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Dr. J. Lederberg
Department of Genetics
Stanford University Medical Center
Palo Alto, California

Dear Josh:

My apology for the delay in sending the bibliography on spectrophotometry of turbid suspensions. Most of the papers were "lost" in my excellent system of filing reprints. This delay has given me an opportunity to consider the general problem a bit and to make a few simple measurements of the UV absorption spectrum of whole cells of Escherichia coli.

The most sensitive method for aqueous suspensions is that of Bateman and Monk, in which method the liquid sample fills a diffusely reflecting sphere. The effective path length is increased tenfold by multiple reflection; discrimination against losses from turbidity is at least 150 db; and, with suitable arrangement of the detector, this method should not demand excessive illumination from the monochromator. As far as I am aware an apparatus suitable for testing this method is not yet available.

I was able to obtain quite satisfactory spectra of small samples of E. coli dried on paper. This method is not only simple and easily adapted for automatic sampling but also gives better discrimination against scattering than the more conventional technique of re-diffusion of the light scattered by suspensions. I am enclosing the traces obtained by various methods of sample preparation. Traces 1 and 2 are of the same suspension in water with the sample placed in the rear of the cell compartment. Trace 2 was obtained by diffusion of both channels with a piece of paper. Trace 3 is a rather non-uniform film deposited on silica; the poor spectrum probably results from this non-uniformity. Trace 4 is obtained by depositing approximately the same number of cells over an area on the paper just slightly larger than the window of the cell holder. Of course the efficiency with respect to light from the monochromator is low. This can be improved by re-designing the optical path to the detector to increase the effective aperture of the detector with respect to light scattered in the plane of the sample. So much for the problem of recording UV spectra for now. You have probably considered this in more detail than I.

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Since our last telephone conversation I have begun to re-examine the basis for our original "microscope experiment." At first I was reluctant to admit that we could forego differential UV absorption as a primary basis for discrimination between particles like terrestrial bacteria and particles of minerals. Perhaps it is better to obtain detailed UV and IR spectra of the low density fraction and to limit the microscopic observation to size, shape and refractive index of the particles in the low-density fraction. Phase contrast principle seems the method of choice. Your suggestion of phase vs. brightfield

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as differential measurement has the advantage of simplicity; it could be accomplished with a filter replacing the opaque stop at the aperture of the condenser and appropriate modulation of wavelength using broad band filters to conserve the energy of the light source. Another principle, which was suggested by Silverman in the report of the committee on biological aerosol detection, is to discriminate on the basis of the dispersion. This can either replace the brightfield observation or can be incorporated into the previous system using a third spectral band. Barer and Joseph (J. Appl. Bacteriol. 21, 468, 1958). measured the refractive index of a variety of bacteria by the minimum contrast method. I am not aware of measurement of the dispersion by cells but it is probably similar to the dispersion of solutions of albumin (Richards, Tech. Pub. #257, Amer. Soc. for Testing Materials. 1959, p. 6). A somewhat different principle, related, however, by the measuring technique, is the general permeability of cells to glycerol. The refractive increment of cells is not altered by aqueous glycerol while that of particles of minerals is lower in glycerol solution than in water.

One recurring theme which plagues our planning as well as those concerned with detection of aerosols is the ignorance of many of the physical properties of bacteria which might be useful in their detection. I hope we can establish working relations with those in BW who have comparable problems. It may be possible to urge them in a direction which will be of great assistance to us and possibly profitable for them.

I am grateful that the meeting will be held in Palo Alto rather than Eugene. As much as I would like to visit Aaron's institute, I doubt that it would have been possible for me to attend.

Last but not least, I do have a likely candidate for your research associateship, Dr. Edgar H. Nilson, Department of Biology, Redlands University, Redlands, California. Dr. Nilson finished his Ph.D. with me last year; his thesis concerned the maintenance metabolism or basal metabolic rate of E. coli. He has had a rather varied career including work with Carl Lamanna at Detrick during World War II, a tour of duty at the Naval Medical Center (Bethesda), and a year of work in enzymology at the Edsel Ford Foundation. Nilson has an exceptional knowledge of electronic instrumentation for a microbiologist, some experience in construction (turbidostat and microcalorimetry), and a rather good mathematical background (differential equations, vector analysis). He is quite dissatisfied with the opportunity for research at Redlands and would undoubtedly welcome an extended leave of absence. He may have just the combination of qualities which you desire. I will be happy to write a detailed recommendation for him.

Sincerely yours,



Allen G. Marr
Associate Professor

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Encl.