Die Möglichkeiten, daß nach dem Radikalmechanismus formulierter Reaktionen sich als Ionenreaktionen erweisen, wurde berücksichtigt.

Herrn Prof. Dr. Th. Wieland sei an dieser Stelle für seine Ratschläge herzlichst gedankt.

The effect of ether on Newcastle disease virus: a morphological study of eight strains

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(Z. Naturforschg. 18 b, 114—118 [1963]; eingegangen am 15. Juli 1962)

Eight strains of Newcastle disease virus (NDV) have been examined using the technique of negative contrast electron microscopy, before and after treatment with ether. There is considerable variation in the shape and size of particles in any one strain, and the degree of this pleomorphism varies from strain to strain. The two common features of all the strains are the internal ribonucleoprotein and the rosette-like haemagglutinin formed by disruption of the coat with ether. The effect of ether is to disrupt the particles, but the ease of disruption varies from strain to strain. The products of ether treatment are the internal ribonucleoprotein and the haemagglutinin, which consists of portions of the outer coat. Besides these, the coat itself may in some strains appear almost intact, but stripped of its projections, after ether treatment. With the strains whose pathogenicity was known it has not proved possible to relate structure to degree of pathogenicity.

Newcastle disease virus (NDV) consists of particles of irregular appearance in the electron microscope¹. More recently high resolution electron microscopy has revealed an inner component with helically arranged subunits, or capsomeres²,³. This inner component is a ribonucleoprotein⁴. It is seen in particles whose coat has ruptured spontaneously, but it is released in greater quantity from particles treated with ether. The outer layer of the particle is presumed to contain lipid. The outside of the party...
ticle bears numerous projections, and, on treatment with ether, it breaks down into rosette-like structures. These structures have the property of agglutinating red cells, although they were not specifically characterised as haemagglutinin by Rott and Schäfer. Observations on the fine structure of the virus have been limited to a small number of strains, and no exact comparison has been made of strains of varying biological properties. The present paper reports a comparison of the fine structure of eight strains and the results of the disruption of their particles by ether.

Materials and methods

Virus

Eight strains of virus were used. Where it is known, the pathogenicity for the chick embryo is given, i.e. velogenic, mesogenic and lentogenic, according to the speed of killing, velocigen being the most rapidly lethal.

1. "Standard": An unnamed strain received from Dr. A. Isaac (National Institute for Medical Research, London). This is the strain described in earlier publications.

2. California-11914: Received from Dr. P. I. Marcus (Albert Einstein College of Medicine, New York). Velogenic.

3. Italy-Milano-1945 (Milano, M or M2): Received from Dr. R. P. Hanson (NDV Repository, Department of Veterinary Science, College of Agriculture, University of Wisconsin, Madison, U.S.A.). Velogenic.

4. Texas-DK: Received from Dr. R. P. Hanson. Mesogenic.

5. NJ-Roakin: Received from Dr. R. P. Hanson. Mesogenic.

6. Blacksburg (Bl, Hitchner): Received from Dr. R. P. Hanson. Lentogenic.

7. Mass-Hik: Received from Dr. P. I. Marcus. This strain is referred to by Marcus and is the same as that studied by Moore and Diamond.

8. "Beaudette": Received from Dr. P. I. Marcus. The relation of this strain to other "Beaudette" strains is not known. Referred to by Marcus.

Preparation of concentrates of virus

Each strain was grown in 11-day embryonated hens’ eggs, and after one or more passages, a batch of eggs was inoculated with virus in the form of allantoic fluid (0.05 ml. of 10^-3 dilution in trypsin broth). The eggs were incubated for 48 hours. Concentrates were then prepared by clarification of the harvested allantoic fluid at 3000 r.p.m. for 10 minutes, followed by deposition of the virus at 45,000 g. for 45 minutes. The pellets were resuspended in a small volume of phosphate-buffered saline (1.0–2.0 ml) and stored at −70 °C until required. The preparations of the various strains contained comparable quantities of virus, as measured by haemagglutination.

Ether treatment of concentrates

Concentrates in phosphate-buffered saline were shaken in a "Microid" flask shaker at room temperature with an equal volume of freshly distilled ether. Aliquots were taken at three hours and twelve hours and the ether blown off in a stream of nitrogen. These, and a sample of the concentrate itself, were then dialysed at +4 °C against 1% ammonium acetate for four hours, and sprayed for electron microscopy.

Adsorption and elution on red blood cells

50% washed fowl red cells in citrate saline were added to virus or haemagglutinin preparations (1.0 ml. of cell suspension per 5000 haemagglutinating doses of virus) and the mixture left in an ice-bath for one hour. The red cells were then washed with citrate-saline, and about one tenth of the original volume of phosphate-buffered saline (containing penicillin and streptomycin) added. Elution was allowed to proceed for two hours at room temperature.

Haemagglutination

Haemagglutination titrations were done in plastic plates, using volumes of 0.25 ml. of virus and 0.25 ml. of 1% washed fowl red cells, in phosphate-buffered saline, at room temperature.

Electron microscopy

Preparations which had been dialysed against 1% ammonium acetate were mixed with twice the volume of 2% potassium phosphotungstate (pH 7.0) and sprayed onto carbon-coated grids with a Vaponefrin nebulizer. These were examined in the Siemens Elmiskop at an instrumental magnification of 40,000 X, using double condenser illumination.

Results

Preparation of virus and haemagglutinin

Adsorption of virus in citrate-saline rather than phosphate-buffered saline gave appreciably higher...
yields of virus with most of the eight strains used. This is presumably because the absence of available calcium ions hindered the action of the viral neuraminidase, and hence diminished elution of virus during the adsorption period and during washing. Room temperature was used for elution rather than 37°C because there was considerably less haemolysis at the lower temperature, and at the same time a satisfactory recovery.

The addition of red cells to suspensions of virus which had been treated with ether removed a component identical with the rosette-like structures of Rott and Schäfer. These eluted from the red cells in the same way as intact virus, and hence may be characterized as haemagglutinin, corresponding to the same structure in influenza virus. It should be emphasised that this structure is produced artificially from the particle by ether, and is not naturally released from infected cells, as is the non-infective haemagglutinin described by Rott, Reda and Schäfer.

**Electron microscopy**

(a) *Features common to all strains.* In all the unsplit concentrates the majority of the material present in the phosphotungstate droplet patterns consisted of particles recognisably similar to those previously reported (Fig. 1*). Such particles also constituted the entire material in preparations of virus adsorbed and eluted on red cells, and, in one strain (Italy-Milano-1945) purified on a calcium-phosphate column. This leaves little doubt that the particles described in preparations prepared by differential centrifugation are in fact particles of the virus.

While there was considerable variation in size and shape among the strains, and even among the individual particles of strains, most particles, as seen on the grids, were roughly circular in outline, with a maximum diameter between 1200 Å and 3000 Å. Some strains, especially California-11914, (Fig. 2) had many particles whose axial ratio was greater than 2:1, but in these the inner component could frequently be resolved, and they do not appear to be structurally homologous with filamentous forms of influenza virus. In all strains there were some spontaneously disrupted particles, which revealed an inner component of helical form, and diameter approximately 180 Å (Fig. 3). This component was indistinguishable among strains, and was in each case identical with that previously described. The particles were bounded by a coat consisting of a more or less easily resolved membrane bearing short projections (up to 80 Å in length) somewhat less conspicuous than those seen on the particles of influenza virus.

After treatment with ether three structures were seen: (1) The inner helical component (2) Structures with the projections on their surface, derived from the coat. These usually appeared as small spheres, i.e. they were circular in outline (Figs. 7, 8), with a diameter between 500 and 50 Å. They resemble the haemagglutinin described by Rott, Frank and Schäfer. Not infrequently longer "unrolled" forms of these were seen. (3) Smooth, sharply defined rings were also seen, of a diameter comparable with that of the particles. These appear to be the membranes of the particles, freed from the inner component, and stripped of their projections (Fig. 9).

(b) *Special features of each strain*

(i) "Standard". This was the least pleomorphic strain, and consisted mainly of forms with a circular outline, such as that shown in Fig. 1. The surface projections were of medium length and rod-shaped. Membranes were conspicuous and the internal component was seen easily only in particles which had burst open. After three hours ether treatment both the internal component and the haemagglutinin were well seen. The helix was characteristic in that much of it was in the form of long chains. Both components were identifiable at twelve hours but, although the helix remained as intact as at three hours, the coat was more broken up, and there was less to be seen.

(ii) California-11914. This strain had a quite distinctive morphology. Two principal forms were seen. The predominant one consisted of elongated particles, up to 4000 Å long, though there was con-

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*Fig. 1—9 see table pag. 116 a and b.

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Electron micrographs prepared by negative contrast technique, using potassium phosphotungstate. Newcastle disease virus.

Fig. 1. "Standard". Typical particles. X 280,000. Fig. 2. California-11914. Group of particles showing pleomorphism. X 130,000.

Fig. 3. California-11914. Particle which has disrupted on spraying, releasing the inner helical component. X 140,000. Fig. 4. "Beaudette". Particle in which the helix is visible as a series of concentric rings. X 150,000.

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Fig. 5. Italy-Milano—1945. Ring forms. X 160,000.
Fig. 6. NJ-Roakin. Particles illustrating the great pleomorphism. X 170,000.
Fig. 7. "Beaudette". Three hours ether treatment. Free helix, amorphous debris, and rosettes of haemagglutinin. X 150,000.
Fig. 8. Texas-DK. Three hours ether treatment. Similar to "Beaudette". X 190,000.
Fig. 9. Mass-Hik. Twelve hours ether treatment. Membranous part of the coat, stripped of its projections. X 160,000.
considerable variation in length and breadth. The helix was visible within these forms, and ran in parallel strands along the long axis of the particle. The surface projections were long and club-shaped. This type of structure (Fig. 2) was often associated with close packing of the particles, though this was not always so, and was evidently not caused by the close packing. The other form had an irregular, but roughly circular, outline. The membranes in either type were poorly defined. There were a few ring forms.

After three hours ether treatment all particles were disrupted. There were abundant large pieces of the coat and short lengths of helix found mainly in clumps. After twelve hours only the identifiable structures were pieces of the coat.

(iii) Italy-Milano-1945. Particles were fairly regular in size and shape. Occasional giant forms up to 8000 Å diameter were seen. There were a few ring forms (Fig. 5) in which projections could clearly be resolved around the hole in the centre. The projections were short, and were absent in some particles.

After three hours ether treatment both outer and inner components were plentiful. The inner component was all in short lengths. Membranes were not seen. After twelve hours ether only a little of the coat was recognizable.

(iv) Texas-DK. This strain was conspicuously pleomorphic. There were very small forms and giant forms as well as ring forms, which were seen more with this strain than with any other. The surface projections were short and the membrane barely visible.

After three hours ether treatment many particles remained intact. In those which were split, the products were frequently seen to remain together as a mass. The haemagglutinin was well seen and large amounts of helix were present in masses of moderate length (Fig. 8). After twelve hours there were no intact particles left, but both the products of splitting retained their form.

(v) NJ-Roakin. The overall size was fairly constant, but many bizarre forms (Fig. 6) were seen, especially ring forms and dumb-bell-shaped forms. The projections were long and the membrane conspicuous. After three hours ether treatment both components were equally obvious, but after twelve hours only a few rosettes of haemagglutinin remained.

(vi) Blacksburg (Bl, Hitchner). This strain was fairly pleomorphic, but not so much as, for example, California-11914 or Texas-DK. Many particles were made up of a larger spherical part with a much smaller sphere arising on one side. Surface projections were long and the membrane indistinct. Both components were visible after three hours ether treatment, but after twelve hours there were no recognizable viral components.

(vii) Mass-Hik. The particles were fairly uniform. No ring forms were seen. The membrane was conspicuous. Some particles had burst to release the internal component. The projections were conspicuous. After three hours ether treatment only a few intact particles remained. The haemagglutinin was plentiful. No helix was seen at all at this time. By twelve hours the structures formed from the coat were much smaller. Stripped membranes which still retained the form of the particle were particularly conspicuous (Fig. 9).

(viii) “Beaudette”. The particles were fairly uniform in size and shape. A few had an elongated “tail” at one end. The internal component was seen lying in concentric rings in many intact particles (Fig. 4). The membranes were conspicuous. After three hours ether treatment all three products of splitting (helix, haemagglutinin and membranes) were plentiful. The helix was in short lengths (Fig. 7). After twelve hours only the outer component remained recognizable.

Discussion

It is clear that the first strain of NDV to be examined in the electron microscope by negative staining (“Standard”) was particularly uniform in its particle form, whereas others may be very pleomorphic (e.g. California-11914 or Texas-DK). However, in spite of this variation between strains, there is one constant feature of the particle common to all those studied, and indistinguishable by available techniques of microscopy, i.e. the internal helical ribonucleoprotein of the various strains. The fact that this is released from within the lipid coat of the virus by ether treatment shows the analogy of structure with the influenza virus. The haemagglutinin formed from the coat is also indistinguishable from strain to strain, though strains vary in the susceptibility of the coat to the destructive action of ether. This uniformity of these component parts,
especially the ribonucleoprotein, outweighs the variation which is at first sight to striking. Pathogenicity for the chick embryo is not related to any particular form of the particle, nor to the ease of disruption by ether, at least in the strains studied. It is clear from these findings that if it is desired to prepare any one particular component of the particle, e.g. the disrupted outer coat (haemagglutinin) or the ribonucleoprotein helix, the strain of virus and the length of ether treatment must be selected carefully.

We are indebted to the Medical Research Council for a grant in aid of this work.

Studien über die uv-induzierte Mutabilität des Serratia-Phagen KAPPA durch Versuche mit uv-bestrahltem Indikator und Phagenkreuzungen

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UV inaktiviert KAPPA can be reactivated like other temperate phages by plating on unirradiated host cells (indicator). The capacity of the indicator Serratia HY for multiplication of unirradiated KAPPA was about 0.1% survivors (colony formers). The induction of clear plaque (c-) mutants by irradiating extracellular KAPPA and plating on unirradiated indicator can be increased further about 2 to 4 times by using UV irradiated indicator. The increase of the number of c mutants under the latter conditions, with increasing UV dose given to the phage, was never a first-order reaction. The highest frequency of c mutants obtained was about 4.5 per cent. Plating of unirradiated KAPPA on irradiated indicator (lowest survival fraction was 0.01%) never increased the spontaneous mutation rate to c. Two c mutants studied in detail belong to two different cistrons as shown in a complementation test (map distance about 5.3%). Only one of both was revertible to the phenotype c+ spontaneously and with a higher rate by UV. However, as shown in crossing experiments with the wild type, the backmutants do not have the original genotype but originated from mutations in at least two different intragenic suppressor loci; the map distances between them and the original c mutation were 0.64% and 0.13 per cent. Host range (h) and virulent (v) mutants could not be induced by irradiation of the free phage and plating on unirradiated indicator. This indicates that the UV induced high mutability of the c loci in KAPPA represents an exceptional case of behavior (UV-hot spot). Some unstable h mutants could be isolated by plating irradiated phage on irradiated indicator.

Der aus dem lysogenen Serratia-Stamm K gewonnene 1 DNS-haltige 2 Phage KAPPA bildet auf dem rot pigmentierten Stamm HY im allgemeinen trübe Plaques mit violettem Hof. Gelegentlich findet man aber auch Klarplaques (c Plaques)**. Ihre Häufigkeit wächst mit der auf den freien Phagen eingeschalteten UV- oder Röntgendiagosis, ohne daß Selektion dafür verantwortlich gemacht werden kann 1, 3.

Versuche mit KAPPA und uv-bestrahlten Wirts-(Indikator-)Zellen waren von Interesse, weil bei dem am besten untersuchten temperierten Phagen, nämlich LAMBDA von E. coli, und auch anderen nur dann c-Mutanten durch UV ausgelöst werden können, wenn nicht nur die Phagen, sondern auch die Wirtszenlen bestrahlt worden sind 4. Bei KAPPA dagegen erhält man 1—2% c-Mutanten von bestrahltem Phagen auch auf unbestrahlten Wirtszenlen. Die erste Frage war also, ob die Häufigkeit der c-Mutanten von KAPPA durch Wirtsbestrahlung noch zu erhöhen ist oder nicht. Wie später gezeigt werden kann, liegt die Mutantenhäufigkeit auf bestrahltem Indikator höher als auf unbestrahltem.

Bei den Phagenkreuzungen sollte insbesondere ermittelt werden, ob die von einigen c-Mutanten isolierbaren c+-Rückmutanten mit dem Wildtyp gene-

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** Sie werden im folgenden aus Gründen der sprachlich einfacheren Darstellung als c-Mutanten bezeichnet, auch wenn ihre mutative Entstehung noch nicht sicher bewiesen ist.