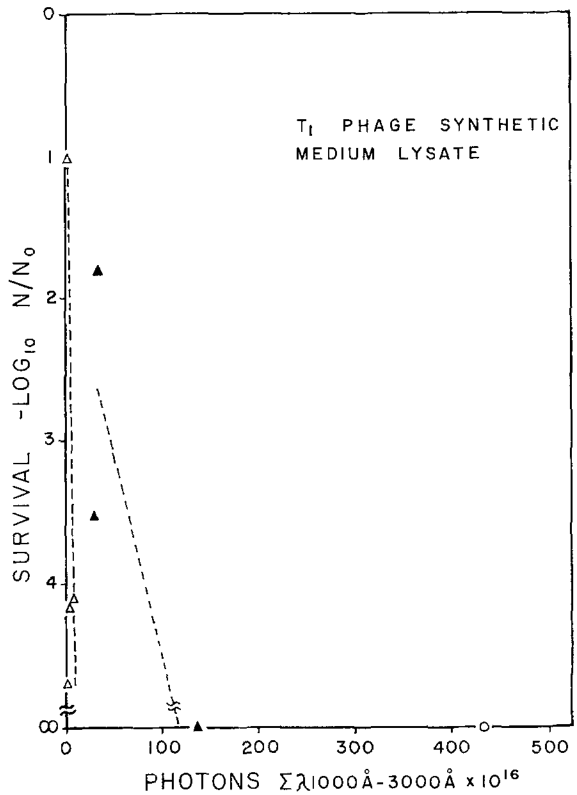


Fig. 4. Survival of $T_1$ phage synthetic medium lysate is compared with incident solar photons of wavelengths 1000 Å to 3000 Å. See legend of Figure 1 for further explanations.

exponential curves. The steeper curve was obtained from sounding rocket and satellite experiments. The more shallow curve resulted from the balloon experiments. When the survival is compared with solar photons of wavelengths 1000 Å to 3000 Å, results from rocket, balloon, and satellite experiments can be expressed by one curve (see Figure 2). This curve shows an initial steep component which accounts for the loss of approximately 80% of flown micro-organisms, then develops into a more shallow exponential slope. A similar curve was obtained with $T_1$ phage broth lysate (see Figure 3). The curve runs parallel to that of the broth concentrate at an approximately 10-fold lower survival level. For reasons described elsewhere (Lorenz *et al.*, 1968c), 2 different preparations of broth lysate were flown on board Gemini-XII. Complete inactivation of $T_1$ phage broth concentrate was found after 4 months of direct space exposure on the Gemini-VIII Agena spacecraft. In this experiment, the micro-organisms were possibly exposed to heat and air blast phenomena during launch (LORENZ *et al.*, 1968c).

$T_1$ phage synthetic medium lysate and *Penicillium* were rapidly inactivated on board rockets. Approximately 25 to 100 times as many photons of wavelengths

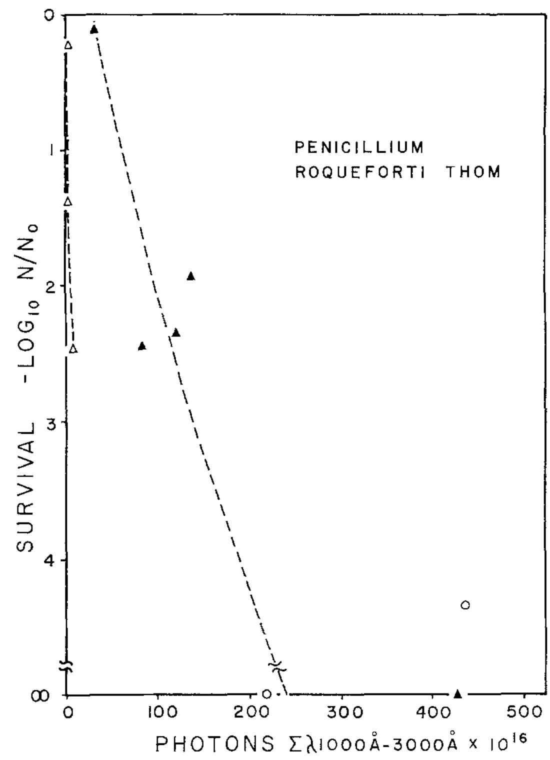


Fig. 5. Survival of *Penicillium roqueforti* Thom is compared with incident solar photons of wavelengths 1000 Å to 3000 Å. See legend of Figure 1 for further explanations.

below 3000 Å were necessary for inactivation during balloon experiments (see Figures 4 and 5). Survival of TMV is shown in Figure 6. Data obtained in the satellite experiments appear inconsistent. Comparison of the survival obtained in balloon, rocket and the Gemini-XII experiments suggests an exponential inactivation curve.

## 4. Discussion

The results reported above indicate that space-exposed micro-organisms are inactivated by solar UV, and confirm earlier conclusions based on experiments with optical filters (LORENZ *et al.*, 1968b). Comparison of survival of the more resistant preparations of micro-organisms with the numbers of incident solar photons shows that the chance of survival can be represented by exponential functions, and complete inactivation of all types of directly exposed micro-organisms was observed. This is important since it was reported that deep space conditions will probably not cause total inactivation of a population of terrestrial micro-organisms (BRUCH, 1967). However, previous experiments have shown up to 100% survival of test organisms when these were shielded or shadowed against solar radiation (HOTCHIN *et al.*, 1965;

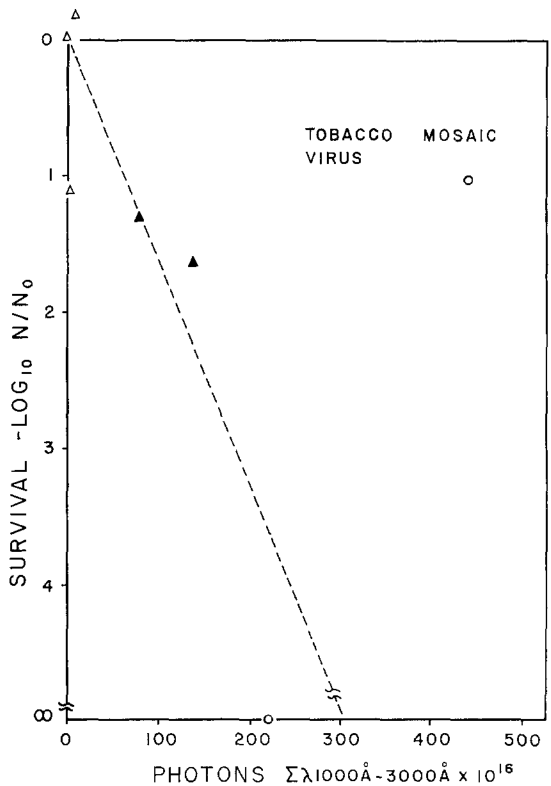


Fig. 6. Survival of tobacco mosaic virus is compared with incident solar photons of wavelengths 1000 Å to 3000 Å. See legend of Figure 1 for further explanations.

LORENZ *et al.*, 1968a, b, c.; ORLOB and LORENZ, 1968). Sterilization of spacecraft for interplanetary missions therefore appears necessary (SAGAN *et al.*, 1968).

The difference in the inactivation curves obtained in the balloon and satellite experiments shown in Figure 1 was eliminated by neglecting the spectral range from 3000 Å to 50000 Å. As we had expected, the biological effectiveness of this range was not significant. At the balloon altitude of 34 km radiation of wavelengths shorter than 2000 Å is completely absorbed by oxygen (LADENBURG and VAN VOORHIS, 1933), and the spectral range of 2000 Å to 3000 Å is partially absorbed by the ozone layer (VIGROUX, 1953). Correction of the flux intensities received in balloon and satellite experiments for exposure time yielded comparable results. This supports our conclusion that solar

UV of wavelengths 2000 Å to 3000 Å was the sole cause of inactivation of broth $T_1$ phage and, probably TMV. Involvement of UV radiation in the inactivation of viruses was also indicated by observing morphological change in TMV exposed to space during the Gemini-XII flight typical of UV damage (ORLOB, unpublished data).

Radiation damage by wavelengths of the 2000 Å to 3000 Å range can be photoreactivated (DULBECCO, 1950; LATARJET, 1956; SETLOW, 1957; ECKART and ESCHKE, 1962). It has been reported that dried $T_1$ phage does not show photoreactivation (HILL and ROSSI, 1954), but this phenomenon may be important for other micro-organisms. PETRAS and BISA (1968) have demonstrated photoreactivation of *B. subtilis* after exposure to space. It is conceivable that such micro-organisms may be deposited by interplanetary rockets on other planets and regain biological activity.

Survival also depended on the suspension medium and on the type of micro-organism. $T_1$ phage in synthetic medium is rapidly inactivated even during short-term rocket exposures. Exposure on board balloons required 25 times as many photons of wavelengths shorter than 3000 Å for comparable levels of inactivation than exposure on board rockets. A similar trend was observed for *Penicillium* spores. This indicates that radiation of wavelengths shorter than 2000 Å, which is completely absent at the balloon altitude, may have been responsible for the higher rate of inactivation in the rocket experiments. Broth medium provided effective protection for $T_1$ phage against radiation below 2000 Å while exposure to radiation of wavelengths 2000 Å to 3000 Å led to complete inactivation. TMV data indicate that this virus may have been resistant to radiation of wavelengths below 2000 Å.

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