W Stanley 6/23/93

# HANDBUCH
## DER
# VIRUSFORSCHUNG

HERAUSGEGEBEN VON

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## ERSTE HÄLFTE

DIE ENTWICKLUNG DER VIRUSFORSCHUNG UND IHRE PROBLEMATIK · MORPHOLOGIE DER VIRUSARTEN · DIE ZÜCHTUNG DER VIRUSARTEN AUSSERHALB IHRER WIRTE · BIOCHEMISTRY AND BIOPHYSICS OF VIRUSES

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MIT 71 ZUM TEIL FARBIGEN ABBILDUNGEN IM TEXT

WIEN
VERLAG VON JULIUS SPRINGER
1938

## Vierter Abschnitt.

## Biochemistry and Biophysics of viruses.

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With 20 illustrations.

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# Biochemistry and biophysics of viruses.

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## I. Inactivation of viruses by different agents.

### Introduction.

Studies on the effect of different chemical and physical agents on the activity of viruses were in progress even before viruses were recognized as a separate group of infectious entities and have been continued to the present time. During the earlier work two objectives were sought, one the preparation of immunizing antigens and the other the elucidation of the nature of viruses. These have continued to remain as objectives and recently a third has been added; during the past few years studies on the effect of different agents on viruses have been made with a view towards establishing conditions and reagents that could be used in the purification and concentration of viruses. Considerable difficulty has been encountered, not only during the progress of the studies but also in the interpretation of the results that were obtained. Much of this has been due to the great variation in the physical and chemical properties of the different viruses and to an apparent variation in the properties of the same virus in different preparations. The latter appears to have been due to the presence of varying amounts of extraneous material in the different virus preparations. For a great many years the presence of extraneous material made it impossible to be certain that any given physical or chemical property was one of the virus itself. This point is discussed at somewhat greater length in the third section of this chapter. Recently the effect of enzymes on viruses has been studied in an effort to learn something of their nature. However, until very recently only crude enzyme preparations containing a mixture of materials were available, and there is considerable doubt concerning the significance of results obtained with such preparations, for PIRIE's work indicates that the inactivation of some viruses was due to extraneous material rather than to the enzymes. The isolation within the past few years of several enzymes in crystalline and apparently pure form has made it possible to study more accurately the effect of enzymes on viruses.

Although a vast amount of work has been done in attempting to achieve the three objectives mentioned above, no effort will be made to consider all the work in this section, because the general situation has been considerably altered within the last few years by the isolation of several viruses in apparently pure form. It is obvious that studies on the nature of viruses and on the preparation of immunizing antigens should be made with such purified preparations of virus rather active protein isolated by fractional precipitation with ammonium sulphate between 0,2 and 0,4 saturation at $p_H$ 7. NORTHROP was able to isolate about 50 mg. of protein, representing about 25 per cent of the activity in the starting material, from 200 liters of lysed culture. The detailed procedure for an average experiment is shown in table 3. It may be seen that the protein nitrogen could be reduced from about 4000 mg. to about 40 mg. without any great loss of phage activity, but that further purification was accompanied by loss of activity. NORTHROP found the preparations to become increasingly unstable as purification proceeded and encountered considerable difficulty in achieving final purification. However, he has subjected the purified phage protein to extensive studies in which the activity and protein have been correlated by several different procedures, and has secured good evidence not only that the phage activity is a property of the protein but that the phage protein is essentially pure. The phage activity was found to be greater than that of any preparation previously reported, for only $10^{-16}$ gm. protein nitrogen of purified phage was found sufficient to cause lysis. The physical and chemical properties of the phage protein will be considered in the next section.

## The transformation agent of the pneumococcus.

In 1928 GRIFFITH found that he could transform one specific S type of pneumococcus into another specific S type through the intermediate stage of the R form. He effected the transformation by injecting mice with nonvirulent R forms, together with large amounts of heat-killed S pneumococci of a type other than that of the organisms from which the R cells were derived. Living virulent S organisms of the same type as the heat-killed S forms were then recovered from the animals. These results were confirmed by NEUFELD and LEVINTHAL and by DAWSON. Later DAWSON and SIA demonstrated that the transformation in type could be accomplished *in vitro* by inoculating small amounts of R organisms derived from S organisms of one type into blood broth containing anti-R serum and heat-killed S cells of the other type, or more strikingly by the use of an extract of the cells of several times frozen type-specific pneumococci. The latter finding was confirmed by ALLOWAY, who found that cell-free, heated and filtered extracts of one type of S pneumococci could be used to induce the conversion of R forms derived from another S type into the same type as that of the cells used to prepare the extract. It is obvious that there is a factor which may be obtained from any one of the S type of organisms that is normally absent from R type cells, but that when added to such cells induces their conversion into the same type of S organisms from which the factor was derived, with the very important result that more of the factor is produced in the induced S cells. This phenomenon is virus-like, and it is because of this and the fact that it may become important from the standpoint of the chemistry of viruses that a discussion is included here. The various type-specific pneumococci may be regarded as cells infected with different "virus" strains and only the R organisms as healthy. The R organisms may be converted into any one of what we refer to as type-specific organisms by "infection" with any one of the different "viruses". By appropriate treatment it is again possible to free the pneumococci of "virus" and secure the healthy R type. It is of interest, therefore, to examine the nature of this factor or "virus". The type-specificity of the pneumococcus is determined by its capsular polysaccharide, hence it might be assumed that the type of soluble specific substance or polysaccharide isolated by HEIDELBERGER and AVERY or the acetyl derivative isolated by AVERY and GOEBEL from pneumococcus type 1 might be responsible for this conversion. However, Dawson and Sia found that the specific capsular polysaccharide in chemically pure form would not induce the transformation in type. It seems probable, therefore, that, if the polysaccharide plays a role in the transformation, it does so only when in combination with some other substance. Alloway, in attempting to purify the active agent, found that considerable inactive material could be removed by dissolving heat-killed S organisms with sodium desoxycholate, precipitating with cold alcohol, and extracting the precipitate with salt solution. The extract was then heated to 60° C., centrifuged, the supernatant liquid filtered through charcoal, and again precipitated with alcohol. The precipitate was dissolved in water and centrifuged to give a colorless, water-clear supernatant liquid containing practically all of the original activity. No chemical tests were made on these purified preparations, hence nothing is known about the nature of the active agent. It is to be hoped that the study of this agent will be continued because of its virus-like nature.

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# III. Chemical and physical properties of viruses.

## Introduction.

For some years attempts to study the chemical and physical properties of viruses have consisted of experiments designed to yield information concerning their nature and size. Extracts containing virus plus greater or smaller amounts of extraneous material were subjected to the action of different chemical and physical agents, to filtration through membranes of known porosity, and more recently to centrifugation in known fields of force. These studies, some of which are described in the two preceding sections, were very valuable from the standpoint of serving to increase our general knowledge of viruses. Frequently, however, they did not yield information concerning the chemical and physical properties of the viruses themselves, despite the fact that the results were usually so interpreted, for the viruses were always accompanied by extraneous material, the effect of which it was impossible to evaluate. In some virus preparations the inert extraneous material probably comprised over 99 per cent of the solids, whereas in others it probably comprised less than 20 per cent of the solids. The nature and amount of extraneous matter varied with the host from which the virus extract was prepared. The presence of this extraneous material was either a real or a potential source of interference in the establishment of the true properties of a given virus, and before viruses were concentrated and purified it was practically impossible to be certain that a given property was really characteristic of a given virus. For example, the thermal inactivation point of tobacco mosaic virus is usually given as 93° C., because the virus in freshly expressed juice from diseased Turkish tobacco plants is usually inactivated on heating to 93° C. for 10 minutes. However, this point varies from sample to sample, depending upon the concentration of virus and upon the host from which the virus was obtained, for these two factors affect the relationship between virus and extraneous material. It has been impossible to determine the thermal inactivation point of the virus itself in such preparations because of the effect of the extraneous matter. When this extraneous matter is removed and the tobacco mosaic virus is obtained in the form of crystalline virus protein, the thermal inactivation point is found to be, not 93° C., but about 75° C., and the point remains the same regardless of the source of the virus.

The filtration experiments on the virus of latent mosaic of potato may be given as another example of the erroneous impressions that may result from work with unpurified preparations of virus. These filtration results indicated that the particle size of the latent mosaic virus is 75–112 m$\mu$, or about 3 or 4 times that of tobacco mosaic virus. This virus has recently been concentrated and obtained in the form of a homogeneous purified virus protein having a sedimentation constant of 113, a value that is only about 60 per cent that of tobacco mosaic virus protein.