

PROGRESS REPORT ON WORK AT STANFORD ON EXOBIOLOGY

This will be rather brief although we have had notification of support from NASA since about mid-May. Procurement has been slow and components have only recently been assembled so that we can begin to check out a system of UV Vidicon microscopy. The new Zeiss achromatic UV objectives have just recently arrived and these appear to be far superior to the previously available mirror objectives and catadioptric lenses that were previously available. We have assembled a system for microscopy at 2537 Å using a low pressure mercury source, interference and chemical absorption filters (neither of them very satisfactory), the Zeiss objectives, and a television chain incorporating a UV-sensitive Vidicon tube (two samples of the RCA Vidicon and one from EMI in England). The latter seems to give a superior picture with better resolution, short lag and, probably, higher sensitivity. However, both tubes, although designated as UV-sensitive, are rather disappointing in their performance at <3000 Å and probably have not been designed primarily for such an application. They would be relatively satisfactory if we could operate at considerably higher intensities of monochromatic UV illumination but this is difficult to obtain in any event and should probably be avoided because of its potential damage to viability and to organic materials. The present microscopic arrangement is, however, already quite satisfactory for photographic recording. The answer to our present problems might lie a) in improvement of the light source and filters, or perhaps better the incorporation of a simple monochromator and b) the use of the much more sensitive image-intensifying UV tube, the Ebicon. In any case, this development should be just the first step of a system in which images can be compared as between the ultra-violet and the visible. (The flying-spot microscope design is capable of much higher intrinsic sensitivity than the Vidicon image tube, since a photomultiplier can be used as the detector; however, the Ebicon may give a comparable result.)

As an alternative to the use of ultra-violet for the visualization of transparent objects, the now technically much simpler phase contrast optics might be employed. Again these would be especially advantageous if a simultaneous recording could be made of the form of a particle in phase contrast and its transparency by transmission optics. This might be converted into color rather readily by the use of narrow pass filter elements in place of opaque diaphragms in the phase optics ("color phase contrast").

Much more information can then be obtained from UV optical methods by obtaining an absorption spectrum, particularly in the range between 2400 and 3000 Å. This UV information would be parallel to the image formation, and both sets of data might be collected with the same optical system, the scan alternating between wave length and position of the beam.

As far as I can determine, UV spectra of individual bacteria obtained in such a fashion have not been published, but this partly reflects the limitations of the microscope optics available until now. The Zeiss achromatic objectives were designed for just such analytical purposes at the instance of Caspersson in Stockholm. He believes that it would be technically feasible to obtain such spectra, and has agreed to run some representative cultures on his own instrument during the next few months. Serious problems, e.g. correction of scattering

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losses, and especially the installation of satisfactory light sources must be dealt with, but this approach is potentially the most powerful that could be devised for the analysis of micro-organisms by remote technique. If the technical development is successful, particles could be scanned for their spectra, and this information then used to monitor the transmission of selected images which consume more communication time.

Some of the most encouraging results obtained so far have been in the fractionation of organic material from soils. After a considerable search for suitable solutions of adequate density, most of which were for one reason or another rather unsatisfactory, we tested "Ludox" at the suggestion of Kopac from NYU. Ludox is a colloidal suspension of silica in water and can be made to a density as high as 1.20, which is more than adequate for the flotation of living cells, while almost all minerals still sediment in it. The silica being chemically almost inert and having no appreciable osmotic effect is relatively harmless to the cells and leaves them quite viable. A number of types of soil sample have been fractionated by flotation in Ludox and the method has proven to be startlingly efficient in the detection of micro-organisms even from rather unpromising sources, for example, volcanic sand collected at 14,000 feet from Mt. Popocatepetl; old dried mud from a core dredged from the Mariana Trench, and dust that had settled on the roof of a car and exposed for appreciable time to California sunshine. In each case, the supernatant fraction was highly enriched for bacteria and these could readily be seen at the very first glance through the microscope. The same samples had only a marginally detectable microbial population when examined directly. Samples from richer soils also displayed an abundance of other kinds of organisms, with relatively little interference from residual adherent soil particles. We are now seeking even more marginal samples to see whether there is any likely sample from the earth's surface in which we would not readily detect microbial life by this method. The flotation-separation is facilitated by spinning the samples in a centrifuge and larger samples could be efficiently processed by a continuous flow sedimentation. However, smaller samples will sediment if merely allowed to stand several hours. We are now designing a cell which will allow for microscopic observation with no handling after the sample is introduced into the cell. The relatively low density of living particles, having an aqueous base, should be a fairly general principle for the preliminary separation of them from mineral fragments. More refined criteria are of course needed for a final conclusion.

(This method of separation promises to have great value as a general laboratory procedure and may have practical applications in other areas of medicine.)

The basic feasibility of the proposal to grow bacteria on permeable tapes has been verified; flattened tubes of cellophane dialysis tubing were impregnated with nutrient broth and would then very satisfactorily support the growth of various bacteria and fungi. These were then very informative when examined under the microscope. The chief problem appears to be the loss of moisture by evaporation so that the tapes will have to be maintained in a closed humidified chamber. In view of the long transit time of the space craft, the tapes may have to be flown dry and filled with water only at the time of the experiment; alternatively nutrients may be prevented from diffusing from one site to another by mounting the

culture spots as blisters on an impermeable, e.g. quartz, ribbon. We have not succeeded in obtaining quartz ribbons as might be extremely useful as tapes for the indicated purpose but they should not be too difficult to fabricate. More mechanical skill and ingenuity than we can readily muster should be centered on the production of prototype hardware along these lines for testing and improvement.

Recommendations

Our experience to date suggests that the basic strategic principles that had been formerly enunciated are sound and should be exploited further as the basis of exobiological detection. However, as we have now reached the stage where considerable new hardware, both electronic and mechanical, must be designed and built, it is essential that we have the cooperation of an engineering facility. Needless to say, we would be very happy to maintain a close continuing contact with such a facility and in particular to assess the results for their biological usefulness.

In view of the pressure of vehicle schedules, it would be foolhardy to delay the development of prototype hardware for the exploration of every worthwhile accessory. New technical advances and new insights into scientific goals would lead to indefinite postponement of any actual experiment.

Enough information is now at hand to justify the construction of the basic mechanism of an automatic microscope. This should have provision for the transport of specimens, focus, photoelectric conversion, color discrimination, signal reduction and telemetry. The first version might be designed to use color phase contrast, which is technically simpler than UV microscopy. This basic instrument would be a valuable tool even if time did not permit the addition of the accessories to improve its sensitivity and precision. Concurrently, design work should proceed on the technique of collection of samples, and on their centrifugal fractionation, to furnish the input to the detector system. This much of the system should lie within the reach of present art. Study work and preliminary trial are also required for the microspectrophotometer accessory to the microscope, which does pose serious technical problems, especially the provision of a suitable light source. A bench model should be constructed for tests on terrestrial samples as a preliminary to its adaptation to the system for spaceflight. By concurrent work on the particularized problems thus indicated, we can be sure of having the best instrument that we had the means to develop in time for schedules of planetary flight. There are cogent reasons besides the relative scientific impatience of biologists why such instrumentation should accompany the earliest planetary explorations. Laboratory work in biology does not now have the benefit of a well-established tradition of development of instrumentation. Much more than in the physical sciences, spaceflight research in biology will have to develop new instruments for which only the principles, and not their technical realization, are already current in laboratory practice.