Memo to File

Resumé of discussion with Caspersson, Feb. 14-18, 1961.

A tentative agenda was set up for Tuesday morning, Wednesday afternoon and Thursday morning to cover the following items:

1) His measurements already conducted of bacterial spectra.

2) Plans for continued collaboration.

3) The design of his instrument.

4) Fundamental measurements that may be required for background.

5) Our technical plans.

6) Organizational problems including suitable staff.

Most of these topics were, in fact, discussed during succeeding conversations to varying depths. This memo is in draft form and should summarize most of the items although not necessarily in a completely connected fashion.

1. We discussed our progress in the development of selective filters for the ultraviolet. He had not been acquainted with cation X nor with the G4S11 lamp. He was not deeply impressed with the possibility of increasing intensities from an extended source. However, increases of the order of a factor of 3-4 were conceded and this might well become quite important for us. Caspersson does not seem to be too concerned about intensity limitation for his own studies. He uses primarily a high pressure mercury lamp, Phillips SP500, which he believes to be essentially equivalent to the GE lamp AH6.

2. Some aspects of his UMSP, the ultramicrospectrophotometer. The high pressure lamp shows a fall off at the reversion interval but this is mainly well below 250 nm. His system now uses a Zeiss prism monochromator, I believe. He has never measured the insertion losses of the optics and at lower wave lengths, they vary with respect to frequency and also in time. This is one reason that he does not consider a double path system to be quite satisfactory and prefers instead to make intermittant recordings of a clear area and of the sample area. He finds it necessary to make the reference recordings within the same microscope field. Although he has never made precise measurements, he would consider that the optical efficiency of his system is at least 25% even down to 240 nm. In order to reduce glare, the illumination spot should not be many times larger than the object being studied. In our case this could well be of the order of 1 micron. The measuring spot in the image plane can be reduced further to about 0.3 microns. Some of his very elegant spectra are obtained by direct spectral scanning of a selected spot in the center of the bacterium. However, for further precision he will often make a zig-zag line scan across the bacterium at each wave length and use the intensity at the central saddle of the traverse as best representative of the actual absorption. There is a halo effect at the edges of the bacterium due to the refraction of the light by the cell. The refractive index of the cell material may be in the

range of 1.42-1.45.

3. We had some discussion of the distorting effects of incomplete obscuration of the measuring spot by the organism and he feels that it may be important to allow for mechanical scan in order to optimize the absorption. However, the main effect of this is not to introduce any spurious qualitative results in the positions of absorptions but to add a considerable amount of what does amount to stray light so that the absorption differences will become a smaller percentage of the total beam.

4. He proposes the, and in fact has used, optical systems for further calibration.

a) The separation of apair of convex and concave lenses for focus and

b) The horizontal movement of a thin lens for centering.

5. He believes that we should use a 100:1 projective lens for our measurements. At the present time we have a 10:1 lens and this may not give adequate magnification.

6. Caspersson was not too optimistic about the possibility of keeping several objects in focus at the same time for the multiple rapid scan that we had envisaged. However, this will have to be determined by experience.

7. Caspersson uses polaroid film for routine photography. For more careful work he uses Ilford Photomechanical film.

8. Although in the past Caspersson had expressed some diffidence about this project, he now indicated that this was mainly because he did not know how seriously it was being undertaken and in the present circumstances, he is ready to furnish the most detailed assistance. We therefore had a preliminary discussion on the kinds of materials that he might fruitfully examine. We concluded that for the most part, it might be best if we sent him the appropriate samples. The problem of how to fix the material arose and we considered that this might be a rather serious problem. However, for the time being, suspensions in alcohol can be shipped and he can then prepare the necessary slides. The possibility of handling the material in polyvinyl alcohol films was also discussed. There was some persistent confusion about this until it was clarified that this is a water soluble material. Caspersson raised the point that it should be possible to use oils in place of glycerin as the immersion fluid and if necessary that Zeiss could make any required re-adjustments in the correction of the lens for the purpose. The present Zeiss lenses are not homogeneous immersion lenses. If such an oil can be used, then as we discussed, it might become practical to have the entire sample holding mechanism immersed in the oil. This might simplify the problem of how to conduct the immersion observation.

9. For more detailed work a suitable precipitant for DNA should be found and possibly lanthinum in acetic acid may serve this function. He was not well acquainted with the properties of the lanthinum salts in aqueous media and this should be further studied. His own approach to this problem with tissue

work has been to avoid aqueous media that might be capable of extracting nucleic acids. He has done considerable calibration work on this and finds that the absorbtivity of his specimens in the ultraviolet does not change over long periods of time when they are immersed in water-free glycerin; however, small amounts of water do distort the picture.

10. The Zeiss lenses are calculated for quartz slides of .35 mm thickness. The same is used as a cover glass. We have to investigate sources of such slides for mounting our specimens. Meanwhile, however, Caspersson should obtain these for us although it might be well to remind him.

11. We discussed the density separation technique in Ludox which on further discussion may become not only desirable but essential for the differentiation of mineral from organic particles. For example, iron oxide which may well have an embarrassing cutoff at around 280 nm should have a density of 5.2. We still have to determine whether a thin layer of Ludox will cause an inordinate amount of scattering for the microscopy.

12. Recapitulate on materials to be exchanged: alcohol suspensions of _B. subtilis_, _E. coli_, low density fraction from soil. In addition, some of these samples can be mounted in PVA, then preparations of particles of ferric oxide and other materials and other soil components that perhaps should be scanned on a routine basis.

13. Our agreement to furnish $5,000 towards the stipend for a technician in Caspersson's laboratory. The detailed way of arranging this will have to be cleared through NASA and Stanford but should afford no problems.

14. Possibility of replacement of glycerin as an immersion fluid. Caspersson is looking into this but we should be checking the refractive index on various oils also.

15. Question of source information on mineral absorption spectra. The Landahl-Bernstein handbook and then there is a tabulation some time ago in Tabulae Biologiae by Elsevier Press by Ellinger. Caspersson also thinks that Hilger has a booklet on this. Perhaps this is referred to in some of the other Hilger books for example, _Lothian_. (Perhaps see also the University of Michigan summary on optical materials.)

16. Caspersson placed particular stress on the need to localize the optical axis on the particle and it may be necessary to scan for maximum absorption, perhaps in the low ultraviolet.

17. The salary stipend would represent about half of Caspersson's costs and he would like to consider this as a mutual collaboration.

18. We are still somewhat confused as to the present market status of the Zeiss instruments. According to my notes, Caspersson said that Zeiss was marketing 10 instruments perhaps by the end of this year. In any case, it probably would be best if either Elliott or I saw the instrument in operation in Stockholm before making a firm order. On the other hand, it might be well to place a tentative order in order to be on the priority list.

19. Caspersson's plans are somewhat indefinite. He will be returning to Stockholm in about a week and he then has a firm date in Geneva on March 25. He had previously planned to spend 3-4 weeks in Boston after the Geneva meeting. This would have precluded my own seeing him after Florence. He will inquire in Boston (Farber) about the possibility of altering his schedule and will write me by the end of February. If he can alter his itinerary, then he could be back in Stockholm by the 23rd of April. This could match my own plans as I should be back by the 29th.

20. Fundamental measurements. Caspersson can investigate the light scattering envelope of bacteria by looking into the effect of the aperture of a field diaphragm in his microscope on the amount of light collected (a similar procedure is indicated in a paper by Brackett in the JOSA, 1959). For tissue materials, the largest part of the "scattered" light is found within 20 degrees of the forward beam. From the shapes of the curves obtained by scanning across a bacterium, he anticipates that much the same will be true in the ultraviolet for bacteria and that there should be no real concern as far as the microspectrophotometry is concerned. However, the problem of bulk samples of turbid materials may be much more difficult: 1) in dilute suspension only a fraction of the beam is attenuated and the signal is a rather weak one superimposed on a rather high pass 2) With more concentrated suspensions, one must deal with considerable losses due to multiple scattering before the entire beam is obscured. We agreed that it would be desirable to measure the scattering envelope of our specimens as a necessary basis for designing the optical attachments to a conventional spectrophotometer (in subsequent discussion with the Beckman people, they agreed to furnish the optical constants so that we will not have to measure the collecting angle ourselves).

21. Caspersson brought with him several examples of spectra on individual bacteria. Most of these cover the range between 250 and 300 or 310 nm. They very considerably from one specimen to the next, in some cases, absorbancies of as much as .5 at 260 nm compared to .1 at 300 are seen.

22. Caspersson agreed in principle that he might specifically look for a technician that could be used on this program in his laboratory and after perhaps a year's work might be available to come here on loan for continuation of the program. He does not know whether he will be able to find the appropriate person.