ability of occurrence of such natural satellites is quite dependent upon the magnitude of the drag coefficient. Thus, the inclusion of the transitional-flow correction in the analysis becomes extremely significant. Investigation of the Canadian fireball procession of 9 February 1913 (*4*) is now under way in order to determine whether or not those fireballs were natural, ephemeral, meteoritic satellites of the earth (*5*).

ROBERT M. L. BAKER, JR.
Aeronutronic Systems,
Glendale, California

References and Notes

1. R. M. L. Baker, Jr., "Drag Interactions of Meteorites with the Earth's Atmosphere," doctoral dissertation, University of California, Los Angeles (May 1958).
2. A meteoritic "dust-ball" or "stone-flake" might, however, be fragmented during such a deceleration.
3. The curves, in other words, are drawn for different distances of closest meteoritic approach to the earth's surface.
4. A. D. Mebane, "Observations of the great fireball procession of 1913, February 9, made in the United States," *Meteoritics* 1, No. 4, 405 (1956).
5. It is noteworthy that F. L. Whipple, in a personal communication (8 Mar. 1957), mentioned the possible observational evidence of an ephemeral, natural, meteoritic satellite. I wish to acknowledge the many helpful criticisms and suggestions of Professors Frederick C. Leonard and Samuel Herrick, of the department of astronomy, University of California, in connection with this report.

14 July 1958

Semiquantitative Evaluation of the Gram Reaction

The Gram reaction is a special staining procedure applied especially to bacteria and certain other microorganisms. Essentially the technique consists of applying the dye, crystal violet, to a smear or tissue section, and then applying an aqueous iodine-potassium iodide solution. Subsequent washing with ethanol removes the dye-iodine complex from some species of bacteria (called Gram-negative) but does not remove it from others (called Gram-positive) during the customary time of application, 30 to 60 seconds. A counterstain of a contrasting dye, usually safranin, is then applied to color the Gram-negative cells and make them visible under the microscope. Since this procedure results in a dichotomous separation, it is one of the first and most important steps in the identification of bacteria and in diagnosis of bacterial diseases. The Gram reaction is also correlated with numerous physiological characteristics of the bacteria, particularly their sensitivity to various chemotherapeutic agents.

The advantages of a quantitative determination of the Gram reaction would be substantial and could possibly eliminate other more tedious steps in identification. Bartholomew and Mittwer (*1*) have discussed early methods for obtaining quantitative data which were based chiefly on microscopic examination of many hundreds of individual bacterial cells after various periods of time in the alcohol decolorization step. More recently, Barbaro and Kennedy (*2*) have described a different technique based on micro-Kjeldahl analysis of reagents and cells, but various difficulties in the interpretation of results have been pointed out by Bartholomew and Finkelstein (*3*). The quantitative Gram reaction techniques presented to date have been useful for research purposes and have yielded valuable data; however they are so time-consuming that they have never been adopted for routine use. The data do show that Gram-stained bacteria are all decolorized by alcohol if the latter is applied for a sufficiently long time. Thus Gram positivity can be measured as an inverse function of decolorization time, and all bacteria lie somewhere on a scale between the most Gram-negative and the most Gram-positive.

The present investigation uses the techniques of filter-paper chromatography to determine directly the relative Gram positivity of various species of bacteria (*4*). Bacteria were harvested, suspended in distilled water, and stained 1 minute with 0.1 percent crystal violet while in suspension. The stained cells were centrifuged, washed with distilled water, and recentrifuged (*5*). Lugol's iodine was applied to the cells for 1 minute, and they were again centrifuged and washed with distilled water. Small amounts of stained cell suspensions were applied as spots to Whatman No. 1 filter paper as in standard paper chromatographic techniques. The paper was then placed in a chromatography jar, dipping into 95 percent ethanol. The alcohol was permitted to ascend by capillarity, passing over the spots of stained bacteria. The dye was extracted from the stained cells in proportion to the time of contact with the continually fresh alcohol passing over the cells. This time was usually 6 to 8 hours, but sometimes overnight in the case of exceptionally Gram-positive cells.

This procedure resulted in streaks of dye which varied in length depending upon the Gram positivity of the strain of bacteria, the most Gram-positive species showing the longest streaks, as is shown in Fig. 1. Gram-negative bacteria which are very close to each other with respect to length of streaks could be compared by use of a slower-acting solvent such as propanol for 2 or 3 hours. Similarly, acetone or other fast-acting solvents could decrease the time required to obtain a comparison of a group of exceptionally Gram-positive bacteria. In any case, a direct, simultaneous comparison of several species or

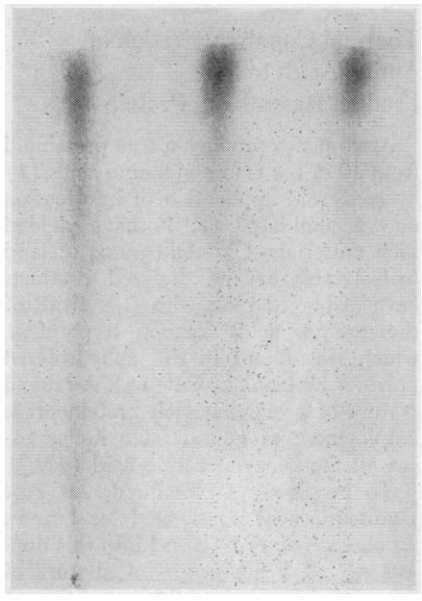

Fig. 1. Comparison of three species (left to right, *Staphylococcus aureus, Escherichia coli, Serratia marcescens*) of bacteria by the method described in the text. The top edge of the streaks represents the solvent front. Note that *Escherichia coli* and *Serratia marcescens,* although both are decan be dried and kept as permanent scribed as Gram-negative, are not of equal Gram positivity.

strains can be obtained. The filter papers can be dried and kept as permanent records. A typical series of results showed the following species to be arranged in an ascending order of Gram positivity: *Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli, Neisseria catarrhalis, Bacillus subtilis, Saccharomyces cerevisiae, Staphylococcus aureus.*

Absolute numerical parameters based upon Gram positivity cannot yet be assigned to bacteria. The streaks on filter paper can be measured as to length, dye content, and in other ways; but numerous factors affecting the Gram reaction (*1*), such as age of culture, culture medium, fixation methods, and temperature, have not yet been evaluated with this new technique. It is expected that complete standardization of all necessary details will require additional time.

TOD MITTWER
Bacteriology Department, University of Southern California, Los Angeles

References and Notes

1. J. W. Bartholomew and T. Mittwer, *Bacteriol. Revs.* 16, 1 (1952).
2. J. F. Barbaro and E. R. Kennedy, *J. Bacteriol.* 67, 603 (1954).
3. J. W. Bartholomew and H. Finkelstein, *ibid.* 67, 689 (1954).
4. This investigation was supported by research grant E-1329 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.
5. This washing step must be standardized and kept as brief as possible, since it exerts a strong influence on the results of the Gram reaction [J. W. Bartholomew and H. Finkelstein, *J. Bacteriol* 75, 77 (1958)].

14 April 1958