Section of Comparative Medicine

President—S. P. BEDSON, M.D., F.R.S.

[*November* 24, 1937]

DISCUSSION ON RECENT WORK ON HEAVY PROTEINS IN VIRUS INFECTION AND ITS BEARING ON THE NATURE OF VIRUSES

Dr. J. Henderson Smith: There is nothing new to the plant pathologist in the idea that virus is associated with crystals. Iwanowski pointed out over thirty years ago that tobacco mosaic disease was characterized by the appearance in the cells of flat plate-like crystals which were not found in the cells of normal plants, and these exhibited marked cross striation, especially under the action of acid. It was not then suggested that these crystals were anything more than a regular concomitant. Very much later (in 1931) when attempts were being made to obtain virus in a purified state, freed from the non-specific ingredients of the plant juices, the idea was mooted that the virus itself might be crystalline. True crystals which had infective properties were in fact actually obtained, and the conception of a crystalline virus became familiar. But these crystals were soon shown to be really crystals of the phosphate used in the method of purification, and their infectivity was due to virus entangled as they formed. It was not till about two years ago, when Stanley brought out his paper on " The isolation from diseased tobacco plants of a crystalline protein possessing the properties of tobacco mosaic virus " that the possibility of a genuine crystalline virus had to be seriously entertained.

This conception was abhorrent to the average biologist, who had looked on a virus as a living thing, and was convinced that there is a sharp demarcation between the living and the non-living. For many years nothing was known about viruses that was definitely incompatible with the view that they were essentially living organisms, rather like small bacteria—very small, since they were filtrable, and with special properties associated with the large surface they possessed relative to their bulk. They were obligate parasites, and it was thought that because of their small size their food had to be prepared for them by the host; but apart from that condition of their existence they were organisms like any other organism. They varied in size in a descending scale, but even when so small that they could be photographed only by short wave-length light they looked like organisms. With further research, however, the position became rather difficult to maintain. Elford's gradocol membranes showed that some at least of the viruses were smaller in size than single molecules of known proteins, such as the hæmocyanins, and Laidlaw found a sewage organism,

whose dimensions entitled it to the name of virus, but which grew on artificial or at least cell-free media. It was difficult to believe that a thing the size of a single molecule could possess all the properties of life—whatever that may mean. The diehard vitalist began to feel that his position was not so secure as he had believed.

Then Stanley claimed to have isolated a crystalline protein with all the properties of the tobacco mosaic virus. The assertion that the virus was crystalline was responsible for much of the attention at first given to the new claim, for those of us who had been brought up in the older order of ideas attached a kind of sanctity to the word " crystal ". It was a kind of guarantee of purity, and Stanley himself cited as evidence of the purity of his protein the fact that it could be recrystallized as many as 15 times without change of properties. Whilst Stanley was working on tobacco mosaic, Bawden and Pirie in Cambridge had been isolating virus proteins from plants infected with potato mosaic. The potato mosaic viruses do not give, or at least have not yet given, the needle fibres, but when Bawden came to Rothamsted and applied to the tobacco mosaic virus the methods used on potato, the same needle crystals as Stanley's were obtained, and most of his results were confirmed. The highly purified proteins, however, were shown to give liquid crystalline solutions, and it was suggested that the needle structures were not true crystals. Bernal has now shown that they are not really crystalline in the old sense of the word, but have a 2-dimensional regularity instead of a 3-dimensional, and are better described as fibres or paracrystals. It was also shown that their repeated production is no guarantee of purity. But the crystalline property attributed to tobacco mosaic undoubtedly caught the attention of many who might otherwise have been slow to appreciate the significance of the new step.

I do not propose to go much into detail, or touch, except incidentally, on the animal virus work. I will give only the broad outline of the progress made to date on the plant side, taking tobacco mosaic virus as the chief example.

There is no doubt that the material isolated from the juice of infected plants has most of the properties of tobacco mosaic. It is infective in concentrations of the order of one hundred-millionth of a gramme per c.c., increases very rapidly in the infected plant, and is transmissible in series indefinitely. The disease produced is identical in every respect with the disease of the plants from which the material was obtained in the first instance. With one important exception—with which I will deal in a moment—it has all the properties of the virus found in naturally infected plants, such as resistance to chemicals, ageing, heat, enzymes, &c.

There is also no doubt that it is a protein. It gives all the usual reactions, and its analysis conforms to that usual in known proteins. Bawden and Pirie consider that it is a nucleoprotein, but the American workers do not accept this view. The discrepancy is important, because of the implications and associations which a nucleoprotein-nature suggests, but that is a technical matter and will no doubt soon be cleared up. Its composition is constant, whatever the source of the material analysed and the concentration of the substance it contains. It can be obtained from every host plant that the virus can actively infect. It is, therefore, not a substance peculiar to the tobacco plant or the tobacco virus complex, but appears wherever the virus is able to multiply—even in hosts in no way related to the tobacco plant, such as phlox or spinach.

It has not been found in any normal plant, even in very small amounts, and the methods of extraction are delicate enough to reveal it, even when it constitutes only a millionth part of the plant tissue. In the infected plant, on the other hand, it occurs in surprisingly large quantities. The juice of an infected plant contains from five to ten times as much soluble protein as that of a normal plant, and about 80%

of this soluble protein consists of the abnormal substance. From one to two grammes can be obtained from a litre of sap, the amount varying with the condition of the infected plants and the duration of their infection. Where all this excess protein comes from is unknown. It is conceivable that it is a modification of the protein already existing in the normal plant. Possibly the existing protein, perhaps the non-soluble portion, is converted into the new soluble form, and the cells, requiring the normal protein for their own purposes (and one must remember that the diseased plant, though damaged, is still a functioning organism, growing to a large size), are stimulated to replace the converted material by more of the original, which is in its turn converted ; and so we get an accumulation of the new form, giving a total content of soluble protein much greater than is normally found. There is indeed a small bit of evidence that there is actually less of the normal protein in the diseased plant than in the normal plant. This theory would imply that the creative force, the synthetic or constructive power which converts the normal material into the abnormal, resides in or is a property of the abnormal substance itself. But there are other obvious possibilities. It may be, for example, that the *cell* produces the abnormal protein under the stimulus of the abnormality, a theory which dodges the necessity of giving reproductive powers to the protein itself. The plain fact is that we have as yet no evidence at all as to the mechanism or the source of this huge development of a foreign substance.

From solutions of the protein can readily be obtained needle-shaped bodies resembling crystals, which are easily visible under the microscope. They average in length about two- to three-hundredths of a millimetre. They can be easily dissolved and again obtained, and the process can be repeated indefinitely. In spite of this it has been shown by Bawden that these " crystals " are not necessarily pure, even after repeated recrystallization but, as ordinarily prepared by precipitation with ammonium sulphate, contain a fraction which is not virus protein and can be removed by tryptic digestion. This has been beautifully demonstrated by the anaphylactic reaction by Chesters, who showed the presence of the impurity in the American ammonium sulphate crystalline protein, and its absence in the purified material prepared by Bawden.

These crystals are not true crystals in the ordinary usage of the term. They should rather be described as fibres or paracrystals, and under certain conditions the protein can be obtained in the solid form as long mesomorphic fibrils visible to the naked eye. These have the orderly molecular arrangement found in crystals, but so have many other things, such as muscle fibres or hair structures. They have a 2-dimensional regularity instead of a 3-dimensional, and the use of the term crystalline is misleading, at least to those who are not familiar with the modern extensions of this word. That they are better described as fibres is shown by the mode of their formation, which consists in the breaking-up of a gel into slivers or splinters.

When a solution of the protein reaches a certain concentration it reveals a new property, becoming birefringent and showing anisotropy of flow. This property indicates that the constituent particles are rod-shaped. When the concentration is high, so that the rods cannot move about freely but are necessarily arranged in parallel bundles owing to lack of space, the solution is permanently birefringent. When the concentration is of a lower order, the rods are able to move comparatively freely, but on the formation of currents or eddies they assume the parallel orientation, and there is anisotropy of flow. The property is greatly affected by impurities, notably by the presence of breakdown products of the virus protein, and it is not possible at present to estimate the length of the rods with any precision, though it seems that this must be at least ten times as great as the width. The width can be estimated accurately from X-ray analysis. This will be discussed by Bernal later

on, and I will not anticipate his remarks further than to say that the cross diameter is 150 Ångströms, that the cross sectional area amounts to 20,000 square Ångströms, and is probably triangular, and that these dimensions are constant for all concentrations of the protein.

The molecular weight and the particle size could be derived from the sedimentation constant if the particles were spherical. Since they are rod-shaped, it is scarcely possible to arrive at a really reliable value for the particle size. The cross-section shows that the molecular weight is large, and if we take the length as ten times the width and the specific gravity as 1·37, the minimum molecular weight must be of the order of 20 millions—not grossly different from the 17 millions calculated by Svedberg for the American preparation. This is enormous. It is larger than the largest hæmocyanins, and is approached by only one known substance, a thyroglobulin polymer, which is estimated to have a weight of 15 millions. The new protein is, at least, a very unusual substance—unlike anything found in the sap of normal plants.

There is reason to believe that these rods are not the ultimate constituents, but are aggregates of sub-units arranged in linear form. The chief evidence for this is the fact that in the purified state the protein has lost the outstanding character of a virus, namely filtrability. Every operation which precipitates the protein, such as simple high-speed centrifugalization, precipitation by alcohol, by acid, or by ammonium sulphate, entails this loss of filtrability. We know that in untreated sap the virus can be filtered through membranes of a pore size of 53 mμ, but when the virus is purified, it will no longer pass a membrane with 450 mμ pores. It looks as if in the juice, and probably in the living plant, the virus exists in a smaller and more discrete form, but in the isolated condition it has undergone an aggregation or polymerisation. The aggregation must be linear, because its width remains the same. The fact that such rods of unaltered width do not pass the filters endways on is perhaps a phenomenon similar to the jamming of logs when lumber is being floated down a river. There is other evidence that in the crude sap the protein is present in a different molecular arrangement—for instance, the absence of anisotropy in sap which is a solution of 0·2%, and the smaller infectivity of the isolated virus, whose serological titre nevertheless remains the same as that of the virus in the sap. Up to the present it has proved impossible to disaggregate the protein by any method tried.

Proteins of this type have now been isolated by Bawden and Pirie from three strains of tobacco mosaic and two strains of cucumber mosaic having a serological affinity to tobacco mosaic. The diseases are clinically distinct, and the corresponding proteins are also characteristically distinct. The protein varies as the virus varies. From other diseases, such as certain of the potato mosaics, these workers have obtained infective nucleoprotein precipitates which are amorphous and do not give the needle crystals, and which have a different composition on analysis. These proteins are susceptible to tryptic digestion and so cannot be purified by its use, but they can be obtained by high-speed centrifuging. Other viruses examined in America have yielded similar proteins. But we have no reason to suppose that all viruses, even all plant viruses, will conform to any of the types as yet investigated, and it is unwise to generalize from the small number examined. All we can say is that so far the viruses examined *have* given infectious proteins with the properties I have been describing.

There remains now the question—is this protein actually the real virus, the *virus ipsissimum ?* If we could be quite certain that the purified protein is really homogeneous, really pure, the question would not arise. So far as we have been able to determine, virus proteins have been prepared which do seem to be really homogeneous,

but it can always be asserted that they are not, and that the virus is present as an impurity in the protein preparations. Although the protein is found only when the virus is found and is specific, it may yet be a reaction product produced by the plant in response to the virus, a symptom in fact just like any other symptom, and its infective properties are due to the presence in it of the true virus, from which it has not yet been separated. This theory cannot be directly disproved for one remembers the presence of argon in what was believed to be pure nitrogen and of heavy water in pure water. Moreover, there is always the possibility that some test as yet unthought of, or some increased refinement of the existing tests, may reveal such impurity. This theoretical possibility must always remain, but the mass of evidence against it is now so large that we are entitled to disregard it and the onus of proving its existence as a fact is transferred to those who assert it.

Certainly there can be no gross impurity or inhomogeneity. The constancy of the product obtained from the most varied sources is enough to show that much ; and also the infectivity, which is regularly demonstrable in a concentration of 10^{-8} to 10^{-10}. Neither test is very refined. Analysis would not reveal an impurity of less than 1%, and the infectivity test, owing to the variability of the test plants and the unavoidable uncertainty as to the exact amount of effective inoculum, leaves a considerable margin of variation. It is affected, too, by the difference in the molecular state in the sap and in the isolated protein, and this makes precise comparison between the infectivity of the sap and the protein impossible. We can, however, be sure that the protein is many times more infective than the original material.

Any procedure that removes protein lowers the infectivity, and the activity declines *pari passu* with degradation of the protein. The temperature or the degree of acidity or alkalinity which destroys the protein, also destroys the activity, and it has not been found possible in any way to dissociate the protein from the virus. The hypothetical virus contaminant must have the same iso-electric point. It must also have the same molecular weight, since in the analytical centrifuge the pure protein gives the sharp sedimenting boundary of a single molecular species. In short, the contaminant must have the same physical properties as the protein, and, applying the razor of Occam, it is gratuitous to postulate the presence of two substances where one is enough to satisfy the data.

So far as can be seen at present, the evidence is all in favour of the view that the protein is the actual virus, and whatever the implications involved one must proceed on that assumption.

Dr. C. H. Andrewes: Workers with viruses pathogenic for animals—animal viruses, for short—must have watched with interest and excitement the recent work on plant viruses, and must have wondered how far the results would be applicable to their own subject. My part in this discussion is to review the work so far published on " heavy proteins " in animal virus infections. As yet there is little to review—only a few short notes by Wyckoff and Beard.

These authors, working at Princeton in close contact with Stanley, first studied the virus of the rabbit papilloma (Shope), a virus remarkable for its great resistance to heat and other agents. Suspensions of warts were purified by repeated fractional centrifugation in the ultracentrifuge, coarse particles being deposited at low speeds and discarded, and then the virus bodies thrown down at high speeds and repeatedly washed. Beard and Wyckoff (1937) thus obtained suspensions of bodies which, from the evidence they offer, almost certainly represented the active agents of the papilloma. When observed by means of the Svedberg optical apparatus in connexion

with their ultracentrifuge, the bodies were found to deposit in a manner indicating great uniformity in size, the particle size corresponding to a molecular weight of 20 million or a diameter of 40 mμ, a figure which agrees well with that obtained by filtration. The material gave the chemical reactions of a protein and contained 15% of nitrogen. Beard and Wyckoff concluded that they had obtained a "high molecular weight protein with which is associated the infectiousness of the disease".

This sounds dramatic, but I wonder if it really means much. May it not be that the novelty of the conclusion lies not so much in the technique or in the experimental results, as in the attitude of mind of the workers? If we ourselves should carry out washings of a virus in a centrifuge and find that our deposit consists of bodies of uniform size giving the chemical reactions of a protein, we should state that we have obtained a suspension of purified virus bodies, which contain protein, and nobody would give us credit for having done anything important. If, however, we called our product a high molecular weight protein, we should produce a profound impression on the scientific world.

Later, Wyckoff (1937) carried out similar experiments with the virus of equine encephalomyelitis, and found that it deposited about as readily and as uniformly as that of the rabbit papilloma. The molecular weight of this heavy protein was estimated as 25 million. As this virus is very unstable at room temperatures, all manipulations had to be carried out in the cold; the virus rapidly disintegrated on standing, and the uniformly sedimenting particles shown by the Svedberg optical system were no longer recognizable. Further observations on the sedimentation of the rabbit papilloma (Wyckoff and Beard, 1937) now revealed that at a pH of 10 on the alkaline side and 3 on the acid side the virus-heavy protein broke up into smaller fragments; these points correspond with the pH values at which infectivity of the virus was very rapidly lost, though it is true that virus held between pH 7 and 10 was slowly inactivated though its sedimentation constant was unaltered. Experiments of this sort are clearly likely to yield information of great interest, though in the present state of knowledge I do not think that they justify definite conclusions about the nature of viruses.

Beard, Finkelstein, and Wyckoff (1937) have quite recently carried out similar experiments with vaccinia virus—a virus whose diameter is about five times that of the two previously studied. Again particles sedimenting in a very uniform manner were obtained, and it was again found that this uniform sedimentation was only to be observed over a pH range in which the infectivity of the virus was well preserved. I do not know whether these workers conclude that vaccinia virus also is to be described as a "heavy protein". If uniformity of sedimentation and positive chemical tests for proteins are the sole criteria, presumably they should logically do so. Possibly they hesitate because Hughes, Parker, and Rivers (1935) found that their preparations of vaccinia, purified by similar means, contained, in addition to protein, some fat and also carbohydrate which was readily washed away.

Workers with animal viruses have made valuable progress on the basis of a working hypothesis that viruses are micro-organisms owing their peculiar characters first to their small size and secondly to their highly specialized intracellular parasitism. What is to be their attitude in view of the recent work on heavy proteins, particularly in relation to plant viruses? They may, I conceive, adopt one of four attitudes:—

(1) They may disclaim any interest in botany, holding that the work on tobacco mosaic has no bearing on their own problem. There seems no logic in this; the most important properties of viruses appear to be held in common by those attacking animals and those attacking plants.

(2) They may suspend judgment pending further proof that the crystallized product really represents the virus. This attitude is more reasonable. At first the identity of the crystals and the virus seemed very doubtful, but the evidence in its favour is in my opinion gradually increasing.

(3) They may abandon their defences and cease to think of viruses as micro-organisms and regard them as auto-catalytic enzymes.

(4) They may hold that what Stanley has done is, after all, primarily to show that the infective particles of a virus under certain conditions arrange themselves in regular rows. In my opinion this fact does not solve the problem of whether they are micro-organisms or autocatalysts.

I shall not discuss whether these viruses are alive or dead, because I think it obvious that according to the criteria we have held in the past they possess some of the properties hitherto associated with autonomous living things and some hitherto associated with non-living chemical substances. But I should *not* conclude from that that they are in process of evolution from the non-living to the living. I find it far easier to think of them as small micro-organisms which in the course of evolution have gained by becoming smaller, losing some of the chemical complexities of larger beings, and perhaps thereby becoming subject to physico-chemical laws which may on occasion cause them to do such things as form paracrystals. I know that viruses are of a graded series of sizes, larger in some instances than cultivable organisms, that their immunological behaviour is like that of bacteria in that an animal once infected becomes more or less immune to subsequent infection, that some of them can form soluble haptenes, or multiply in an insect intermediate host, that they can mutate and adapt themselves to altered conditions. If I am asked to forget all that and adopt a new philosophy of life because the bodies in question can arrange themselves in orderly rows, I entirely refuse to do so.

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Dr. F. C. Bawden: It is extremely difficult to prove that something does not exist, and for this reason it is impossible for us to state dogmatically that the nucleoproteins we have isolated from virus-infected plants are the viruses themselves. Many substances long regarded as homogeneous have ultimately been shown to be heterogeneous, and it is well to begin by admitting the possibility that our purified virus preparations consist largely of an inert nucleoprotein contaminated with small amounts of active virus. There is no evidence at present to support this view, and I wish to give some of the reasons why we consider it to be improbable.

In the first place, the characteristic proteins which we have isolated have been found only in virus-infected plants, and they can be found in all species of infected plants, whether these are taxonomically related or not. An exhaustive search of healthy plants has not revealed anything at all similar to them, and the amount of protein that can be isolated from infected plants is directly proportional to the infectivity of the sap. Also, the particular protein isolated depends only on the virus used for infecting, and not at all on the host plant infected. This, of course, does not prove that the proteins are the viruses, for they may merely be specific by-products

of virus activity. If this view be adopted, however, the problem is greatly complicated, for the virus again becomes a mysterious something, with the added complication that it now not only itself increases but also causes the production of a specific and extremely unusual protein.

It has been known for some time that plants infected with certain viruses contain specific antigens, and a great deal of evidence has been accumulated indicating that the antigens are the viruses themselves. There can be no doubt that the nucleoproteins we have isolated are these specific antigens. One cubic centimetre of solution containing from one five-millionth to one ten-millionth of a gramme gives visible precipitates with antiserum, and the serological titres are therefore of the same order as those of other highly purified antigens.

Stanley describes the acid and ammonium sulphate precipitates from tobacco mosaic virus preparations as crystals, and argues that as repeated "recrystallizations" do not affect the properties his preparations are homogeneous. Even if the precipitated needles were true crystals it is doubtful if this argument is valid, but, although the protein possesses some properties that are characteristic of crystalline materials, the needles appear to be merely pieces of jelly containing 50% of water. They lack the 3-dimensional regularity of true crystals, and are more accurately described as fibres or as paracrystals. We have found that the appearance of the precipitated material is not greatly affected by the presence of comparatively large amounts of certain impurities, and the apparent physical uniformity cannot be taken as conclusive evidence of purity. We find that virus preparations which will not form dilute, spontaneously birefringent solutions can always be further purified, but when they do we have been unable to demonstrate any heterogeneity. Fractionations which often cannot be made by further precipitations with acid and ammonium sulphate can readily be made by incubation with trypsin. The further purification affected by this method is shown by some anaphylactic tests made by Chester, who finds that tobacco mosaic virus stimulates the production of good precipitating antisera but is not anaphylactogenic, whereas normal tobacco protein is strongly anaphylactogenic. Chester demonstrated the presence of normal tobacco protein by the anaphylactic test in all the preparations of tobacco mosaic virus prepared merely by precipitation methods, but he was unable to demonstrate the presence of such protein in our liquid crystalline preparations which had been treated with trypsin.

In addition to the virus causing ordinary tobacco mosaic, we have worked with the related strains causing Enation mosaic and Aucuba mosaic of the tomato, and with cucumber viruses 3 and 4. Although each of these viruses causes a characteristic disease, and the cucumber viruses have distinct host ranges, all five are serologically related. From different plants infected with each of these viruses we have isolated proteins which, when highly purified, form dilute liquid crystalline solutions. The analytical figures of these preparations are constant, and from the chemist's viewpoint they can probably be regarded as homogeneous. Dried preparations of all five viruses contain about 50% of hydrogen, 16% of nitrogen, 0·5% of phosphorus, and 2·5% of carbohydrate. When denatured by heating to 90° C. they all break down, and give a denatured protein free from phosphorus and carbohydrate, and a free nucleic acid of the ribose type. We have never obtained fully active preparations of any of these viruses free from nucleic acid, and we therefore describe our products as nucleoproteins.

Denaturation of the protein by heating, by acid, or by any other means, is accompanied by loss of virus activity, and the falling-off in the infectivity of a preparation is directly proportional to the amount of protein denatured.

The five viruses mentioned are obviously closely related to one another, and in any natural system of classification would be placed in the same group. The nucleo-

proteins isolated from plants infected with these viruses possess similar physical, chemical, and serological properties, but we have found sufficient differences between them to show that each is a distinct protein. Furthermore, the order of the differences between the proteins is that which might be expected from a knowledge of the pathology of the viruses, for the greater the differences shown by the viruses in their host ranges or in the symptoms caused, the greater are the differences that can be found in the serological reactions, X-ray diffraction patterns, and physical properties of the isolated proteins.

Recently we have been working with potato virus " X ", a virus not related to tobacco mosaic virus in any way, and very much less stable *in vitro* and more susceptible to most physical and chemical treatments. From plants infected with this virus we have also isolated nucleoproteins with a chemical composition similar to that of the tobacco mosaic virus. When highly purified, this nucleoprotein also forms dilute liquid crystalline solutions, and shows the phenomenon of anisotropy of flow quite as strongly as preparations of tobacco mosaic virus. When precipitated with acid and ammonium sulphate it does not give paracrystalline needles, but a flocculent amorphous precipitate. The properties of this protein differ from those of the tobacco mosaic protein in all the ways in which the two viruses are known to differ. Treatments which have no effect on tobacco mosaic virus, such as heating to 70° C., storing at pH 3 and incubating with trypsin, destroy this protein, and the destruction of the protein is again quantitatively related to the loss in infectivity suffered by the preparation.

There are many other reasons for thinking that the highly purified preparations of tobacco mosaic virus are relatively homogeneous. When they are centrifuged at high speeds sharp boundaries are given. The material also behaves uniformly in an electric field, and the X-ray patterns give no indication of the presence of more than one type of molecule. Also, the composition of the sediment is the same, whether the virus preparations are centrifuged at their iso-electric point or on the acid or alkaline side of it. Further, if the preparations were a mixture of virus and protein, it might be expected that precipitation with antiserum would alter the proportion of the components, but this does not happen. Protein which has been recovered from inactive mixtures with antiserum has the same chemical composition, and the same virus activity, as that obtained straight from the plant.

The virus in our purified preparations will not pass through fine filters, and this fact can be explained if it be assumed that the virus aggregates during purification, and it can be shown that the protein particles actually do aggregate. The amount of anisotropy of flow shown by a given weight of protein, other factors being equal, will depend on the length of the particles, for the longer the particles are in relation to their width the more easily will they be orientated by streaming. From the yields of protein obtained it is apparent that clarified infective sap is about a 0·2% solution, but the amount of anisotropy of flow shown by infective sap is much less than that shown by a 0·2% solution of the isolated protein in clarified healthy sap. This suggests that the particles in untreated sap are relatively small and that during purification they aggregate linearly to form long rods. One precipitation of the protein from sap is sufficient to bring about an increase in the anisotropy of flow, and this effect is always accompanied by a reduction in both the infectivity of the sap and the filtrability of the virus. That is, an observed aggregation of the protein is accompanied by changes in the virus which are readily explained on the basis of an aggregation of the virus particles. Yet another fact closely relating tobacco mosaic virus to the protein is that the width of the purified protein, as measured by X-rays, is almost identical with the width of the smallest virus particles in untreated sap as measured by the filtration end-point through collodion membranes.

At present we require about 10^{-10} of a gramme of tobacco mosaic virus protein to get an infection. Although the molecular weight of the protein is not known at all accurately, it is probable that this amount represents a large number of molecules. Our methods of inoculation are relatively crude, and there is no doubt a considerable wastage of inoculum, but until infection can be brought about by one molecule it is difficult to see how proof can be obtained that the proteins are the viruses. At the present stage of the work this seems most probable, and the results obtained suggest that it is reasonable to assume that if all the particles in our purified virus preparations are not active particles, they are at least similar to active particles in their superficial properties.

I would now like to say a few words about another aspect of the work on plant viruses which, if it can be applied to animal viruses, may be of some value in medicine. In doing this I do not wish to imply that I consider all viruses to be similar chemically, but some of the smaller and more stable animal viruses have properties in common with the viruses with which we have worked, and a claim has already been made that one animal virus has been isolated in the form of a protein. We find that any treatment which denatures the protein causes loss of infectivity, serological activity, and the characteristic optical properties. Certain treatments, however, destroy the infectivity of our purified virus preparations without affecting the serological reactions. These treatments do not denature the protein and have no effect on the ability of the virus preparations to form liquid crystalline solutions and to show anisotropy of flow. With plant viruses these results are of no practical value, but if they can be applied to animal viruses they may be useful in the preparation of vaccines. The methods which we find to be most effective are irradiation with X-rays and ultra-violet light, and treatment with nitrous acid, formaldehyde, and hydrogen peroxide.

Mr. J. D. Bernal: The optical and X-ray investigation of the virus preparations which Dr. Bawden has described answer at least some of the questions raised in the discussion. The physical properties of the majority of the viruses studied—i.e. tobacco mosaic, and cucumber and potato X-virus—all indicate the presence of long particles. These account for the double refraction of flow of the dilute solution, for the spontaneous double refraction of the more concentrated solution, for the formation of double refracting gels and also for the formation of the spindle-shaped tactoid bodies. These are formed as the result of the competing tendencies of surface tension to form a spherical aggregate and mutual orientation tending to form a linear one. There seems little doubt that the " crystals " originally described by Stanley are really microtactoids. We have shown that they are indistinguishable from the preparation we call " wet gel ", containing about 50% of water, formed by drying the concentrated solution. The X-ray evidence has confirmed these observations and added much new information. X-ray photographs of the virus show a pattern which may be considered in two parts : one of scattering at large angles, which corresponds to that of a semicrystallized protein, and one at small angles, indicating the packing of the particles. The latter has received more attention but perhaps wrongly so. The X-rays show unequivocably that the particles of the virus are practically identical and pack together in regular 2-dimensional bundles. There is no evidence of regularity in the direction of the rods themselves, but in the other two directions there is a perfect hexagonal pattern, the scale of which varies quantitatively with the amount of water that is inserted between the particles. The absence of 3-dimensional regularity is, however, probably fortuitous and due to the difficulties of inducing a higher degree of crystallization. Such a degree seems to be achieved in Nature, as true crystals containing the virus material, and probably other material

as well, are observed in the cells of affected plants, and these crystals possess end as well as side faces and undeniably show 3-dimensional regularity. This is, of course, no criterion of purity, especially as such crystals probably contain at least 50% of water ; indeed, Northrop has gone so far as to say that where proteins are concerned the larger the crystal the more impure the material.

If this were all the evidence, however, it would still be possible to claim that crystallization in this case was simply a packing together of essentially similar organisms, and did not involve anything incompatible with traditional views as to the vital nature of the viruses. It is the evidence of the large-angle scattering that makes this view untenable because here X-rays show unequivocally that regularities occur inside the particle. That these regularities belong to the particle is shown by the fact that they persist unchanged from the driest gel to the most dilute solution. The scale of this regularity is relatively small, of the order of 20 Å.—much smaller than any particle hitherto observed that may be claimed as a living organism—and is intermediate in character between that observed in undenatured and denatured protein patterns. If there is any more complex " vital " material about, it must be represented by only a small fraction of the bulk of the particles.

I should like to stress finally that the peculiar liquid crystalline character of the viruses hitherto studied, and also of bacteriophage, must be considered rather a convenient accident than an essential character. Quite recently Bawden and Pirie have obtained a preparation of the virus of the bushy-stunt disease of tomato, which shows no double refraction flow and produces isotropic rigid gels. The internal pattern, as shown by X-rays, proves, however, to be of essentially the same type as those of the other viruses, indicating that here, where the particles are not long but probably quasi-spherical and much smaller, a similar internal structure persists.

The physical and X-ray evidence can, of course, only tell us about the structure of the preparations which the biologists present us with ; whether they are or are not the virus we are not competent to discuss, but if on the ground of biological evidence they should be considered to be the virus, then the statements made as to their internal structure, though by no means final, must be taken as positive evidence for a high degree of internal and external regularity.

Dr. A. S. McFarlane : No one will disagree with Dr. Andrewes' criticism of the recently published work of Wyckoff and his collaborators, which is admittedly incomplete and does not in the end prove very much. Its weakness, however, does not justify bacteriologists in failing to realize the important implications of recent work on the heavy proteins. One main reason for regarding the animal viruses as living organisms is their high infectivity and ability to proliferate in contact with living matter. Now in one particle or molecule of tobacco mosaic there are a number of units—amino-acids or polypeptide chains—which are in the fixed relationship to each other of a crystal lattice. Crystallographic measurements indicate with a high degree of accuracy that there can be no significant amount of water inside each particle, and centrifugal results reveal a great similarity in the size of the particles. One could hardly imagine a state of affairs more unlike what would be expected if these particles were small organisms. Nevertheless, there has been a consensus of agreement by the previous speakers that these particles or molecules in contact with the living plant exhibit all the infectivity and proliferative ability which characterize viruses, and are in fact the virus. In my opinion this discovery casts more than a shadow of doubt on one of the central arguments for regarding the smaller viruses as living organisms. It would be very helpful to know more about the homogeneity in

size and the water content of the animal viruses. A reliable series of measurements of these two properties would no doubt go a long way towards solving the problem of where the heavy proteins end and miniature bacteria begin.

Dr. L. P. Garrod said that although the arguments adduced by Dr. Andrewes in support of the living nature of viruses were formidable, he ventured to suggest that one of them could be answered. This was the point that virus infections produced immunity, as bacterial infections did. Non-living substances could excite specific antibody formation, and it was not difficult to picture a precipitin-like action as the basis of acquired immunity to virus diseases, if the causative agent was in fact a protein. He had listened with great interest to Mr. Bernal's exposition ; he wondered whether his description implied that the particles whose structure had been studied were single molecules.