THE ULTRACENTRIFUGAL PURIFICATION AND STUDY OF MACROMOLECULAR PROTEINS

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THE development of air-driven ultracentrifuges provides a new way of preparing and studying proteins and other substances with large molecules. These biological materials have previously been extracted and purified from the tissues in which they occur by chemical methods that ordinarily involve a salting-out with concentrated solutions of ammonium sulfate or similar salts. The successes of Northrop[1] and others in crystallizing several digestive enzymes and of Stanley[2] in preparing crystalline tobacco mosaic virus protein are examples of the fruitfulness of this salting-out process. There are, however, many unstable substances with specific biological properties—such as protein-linked hormones and viruses—to which these chemical procedures have not been successfully applied. Ultracentrifugation, in the sense of centrifuging at speeds sufficiently high so that molecules are actually sedimented, has already provided a number of these less stable proteins in purified form. Not only can the ultracentrifuge thus serve to isolate substances whose molecules are profoundly altered by strong salt solutions, but it furnishes an alternative and often a better way of purifying those biologically active proteins that are amenable to chemical methods of extraction. For example, the globulins in blood serum, including the antibodies, are readily concentrated in the ultracentrifuge without the molecular damage that seems to be the inevitable consequence of ammonium sulfate precipitation; and we are now finding that the molecular homogeneity of most heavy proteins made in the ultracentrifuge is greater than that of the same protein made by the usual chemical methods. In such cases it is hard to avoid the conclusion that the molecular state of the ultracentrifuged sample is nearer that prevailing in the animal or plant from which it is derived.

The ultracentrifuge was first developed by Svedberg[3] about twelve years ago as a means of studying the rates of sedimentation and the particle sizes of colloidal suspensions. He soon found that his methods were equally applicable to the molecules of protein solutions; during the intervening years he has carried his machine to a high degree of perfection and has used it in a remarkable series of investigations into the sizes and shapes of protein molecules.

In spite of the great possibilities inherent in these methods of Svedberg, they were not adopted by others because until recently nobody else could afford them. The air-turbine[4] has figured in several attempts to develop machines[5] for duplicating the type of measurement made by Svedberg. Ours, which we have been using routinely over the last two years, employs the same optical systems as Svedberg's and has rotors of the same size; over the range to which we have applied it—for molecules not smaller than hemoglobin—it yields results of the same accuracy. Its cost is a few per cent. of that of the oil-driven machine.

A great advantage of the air-driven ultracentrifuge lies in the ease with which large volumes of liquid can be centrifuged. For this it is merely necessary to replace the analytical rotor by a suitably shaped block of metal drilled to hold containers for the liquid and equipped with a vacuum-tight cover. Bauer and Pickels[6] first described a quantity head of this type which they used for the concentration of the activity of yellow fever virus. We employed a similar head for the concentration of the horse pneumococcic antibodies[7] from serum and for the crystallization[8] of the tobacco mosaic virus protein from plant juice. During the last few months we have paid considerable attention to the design of these quantity heads. Those now in use hold 150 cc and can be brought to speed, or stopped, in from five to ten minutes, depending on the desired field. If the need should arise still larger volumes undoubtedly could be handled. These rotors can be used to produce fields of between 250,000 and 300,000 times gravity[9]—50 to 100 times the fields developed in ordinary centrifugation.

The use of the centrifuge to isolate materials the size of the larger viruses is not new. Craigie[10] has developed a method of preparing purified suspensions of the elementary bodies of vaccinia which employs

[1] See J. H. Northrop, The Harvey Lectures 1934–5, p. 229 for bibliography.

[2] W. H. Stanley, SCIENCE, 81: 644, 1935; *Jour. Biol. Chem.*, 115: 673, 1936.

[3] See T. Svedberg, *Chemical Reviews*, 20: 81, 1937, for bibliography.

[4] E. Henriot and E. Huguenard, *Compt. rend.*, 180: 1389, 1925; *Jour. phys. radium*, 8: 443, 1927. J. W. Beams and E. G. Pickels, *Rev. Sci. Instr.*, 6: 299, 1935.

[5] J. W. McBain and C. M. O'Sullivan, *Jour. Am. Chem. Soc.*, 57: 2631, 1935; J. Biscoe, E. G. Pickels and R. W. G. Wyckoff, *Jour. Exp. Med.*, 64: 39, 1936; R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instr.*, 8: 74, 1937; J. H. Bauer and E. G. Pickels, *Jour. Exp. Med.*, 65: 565, 1937.

[6] J. H. Bauer and E. G. Pickels, *Jour. Exp. Med.*, 64: 303, 1936.

[7] R. W. G. Wyckoff, SCIENCE, 84: 291, 1936.

[8] R. W. G. Wyckoff and R. B. Corey, SCIENCE, 84: 513, 1936.

[9] R. W. G. Wyckoff, SCIENCE, 85: 390, 1937.

[10] J. Craigie and F. O. Wishart, *Brit. Jour. Exp. Path.*, 15: 390, 1934.

the ordinary angle centrifuge. Bechhold and Schlesinger[11] have obtained very concentrated solutions of *B. coli* bacteriophage by coating the inner surface of a container with gelatin and centrifuging until the phage particles or molecules had become embedded in the gelatin layer. New methods of isolation are possible with the quantity ultracentrifuge because it produces fields great enough so that substances with very large molecules are sedimented as solids.

Last summer Stanley and I isolated the unstable protein responsible for the latent mosaic disease of potatoes by ultracentrifuging the juice of infected plants. This plant juice contained, besides the virus protein, much colloidal cellular debris and many unsedimentable proteins and other light materials in solution. After a couple of ultracentrifugations had eliminated most of these impurities a faint, sharply sedimenting boundary was photographed with the analytical ultracentrifuge. The protein causing this boundary was purified by further ultracentrifugations guided by additional observations with the analytical ultracentrifuge. This year in Princeton we[12] have applied the same method to the isolation of other plant virus proteins. Our success led Beard and myself[13] to see whether a similar heavy protein was present in the infectious warty tissue of virus-induced rabbit papillomas. We found such a substance and demonstrated that the virus activity was intimately associated with it. During the last six months more than 100 milligrams of this protein have been prepared and many of its biological, chemical and physical properties determined. Similar heavy proteins[14] have been demonstrated in ultracentrifugal concentrates of other virus-diseased animal tissues, so that it is clear that such pathogenic substances are not uncommon among both plants and animals.

The ultracentrifuge has been useful not only as a means of preparing these unstable virus proteins but as a way of characterizing the protein itself, of determining the degree of its purity and the extent of its concentration at each step in the isolation. A pure protein in true solution consisting of molecules all of the same size and shape will sediment at a constant rate in a sufficiently intense uniform centrifugal field. This rate as measured by the sedimentation constant is greater the heavier the molecule. It is determined by photographing with a suitable optical arrangement the sedimenting boundaries that arise between protein and solvent. Especially when dealing with very heavy molecules, those a million or more in weight, the sharpness of the boundary is an immediate index of homogeneity in molecular size. By studying this sharpness it is possible to follow the spontaneous disintegration of an unstable protein or the extent of molecular damage that has been done by extractive procedures and chemicals. In this way we have recorded the changes brought about in the tobacco mosaic[15] and potato X virus proteins by ammonium sulfate precipitations as well as the alterations that occur when these proteins are aged or left in phosphate buffer solutions. Many other things about the purity of a protein solution can be learned from its sedimentation pictures. It is especially instructive to compare in this manner the inactivation of a virus and the disintegration of its protein molecules. Beard and I[16] have done this for the papilloma protein and shown that the two parallel one another. Heavy proteins fall apart in various ways. Some, like the encephalomyelitis protein,[14] break up into many small "unsedimentable" fragments; others, such as the papilloma protein in strong acid, break into one or two large pieces. Under suitable conditions it is possible to observe a splitting up into pieces that get smaller with time. Many protein denaturations undoubtedly could be followed quantitatively and recorded photographically in this fashion.

Protein molecules do not necessarily fall apart when they change. Thus the secondary boundary that appears in the tobacco mosaic and other proteins with aging or mild chemical treatment is probably an expression of altered molecular shape. The gradual increase in boundary diffuseness that also accompanies some mild chemical treatments can be construed as the result of innumerable minute and varied molecular alterations. Carried to an extreme, these changes lead to a heterogeneity and diffuseness of sedimenting boundary such as are characteristic of a colloidal suspension. A new and interesting phenomenon was encountered during the study of solutions of Northrop's[17] staphylococcus bacteriophage. Chemically purified and highly active solutions of this phage showed a moderately sharp boundary which sedimented at the rate to be expected from a protein molecule with a weight in excess of fifty millions. If such a solution is inactivated by heat, this boundary is replaced by an exceptionally sharp but slowly sedimenting one. It seems evident that the material causing this is not, as one might at first think, made up of small molecules; it is instead a dilute protein gel that within certain limits can lose and reimbibe water.

On account of its success in purifying viruses, most of the applications of quantity ultracentrifugation have been to these disease-producing agents. Some

[11] H. Bechhold, *Kolloid-Zeits.*, 66: 329; 67: 66, 1934; M. Schlesinger, *Biochem. Zeits.*, 264: 6, 1933.

[12] W. M. Stanley and R. W. G. Wyckoff, SCIENCE, 85: 181, 1937.

[13] J. W. Beard and R. W. G. Wyckoff, SCIENCE, 85: 201, 1937.

[14] R. W. G. Wyckoff, *Proc. Soc. Exp. Biol. and Med.*, 36: 771, 1937.

[15] R. W. G. Wyckoff, in press.

[16] R. W. G. Wyckoff and J. W. Beard, *Proc. Soc. Exp. Biol. and Med.*, 36: 562, 1937.

[17] J. H. Northrop, SCIENCE, 84: 90, 1936.

exploratory runs already made with bacterial extracts, with undiseased tissues and particularly with the glands and organs of special function indicate clearly that many other new and biologically important substances can be prepared and studied with the ultracentrifuge.

The virus proteins themselves are of interest to the physical as well as to the biological chemist because they provide the largest known molecules. The researches of Svedberg have shown the existence of normal proteins in a graded progression of sizes up to the hemocyanins with a molecular weight of several millions; recently[18] he has shown pictures of a polymer of thyroglobulin which must weigh about fifteen millions. The viruses, in their turn, reach in an apparently unbroken series from particles with a size somewhat less than that of a hemocyanin molecule up to the microscopically visible elementary bodies of the pox diseases. The analytical ultracentrifuge has demonstrated that in the unaltered state the molecules of several of the smaller virus proteins are of equal size and shape. The elementary bodies of vaccinia also give sharp sedimenting boundaries and hence are of uniform size, but existing experiments are inadequate to show whether they resemble molecules in other ways. All the colloid chemist's investigations thus far have been made on suspensions of more or less heterogeneous particles; working with virus proteins he has at hand substances possessing the molecular properties of a solution of a pure compound.

In spite of the possibilities for research which these very large protein molecules offer to the physical and colloid chemist, their greatest interest to chemists as well as to others must lie in their ability to cause disease. Ever since the filterability through stone filters and the consequent sub-microscopic size of the contagious principle of virus diseases was first demonstrated, there has been debate about the fundamental nature of this principle. Most pathologists have held to the belief that it was an especially small living organism, and research on virus diseases has been dictated by this outlook. From such a standpoint it is inevitable that one should look upon a virus protein not as the virus itself but as some sort of a carrier of a small, living viral agent.

This assumption can hardly be disproved in any individual instance, but rapidly accumulating evidence makes it increasingly difficult to maintain. The detailed work of Stanley[19] on tobacco mosaic virus protein indicates that the infectivity is a property of the protein; and no way has yet been found of dissociating viral activity from the less stable virus proteins prepared with the ultracentrifuge. Except for the tobacco mosaic protein which constitutes so large a part of the total protein of diseased plants, the virus proteins are many times more infectious, per unit weight, than the material from which they are derived. During ultracentrifugal isolation their infectiousness rises as long as the heavy protein is being concentrated; when it is pure, the specific infectivity is not increased by additional centrifugations. The virus and the protein therefore sediment at the same rate. They have other physical properties in common. The encephalomyelitis protein spontaneously disintegrates at much the same rate that governs the disappearance of infectivity; and Beard and I have found that the papilloma protein falls apart at those pH's where the activity immediately disappears. There is a corresponding parallelism between the ultracentrifugal analyses of the pH stability of the tobacco mosaic virus protein molecule and its range of infectiousness. In the face of evidence of this sort the only fruitful standpoint is one which admits that the virus activity may be a property of the heavy substance and seeks in every imaginable way to compare the behaviors of the activity and its associated protein. A new field of research into the mechanism and control of disease is opened up by the possibility of treating its cause as a pure chemical compound. Stanley[20] has found that the virus activity of the tobacco mosaic protein can be destroyed by several simple chemicals without alteration of its immunological specificity. Beard and I[21] have shown that under certain conditions the papilloma protein loses all activity without measurable molecular change. It is not unreasonable to hope that experiments of this type will some day indicate a new way in which the body can be aided in protecting itself against disease.

In addition to supplying the method for such definite experiments the ultracentrifuge makes it possible for the first time to examine a number of intriguing speculations. One wonders, for example, whether molcules the size of virus proteins occur naturally in plants and animals without producing disease. Of special importance is the problem of the mechanism whereby a susceptible living host yields much virus after being infected with only a trace. As long as the smaller viruses were pictured as autonomous living agents preying on their hosts, their multiplication could be thought of as the consequence of processes resembling bacterial division. Viruses that are definite chemical molecules can be imagined not as such extraneous predatory organisms but as products of abnormal metabolic processes within the "infected" cells. We still know far too little about the details of protein chemistry to be able to understand how the introduction of a heavy virus molecule into a living cell induces its protoplasm to break down according to the new

[18] T. Svedberg, *op. cit.*
[19] W. M. Stanley, *Am. Jour. Botany*, 24: 59, 1937.
[20] W. M. Stanley, SCIENCE, 83: 626, 1936.
[21] R. W. G. Wyckoff and J. W. Beard, *op. cit.*

pattern established by this molecule, but the idea is not incompatible with what has already been learned about enzymal action. Because they bridge the gap between the "dead" simpler protein molecules and the unquestionably living smaller bacteria, the viruses are irresistibly attractive to all who are interested in attaching precise meaning to the term "alive." By supplying some of these viruses in pure form the ultracentrifuge permits a new experimental approach to this question.

SCIENTIFIC EVENTS

SCIENTIFIC AWARDS IN CHINA

THE following prizes and awards are recorded in "Science Notes" issued by the College of Natural Science, Yenching University, Peiping, China.

At the annual meeting and dinner of the Peking Society of Natural History held in April, Dr. Chenfu F. Wu, chairman of the department of biology of Yenching University, was the recipient of the King Medal. This medal is awarded annually for distinguished work in the biological or geological sciences. The award this year was bestowed upon Dr. Wu in recognition of his work on "The Catalogue of Chinese Insects."

Recent announcement has been made of the following new fellowship appointments by the China Foundation: Cho Ting-wei (M.S., 1937), for work in general physiology at Wu-Han University; Lin Cho-yuan (M.S., 1934), for research studies in ceramics at Pennsylvania State College; Ch'en Shang-yi (M.S., 1934) for work in spectroscopy at the University of California; Ho Ch'i (B.S., 1928) and Chang Tso-kan (B.S., 1932) for research abroad in biology. A research grant has also been made to Hsu Peng-cheng (M.S., 1935), instructor in chemistry, in support of nutrition studies at Yenching University.

A scholarship for study in England has been awarded to Tai Wen-sai (Graduate Yuan) by the British Boxer Indemnity Fund for work in astrophysics. Pu Chih-lun (Graduate Yuan) has received the Sun Yat-sen Memorial fellowship for work at Yenching University in biology.

In physics, Yuan Chi-liu (M.S., 1934) has been appointed a research fellow at the California Institute of Technology, and Lu Ho-fu (B.S., 1936) has received a similar appointment at the University of Minnesota. Kuang Jung-lu (B.S., 1935) has been awarded a fellowship in veterinary medicine at Cornell University.

Of the four prizes offered to B.S. graduates for the best research theses in physics for the year 1936, in a nation-wide contest sponsored by the Sino-Belgian Boxer Indemnity Committee, two of the prizes were awarded to Lu Ho-fu and Ch'eng Li-ch'ang of the department of physics of Yenching University.

ARCHEOLOGICAL PROJECTS

WATSON DAVIS, director of Science Service, reports that government funds, available through the Works Progress Administration, may be used to give unemployed men and women work on archeological projects which meet with official requirements. It is not, however, within the province of the WPA to seek suggestions for such projects, but they are interested in helping scientific research in cases where WPA workers can be employed and they welcome the cooperation of scientific organizations and of scientific men.

A few years ago, Science Service, with the cooperation of the Division of Anthropology and Psychology of the National Research Council, conducted a plan known as the Archeological Minute Men. By that arrangement, rumored discoveries in archeology were reported and were investigated as promptly as possible, and accurate reports were furnished to newspapers. No funds, however, have hitherto been available for excavation.

If an archeological site is discovered which seems worth excavating, a WPA project can be undertaken, provided the project is properly sponsored and supervised, and there is relief labor available in the area. All WPA projects must be sponsored by some public institution or organization such as a public museum, state university, municipal government, state government or board of education. An individual or a private institution must arrange for official sponsorship. The Smithsonian Institution is cooperating with the WPA in reviewing projects.

The WPA will not approve any project which does not have a supervisor with training and ability approved by the WPA coordinating anthropologist. It is requested that names be suggested of individuals who might act in this capacity. It is planned to obtain complete reports on these projects, and to see that the collections obtained are placed in public institutions where they will be properly cared for. A further service that experienced archeologists can render will be to report any vandalism or careless excavation.

Science Service is glad to act as an intermediary in bringing archeologists and the WPA into contact, both because of its desire thus to assist in the promotion of scientific research and because incidentally such excavations should be reported to the public through the service.

A FEDERAL CANCER RESEARCH INSTITUTE

A BILL establishing a Federal Cancer Research Institute at Washington, for which an appropriation of