

The findings showed that the activity of the virus is preserved for at least a week at all pH's between 5 and 9.5, that inactivation proceeds rapidly at pH 4 and pH 10.5 and is practically instantaneous at pH's below 3 and above 11.5.

Elementary bodies, pure and concentrated enough for ultracentrifugal analysis, were obtained after several passages of the virus in rabbit skin. The ultracentrifuge was the same air-driven machine⁴ arranged for absorption measurements that has been used in previous studies. Photographs of the rates of sedimentation of products of the first few passages starting with calf lymph showed no sharp boundary characteristic of particles of uniform size. Instead the pictures gave evidence of much heavy colloidal and light "unsedimentable" ultra-violet-absorbing material. After these preliminary passages, however, a sharp sedimenting boundary with a constant of $ca\ 5,000 \times 10^{-13}$ cm sec⁻¹ dynes⁻¹ could be seen. At first the homogeneous matter was accompanied by colloidal and "unsedimentable" substances, but after two or three additional passages and minor improvements in the technique of washing the bodies, the ultracentrifugal pattern was that of a pure homogeneous suspension. Smears stained according to the method of Morosow⁵ then showed little material other than numerous elementary bodies of uniform size. Undoubtedly the particles giving the sharp ultracentrifugal boundaries are those that serve as nuclei for the stain.

Both infectivity tests and the results of ultracentrifugal analysis indicate that solutions or suspensions of the elementary bodies of vaccinia are stable only in very dilute salt solutions. If fresh elementary bodies in 0.005M buffer are washed two or three times with distilled water, their final suspension no longer produces a good boundary. Some of the elementary bodies are injured by the agglutination that accompanies this washing. This is shown by the fact that resuspension in 0.005M buffer gives both a weaker and a more diffuse boundary. The homogeneity of elementary body suspensions in 0.1M neutral buffer rapidly disappears. After a few days in the cold such a suspension will no longer yield a sharp boundary in the ultracentrifuge nor can one be recovered by resuspension in 0.005M buffer.

Because of this sensitivity to strong salt, the suspensions for ultracentrifuging at different pH's were in 0.005M buffers. At all pH's between 5.5 and ca 10 suspensions of the elementary bodies are stable for days and give sharply sedimenting boundaries with

$s_{20}^0 = ca\ 5,400 \times 10^{-13}$ cm sec⁻¹ dynes⁻¹. At pH's between 3 and ca 5.5 agglutination is so nearly complete that sedimentation pictures show nothing. A boundary is again poorly discernible at pH 2.6 and good photographs can be obtained at pH 1.2. The boundaries in these acid solutions are more diffuse than those from active preparations and correspond to a substance that is perhaps 15 per cent. lighter. Further evidence of decomposition in the inactive acid suspensions is the fact that the quartz windows of the analytical cell become covered with a waxy deposit which interferes with the ultra-violet transparency of the air bubble. The decomposition in strongly alkaline solution is even more interesting. No trace of the sharp boundary with $s_{20}^0 = 5,400$ could be found in a suspension brought to pH 11.8. In its place was much unsedimentable material and a faint but fairly sharp boundary coming down more slowly. Some of the substance causing this boundary was isolated with the quantity ultracentrifuge. In pure solution it gives excellent sedimentation photographs corresponding to $s_{20}^0 = ca\ 1900 \times 10^{-13}$, a value about one third that of the active elementary bodies.

These experiments bring out a general parallelism between the stabilities of the virus and of the elementary bodies. The sedimentation constant of the bodies is uninfluenced by pH in that region where the activity is unaffected. In inactive acid and alkaline solutions the elementary bodies are broken down, leaving fragments some of which are very large and of a degree of homogeneity that compares well with that of the active bodies. The many similarities between these results and those obtained with the papilloma protein are evident.

A further study is being made of the properties of these inactive degradation products and of the loss of virus activity in the especially instructive range between pH 9.5 and 11.5.

J. W. BEARD

HAROLD FINKELSTEIN

DEPARTMENT OF SURGERY
DUKE UNIVERSITY MEDICAL SCHOOL
AND HOSPITAL
DURHAM, N. C.

RALPH W. G. WYCKOFF

ROCKEFELLER INSTITUTE FOR
MEDICAL RESEARCH
PRINCETON, N. J.

REDUCTION OF THE METHYL ESTER OF 2:3:4-TRIMETHYL α -METHYL-d-GALAC- TURONIDE TO 2:3:4-TRIMETHYL α -METHYL-d-GALACTOSIDE

IN order to facilitate the analysis of the structure of the derivatives of uronic acids (such as pectins, aldonic acids, etc.), it seemed desirable to convert the

⁴ 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.

⁵ Morosow, *Centralbl. f. Bakt. I Orig.*, c: 385, 1926.

carboxylic groups of the latter into $-\text{CH}_2\text{OH}$ groups and thus to transform the uronic acid into a simple monose.

This has now been accomplished in the case of *2:3:4-trimethyl α -methyl-d-galacturonide methyl ester*, which was reduced to *2:3:4-trimethyl α -methyl-d-galactoside*. The reaction has been accomplished both by the methods of classical organic chemistry (described by P. A. Levene and L. C. Kreider in an article now in press) and also by the catalytic method, with the aid of copper chromite catalyst in an atmosphere of hydrogen.

The reduction was practically complete. The distilled product (a syrup which crystallized) had the following composition:

Found C 50.98, H 8.6, OCH_3 52.75. ($\text{C}_{10}\text{H}_{20}\text{O}_6$ requires C 50.81, H 8.6, OCH_3 52.55.)

It had $[\alpha]_D^{25} = +198.4^\circ$ (in water); $n_D^{25} = 1.4626$ and m.p. $\approx 30^\circ$.

On hydrolysis the substance yielded *2:3:4-trimethyl d-galactose* having m.p. $78-79^\circ$ and $[\alpha]_D^{25} = +147^\circ$ (initial, in water); $+120.4^\circ$ (equilibrium).

Work in the same direction on aldobionic acids is now in progress.

P. A. LEVENE
R. STUART TIPSON
LEONARD C. KREIDER

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH,
NEW YORK

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE PRESERVATION OF BIOLOGICAL SPECIMENS BY MEANS OF TRANSPARENT PLASTICS

In the September 10 issue of *SCIENCE*, on page 247, Dr. J. H. Hibben published a brief paper on the preservation of biological specimens by means of transparent plastics, in which he disclosed that the idea of imbedding various specimens in methyl methacrylate occurred to him some months before the submission of his manuscript for publication.

In view of the appearance of Dr. Hibben's paper and of certain statements contained therein, it seems desirable to point out that the Bureau of Chemistry and Soils, U. S. Department of Agriculture, has under way a Bankhead-Jones Special Research Fund Project dealing with the preservation of agricultural specimens in as natural a condition as possible. This project, which is under the direct supervision of Dr. Charles E. Sando, senior biochemist in the Food Research Division, consists of studies on the preservation of fresh plant materials such as roots, stalks, stems, leaves and flowers, and studies on the preservation of plant and entomological specimens in polymerized methyl methacrylate.

One process has been studied for many years by Mr. G. R. Fessenden and its further development and improvement are being continued by him in this bureau. It consists essentially in chemically treating fresh plant materials in such a manner as to toughen the tissues and set the natural color, after which the specimens are preserved in transparent mountings. Specimens so treated retain practically the same size, shape and color of fresh material and are therefore to be considered more or less permanent, although considerable research is necessary to make the process

reasonably inexpensive and universally applicable. Mr. Fessenden's finished specimens have been exhibited at the Harvard Botanical Museum, the New York Botanical Garden, the American Museum of Natural History, the Buffalo Museum of Science, the Pennsylvania State Museum, Morton Arboretum and other museums, libraries and horticultural organizations, where they have created marked interest and much favorable comment.

The second phase of the special project dealing with the preservation of agricultural specimens, namely, that dealing with the imbedding in methyl methacrylate, was first begun in November, 1936, by Mr. F. L. Goll, of the Bureau of Plant Industry, at the request of Dr. F. W. Parker, of E. I. du Pont de Nemours and Company, Wilmington, Delaware, and was later modified and improved by Dr. Sando, working in close cooperation with the Bureau of Plant Industry and with Dr. D. S. Frederick, of Rohm and Haas Company, Philadelphia, Pa. The most intensive research on the methyl methacrylate method has been in progress since May, 1937. As a result of such work many difficulties have been overcome, and nearly forty unusually fine specimens have been prepared, including seeds, certain flowers, beetles and iridescent butterflies. The largest of these specimens is an ear of corn which in its imbedded state measures $2\frac{1}{4}'' \times 3'' \times 7''$ and weighs approximately $1\frac{3}{4}$ pounds. These specimens have all been machined and polished and are therefore finished mounts.

One is apt to draw the conclusion from Dr. Hibben's paper that mounting biological materials in methyl methacrylate is a relatively simple and easy matter and that polymerization will take place in a matter of hours. The results of our work have shown, however,