Children's Cancer Research Foundation, Inc.

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Director of Research

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Dear Joshua:

Thank you for your letter of April 16 with the questions about the cytochemistry of human chromosomes. I share your views about the importance of that field and also its potentialities for automated large scale surveys in the future.

During the last 2 1/2 years I have had a projectx running, very close to your ideas. At this moment I am just writing up the results. The evaluated data are unpublished and for you. Confidential.

The basic problem was to find out which accuracy one could get at the determination in ordinary metazooan metaphase chromosomes of 1/ DNA 2/ total mass and eventually 3/ RNA. Regarding 1/ the obvious difficulty is in the fACT that the chromosome dimensions are so close to the wavelength of the light to be used and thus the measurements are on the very limit of the theorethically possible. Physical theory is of no real use here as the theory of the microscope image is not completex in that range of dimensions. We have thus made an empiric approach using Chinese hamster, rye and human chromosomes. The spectrophoto metry has been made with somewhat modified models of our high resolution instruments. Quite a large amount of work has gone into that - a numbe r of technical problems arose - but I believe that we are as close to the physical limit in the measurement as is possible.

I include as an example Feulgen and UV measurements on (RNA-free) hamster chromosomes presented graphically. Feulgen determinations can thus be done with an accuracy of about (or possibly somewhat less than) 5 %. UV gives somewhat lower accuracy due to adhering extrachromosomal substances. However, while Feulgen, because of its inherent irreproducibility, gives only information of which %-age of the DNA of the total metaphase plate which is present in one chromosome, the UV gives fairly good absolute values. Our material on Chinese hamster chromosomes is fairly comprehensive. Total mass determinATIONs can be made interferometrically, however with an as yet only estimated error of more than 10 %. We hope to apply the by us for the purpose several years ago developed electrommicroscopic chromosome-mass-determination procedure in the near future but have not started yet. For RNA we have at long last now a good fluorimetric technique which permits the direct determination of RNA in a mixture with DNA - as you know no digestion procedures work well in such systems. I look forward to apply that on different chromosome problems next year.

Rye was used specifically in order to investigate the possibility to identifyxxx follow the DNA-distribution along the chromosome for the purpose of identification and, in the first line, work just now being started on chromosome changes in virus infected materials. It might amuse you to see in the

enclosed sheet giving the DNA-distribution along four different rye chromosomes how the chromosomes distinctly differ (reflecting also details ~~also~~ not visible to the eye) fromeach other and how chromosomes with the same number give reasonably similar patterns.

Regarding the human chromosomes we have also some material. That object is more difficult than the Chinese hamster and the accuracy which can be reached*) is not enough to characterize more than a limited number of individual chromosomes. It would take too long to go into details here, but I am convinced one could get further here with some technical investments, which I am sorry to say we are not able to make just now. Anyhow we have during the last weeks in fact considerably increased the possibility to get good measurements on the small human chromosomes by a new arrangement for quantitative fluorimetry. This arrangement might possibly come closest to your idea of ~~of~~ automated measurements in human materials.

The above is cited as an indication that one can, I believe, already now fairly well define what is technically feasible on the field you outlined. We are now applying the experiences won as yet on virus problems and on differentiation and ageing problems - a long and tedious work no doubt, but the technical possibilities for work on metaphase chromosomes have in the work cited above proved to be considerably better than I believed 3 years ago.

Thank you also for your reprint which contains many refreshing thoughts.

With best personal regards I am
~~sincerely yours~~

Torbjörn Caspersson

*) in the DNA-determination

Encl

P.S. I believe several people are interested in this type of work and as we are now writing it up after a quite long period of quite hard work I would appreciate your keeping the data confidential.