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PARTITION PHENOMENA IN TWO PHASE POLYMER SYSTEMS AND THEIR SIGNIFICANCE FOR PARTICLE SEPARATION

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SVMMARIVM — Nova methodus, eaque valde simplex et efficax, describitur, qua submicroscopicae cellularum particulae necnon cellularum fragmenta separari possint. Disputat autem praeterea Auctor de huius facti pondere ac momento.

Scientists — and also the laymen — have gradually become accustomed to the idea that significant advances in to-day's research practically always require complicated and expensive apparatus. Still we know that often interesting discoveries are made even in these days using the simplest kind of equipment. I have nothing against adequate first class equipment — on the contrary I believe it is essential for any laboratory who wants to contribute to the advance of science among hand-competition. But it cannot be helped that one feels a certain satisfaction that things *can* be done with simple means, a satisfaction which perhaps is not so easy to explain. But even one of the great masters of experimentation of all times — ERNST

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RUTHERFORD — once said « Now that we have no money, we shall have to sit down and think ».

The experiments which I wish to report in this brief communication were not exactly started against such a dark financial background, but the incentive came from other sources. One of the main problems during recent years in my laboratory in Uppsala has been the development and exploration of methods of fractionation and isolation of submicroscopic particles of biological origin, especially particular elements in the cell or fragments obtained by far-going disintegration of tissues. There are several reasons why research into the structure and function of such submicroscopic particles is attracting so much interest in to-day's biochemical research. First: Essential biochemical reactions seem to be localized in submicroscopic particles occurring in the cells. Thus respiration and oxidative phosphorylation are localized to the *mitochondria* (their size is about at the limit of microscopic resolution) and important biosynthetic reactions in the *microsomes,* which are about ten times smaller (about 15-20 mμ).

Second: the smallest particles showing some essential characteristics of Life: *viruses* and *bacteriophage,* are of similar or even smaller dimensions. In such particles enzymes and other biologically active substances are fixed and probably arranged in a very specific structure which is essential for their function, especially in the all-important sequences of coupled reactions. For isolation of submicroscopic particles ultracentrifugation has generally been used, but there is a great need for more specific methods. Of such new procedures I shall refer to only one, worked out by Dr. ALBERTSSON in my laboratory. As most of you probably know the simplest of all chemical separation methods is the so-called partition method, where one makes use of the distribution of a substance between two phases — that is to say, between two immiscible liquids. Such partition experiments using for ex. water and an organic solvent, like benzene, are very common in organic chemistry.

Now, one may ask if this simple and efficient method could be used for the fractionation of biological particles.

Experiments of this type have been very discouraging, and the reasons are 1) that there is no partition: the particles stay in the aqueous phase or go to the boundary 2) the particles and the sensitive substances they contain suffer from the contact with organic solvents and are more or less damaged. It is easy to see that in order to have any reasonable chance of success one must arrange so that both liquid phases are « friendly » towards the biological material, which means that they both must contain water as the dominant constituent. This is still not enough — the two phases must be very similar in physical properties and chemical composition in order to be able to give a real distribution of the particles. If the phases are too different in their properties the particles will tend to concentrate in one phase only, or at the interphase.

ALBERTSSON now could show that such systems can be made very simply, making use of aqueous solutions not of ordinary substances but of polymers. If we dissolve for ex. 1% dextran and 1% methyl cellulose in water, we will obtain a solution containing 2 phases, separated by a sharp buondary. One phase will contain practically all the dextran, the other practically all the methyl cellulose, but both contain ca 99% water and still form two separate phases. The phenomenon is very striking in itself, but becomes still more striking if biological particles like bacteria, viruses and even whole cells are introduced into the system. Well defined and reproducible partition coefficients can be obtained, including also the boundary region, and these coefficients depend upon easily regulated parameters, as for ex. the salt concentration. In many cases where the partition is of « all or none » type, a change in salt concentration will cause a shift of the particles from one phase to another and vice versa. Obviously this principle offers a wealth of new and highly specific procedures for particle separation.

These experiments require only a few test-tubes and some easily available, commercially produced polymer substances. They have opened up a most interesting field of experimentation, and a number of rather striking phenomena have been observed. I would like to emphasize that our interest in these phenomena is not limited to their application for particle separation. We believe that they may have some fundamental role in the growth and the development of the living cell. We have in these partition phenomena a mechanism by which considerable quantities of biological matter (in the form of microscopic or submicroscopic particles) can be transported from one compartment to another across a phase boundary by comparatively small changes in certain parameters like the salt concentration. It appears quite possible that for ex. the cytoplasm would contain polymers which would tend to give use to such phase phenomena under physiological conditions. This is at least an attractive field for speculation but also — as we hope — for future experimentation.

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