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Present Trends and the Future in Rabies Research*,†

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Until very recently, rabies research had made few notable advances since the time of Pasteur, but during the past few years the use of modern virological techniques has led to rapid progress in research on the rabies virus. This article summarizes the present state of knowledge of pathogenesis, immunology, cultivation in tissue culture, cell–virus relationships, and physicochemical properties of the virus.

It is now possible, and opportune, to undertake the study of basic problems in the biology of rabies, which include the need for an improved method of post-exposure protection. Information gained from work on these problems should lead to important insights into other current problems of infectious processes such as " slow-virus " infections. The most promising directions of future research in rabies are enumerated and suggestions are made on the experimental techniques that might be used for such studies.

Until very recently, rabies research in the laboratory had scored few notable successes. The rabies virus did not easily yield the secrets of its basic characteristics even with the striking advances in virological techniques of the past two decades. Appreciable progress has been made in our fundamental understanding of the virus itself, but much remains to be learned. Such knowledge, with the accompanying requirement of modern quantitative techniques, is a prerequisite for any worthwhile advance in biological prophylaxis, as well as for other biological and epidemiological aspects of the disease that still remain obscure.

We are now at a stage in rabies research where it is useful to prepare a balance sheet of what is known and what work needs to be done. With the recent advances in specific knowledge about the rabies virus, and in virological techniques in general, a singularly unproductive field of research is on the verge of being converted into a rich source of information which may have an important bearing on other currently baffling questions concerning infectious processes, e.g., " slow-virus " infections.

The following account, based in large part, but not exclusively, on research conducted at the Wistar Institute, does not represent an exhaustive review of present trends in rabies research. It gives, rather, a summary of the main aspects along with indications of future lines of development that, in our view, would be most rewarding.

PATHOGENESIS

Routes of infection

Rabies virus is usually transmitted by the bite of an infected animal. It can also be transmitted by aerosols (Atanasiu, 1965), but this mode of spread has been observed in nature only in a cave (Frio cave in Texas) harbouring an immense number of rabies-infected bats (Constantine, 1962). Studies of the spread of rabies by inhalation and of the susceptibility of lung tissue as a primary site for entry and replication of the virus should be made on many species of wild and laboratory animals. Up to the present, there is no evidence that ingested rabies virus may be infectious, except perhaps in massive doses; however, here again an opportunity exists for a more thorough investigation of the problem.

Spread of virus throughout body

Rabies virus spreads from the site of infection centripetally *via* the peripheral nerves towards the central nervous system (Schindler, 1961; Dean, Evans & McClure, 1963; Johnson, R. T., 1965; Yamamoto, Otani & Shiraki, 1965; Baer, Shanthaveerappa & Bourne, 1965). Evidence is available

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that neither perineural structures nor Schwann cells are involved in the centripetal spread of the virus; most likely it " ascends " passively through nerve-associated tissue spaces (Johnson, R. T., 1965). These findings need to be amplified by the search for the infectious virus particles in the tissues involved, rather than by relying entirely on the presence of antigen detected by the fluorescent antibody staining technique (Johnson, R. T., 1965; Yamamoto, Otani & Shiraki, 1965). Furthermore, nothing is known about the physiopathological changes that may take place in the affected nerve during the travel of rabies virus from the site of infection to the central nervous system.

Although there is ample evidence that rabies virus is not transmitted by blood (Baer, Shanthaveerappa & Bourne, 1965), viraemia following experimental rabies infection has been reported (Borodina, 1959; Krause, 1966). It is believed that viraemia is caused by diffusion of the virus from tissues of animals infected with very high doses of rabies virus (Dean, Evans & McClure, 1963; Baer, Shanthaveerappa & Bourne, 1965). In the light of recent reports of infection with rabies virus by inhalation, and of marked differences in susceptibility of various animal species to peripheral infection (Sikes, 1962), studies on the role of viraemia in the spread of rabies may have to be reinvestigated, using those animals (e.g., suckling mice) most susceptible to infection by various routes.

Immunofluorescent studies have revealed that rabies virus after entering the central nervous system is present in the grey matter, replicating within that system almost exclusively in the neurones (Johnson, R. T., 1965); very small amounts of antigen were detected in the white matter by only one group of investigators (Yamamoto, Otani & Shiraki, 1965). It is not known why only certain morphologically defined groups of nerve cells are susceptible to rabies infection. When fractionation of nerve tissue into various cellular components becomes a laboratory tool of investigation, perhaps it will become possible to study this puzzling phenomenon of selectivity.

Latency of the virus in the body

Rabies-infected cells appear not to undergo neuronolysis or neuronophagia (Johnson, R. T., 1965). Apart from this observation, virtually nothing is known about the intracellular events accompanying rabies infection of the central nervous system. Studies on a molecular basis of isolated neurones obtained from rabies-infected animals are urgently needed. The results could throw light on the virus–host-cell relationship in animals, and also might offer an explanation for the fate of the virus during the prolonged incubation period which so often accompanies rabies infection.

Abortive infections with street rabies virus occur in experimentally infected animals (Bell, 1964), and the apparent recovery of the animals may be related to the appearance of antirabies immunoglobulins in their brain tissue (Bell et al., 1966). Although these abortive infections are characterized by the impossibility of detecting any infectious virus after a presumed infection has occurred, it is possible that the complete viral genome may persist in the infected cell; a confrontation with an unknown trigger may activate the mechanism for the replication of this virus and thus result in the expression of all its functions, including infectivity. Another explanation for the prolonged incubation period in rabies may have to be sought through tracing precisely the release of infectious virus by the cells of the central nervous system along with the simultaneous determination of the level of interferon production, although there is no indication that interferon is produced in brain tissue after infection with street virus (see below). If it is found that the output of the virus per cell is very small and that, in addition, any excess of infectious virus is bound by specific inhibiting substances in the brain, then these considerations may account for the prolonged symptom-free period of infection. The development, after this period of quiescence, of the frank disease followed by death is still a major biological puzzle. Investigations in this area are of primary importance, because the results obtained may be of consequence not only for understanding the pathogenesis of rabies infection but also for gaining insight into the same problem that exists in the field of slow-virus infections.

Finally, the apparent presence of a specific rabies-induced antigen on the surface of tissue culture cells, reactive with antirabies serum and leading to lysis of the cells (see below), makes it imperative to investigate the possibility of auto-immune phenomena engendered by chronic rabies infection in the body of the animal.

HOST RESPONSES

Antigenicity of rabies virus

In crude suspensions of rabies-infected brain or fowl embryo, the ratio of non-viral to viral antigens is about $10^9:1$ (Kuwert, 1967). Immunological studies with such preparations proved not to be

feasible, since the results obtained could not be evaluated quantitatively. The recent availability of purified rabies preparations (see below) which contain less than 5% of non-viral components has made it possible to initiate quantitative investigations on the antigenicity of rabies virus.

Rabies virus can be considered a strong antigen because sera produced by 4 injections into rabbits of a total of 250 μg of purified virus, emulsified in Freund's complete adjuvant, contained neutralizing antibody titres of up to 1 : 60 000 (against 20 LD_{50} of virus) and complement-fixing titres of up to 1 : 1000 (Kuwert, unpublished data). Experimental vaccine prepared from purified virus inactivated by beta-propiolactone was found to be highly antigenic in mice, being 30 times as antigenic as the International Reference Vaccine (lot 173) in the NIH potency test (Wiktor, unpublished data). The availability of antibody produced against the purified virus made it possible to show marked serological differences (Kuwert & Wiktor, unpublished data) between various strains of rabies virus. These results confirmed and amplified previous observations (Wright & Habel, 1948; Johnson, H. N., 1965; Vujkov, 1966) which referred to the possible existence of specific antigens for various strains or groups of strains of rabies virus.

The immunologically potent, purified antigen should be tested for its immunogenic properties in man. Judged by results so far obtained in experimental animals, we may look forward to the development of a vaccine which will produce adequate responses in human subjects after 1, 2 or 3 injections rather than after the 14 or 21 injections required with presently available vaccines. Further development of specific serological techniques for the detection of undesirable antigenic by-products of the rabies vaccine may make it possible to produce a human rabies vaccine that would be potent antigenically as well as devoid of the side-effects caused by antigenic impurities of the vaccine.

Refinement of the techniques for production of monospecific immune sera should make it possible to classify known strains of fixed and street rabies virus into definite antigenic groups.

"Soluble" antigens of rabies virus

Virtually nothing is known about the properties and the site of synthesis in the infected cells of the "soluble" antigens. It should be determined whether they represent structural components of the virion not incorporated into the virus particles, or whether they are enzymes induced by viral replication. The possibility that soluble antigens are also formed by degradation of the virus particles should be taken into account.

Using immune serum prepared against crude virus, which also certainly contained antibodies against an undetermined number of cellular and tissue-culture medium antigens, two soluble antigens having sedimentation constants of 12S and 23S have been found (Neurath et al., 1966). These findings, however, require confirmation using serologically well-defined antibodies.

Antibodies produced against purified split products of the virion, i.e., envelope material and internal components, respectively, should be used for the determination of the sites of formation of different virus-specific antigens. The dynamics of the formation of infective virus, of viral haemagglutinin, and of other viral antigen observable by fluorescent antibody staining should be investigated in parallel during a single growth-cycle of the virus (Kaplan et al., 1967). Staining with ferritin-labelled immune globulins produced against the individual viral antigens seems to be the most feasible approach for determining the site of synthesis of the various viral antigens in the infected cell.

Humoral and cellular defence mechanisms in rabies infection

The presence of neutralizing antibodies in the blood of man or animals is considered as an index of protection against infection with rabies virus (Habel, 1945; Koprowski et al., 1950; Balthazard & Ghodssi, 1954; Kuwert & Bindrich, 1958; Sabeti et al., 1964; Habel, 1964; Dean, Evans & Thompson, 1964). The level of neutralizing antibodies protecting monkeys against parenteral challenge with fixed virus has only recently been determined (Koprowski, 1967); in this study, it was shown that low levels of neutralizing antibodies did *not* protect the animals.

The presence of rabies-immune globulin in the brain tissue of animals infected with street virus may account for abortive infection with rabies (see above). Immunosuppression, following administration of adreno-corticosteroids as well as stress (Soave, 1964), converts the state of latency into one of clinically overt disease. Future studies should be directed towards the elucidation of the mechanism of this conversion. It is possible that an upset (hormonal, stress) in the delicate balance between levels of antibody in brain tissue on the one hand, and virus production on the other, may account for the conversion

of the latency state into one of clinically overt disease.

Investigations should also continue on the mechanism of lysis by rabies antibody of cells infected by rabies virus (Wiktor, 1966). Availability of antibody produced against pure virus may make possible the identification of " lytic " antibody as distinct from " neutralizing " antibody. The elucidation of the identity and role of the heat-labile, supplementary serum factor (HLSSF) essential for the lytic reaction may facilitate investigation of the lytic phenomenon *in vivo*. It is possible that absence of the HLSSF in cerebrospinal fluid of animals may make it impossible for the antibody to lyse rabies-infected neurones *in situ*. Artificial introduction of the HLSSF into cerebrospinal fluid of rabies-infected animals injected with immune serum may cause lysis of the nerve cells and precipitate or aggravate symptoms of disease similar to the observations made by Berge et al., (1961) with Venezuelan equine encephalomyelitis virus-infected mice. In such a case, the role of anti-rabies serum may have to be re-evaluated.

At present, at least five types of antibodies, i.e., neutralizing, complement-fixing, precipitating, haemagglutination-inhibiting and cytolytic, may be detected in serum of animals exposed to rabies antigen. The interrelationship of these various types of antibody and their role in immunity is unknown and merits thorough study. Also, dynamics of their production under various experimental conditions such as those observed by Svehag & Mandel (1964) with poliovirus should be followed.

INHIBITION OF RABIES VIRUS BY NON-IMMUNE MECHANISMS

Interferon

Hamsters inoculated intramuscularly with a massive dose of a fixed rabies strain produced large quantities of brain interferon only in the 2 days preceding death, and little interferon was recovered from all other tissues examined (Stewart & Sulkin, 1966). Experiments in progress in our laboratory (Wistar Institute) indicate that the amounts of interferon produced following intracerebral inoculation of hamsters with rabies virus depend on such factors as virus dose, age of the hamsters, strain of virus and incubation time; interferon is detected only in the presence of high levels of infective virus. Some fixed strains of rabies produced no detectable interferon during the course of infection, and no interferon was recovered from the brains of hamsters in either the excited or the paralytic stages of infection with a strain of street virus (Brinton & Campbell, unpublished data). These observations suggest that interferon is a by-product of rabies viral infection rather than an effective host defence mechanism against the virus.

In hamster tissue cultures, production of rabies virus reaches a peak and then falls off sharply during the course of subsequent cell transfers—a phenomenon accompanied by the appearance of an interferon-like inhibitor in the culture medium (Wiktor & Koprowski, 1967). Although interferon apparently appears too late during the course of rabies infection in the intact animal to be of prophylactic value, it can inhibit markedly the replication of the virus *in vitro*. It is rather doubtful, therefore, that the passive administration of homologous interferon into an animal will arrest the course of the disease. Induction of interferon formation in the animal body, however, should be investigated as a possible valuable adjunct to the present regimen of post-exposure treatment (Koprowski, 1967). Apparent inability of street virus to induce formation of interferon should be further critically scrutinized. The role of interferon present either at the site of virus replication or in blood in latent rabies infection of animals should be investigated.

Interference possibly not mediated by interferon

It is a truism that infection with rabies virus almost always follows a lethal course. Animals have been protected from general paralysis and death, however, after intraplantar inoculation of virulent fixed virus by intracerebral inoculation with an attenuated HEP-Flury strain (Wiktor et al., unpublished data). The inoculated animals suffered only from partial paralysis limited to the inoculated limb, and lived for a prolonged period. Since the HEP-Flury strain does not seem to cause production of interferon in brain tissue of infected animals, it is doubtful whether protection of these animals is mediated by the production of interferon in the central nervous system.

Similarly, inhibition of rabies virus in tissue culture systems may be mediated by the presence of non-infectious virus particles. Separation of these particles from the infectious particle may present a major task (see below) but it would clarify the nature of this type of inhibition.

Metabolic inhibitors

Since growth of rabies virus is not inhibited and is frequently enhanced by the presence in the culture medium of actinomycin D, mitomycin C, or fluoro-

deoxyuridine, all inhibitors of DNA or DNA-dependent RNA synthesis (Defendi & Wiktor, 1966), it has been reasonably well established that rabies virus contains RNA. In fact, rabies virus can replicate in the complete absence of all DNA synthesis (Maes et al., 1967). It was intriguing to find, therefore, that replication of rabies virus was markedly inhibited by the potent DNA inhibitor, arabinosyl cytosine (ara-C) (Maes et al., 1967). This inhibition occurred in all cell lines tested; no other RNA virus examined was more than slightly inhibited. The inhibitory effect could be partially or completely reversed by adding actinomycin D, nogalamycin, puromycin or cycloheximide during the first 3 hours of viral replication in the presence of ara-C. This indicates that the inhibitory action occurs probably at a very early stage in viral replication, and that it requires the induction of a cellular protein (Campbell et al., 1968). The half-life of this protein must be extremely short, or the messenger-RNA governing its synthesis very unstable, since pretreatment of cells with ara-C and removal of the latter at the time of infection did not inhibit replication of virus. These investigations should be extended with the aim of labelling, isolating and, if possible, identifying the rabies inhibitory protein (RIP). It will also be necessary to determine exactly at which stage virus synthesis is stopped by ara-C. Since there is a small fraction of viral population which escapes inhibition by ara-C, it should be of extreme interest to isolate this fraction and to use it in genetic investigations (see below) if it represents a population resistant to the inhibitory activity of ara-C.

The usefulness of ara-C as a practical rabies inhibitor *in vivo* is limited by its toxicity when used systemically and by the fact that it is rapidly deaminated *in vivo* to arabinosyl uracil, which is inactive against rabies virus. Nevertheless, it may be possible to find an analogue of ara-C which will induce formation of the inhibitory protein, which is not degraded by deaminases, and which does not have the toxic effect of ara-C. Such an analogue may be of great value, not only in the post-exposure treatment of rabies in man but also as a highly selective inhibitor of a single virus—a phenomenon so far unknown in molecular biology.

PHYSICO-CHEMICAL PROPERTIES

Morphology

Recent morphological studies (Hummeler, Koprowski & Wiktor, 1967) revealed that particles of intracellular rabies virus are not pleomorphic, at least in the early stages of cell infection. They are all bullet-shaped with one rounded and one flat end. The diameter of the particles is 75 mμ–80 mμ and their length is variable, the longest measuring 180 mμ. In cross-section the virus appears to consist of a core with a diameter of 40 mμ. Surrounding this core, except on the flat end, is a dense membrane which itself is surrounded by a fringe of surface projections. An indentation, similar to the nock of an arrow, is visible at the flat end of the virus. The surface projections are 60 Å–70 Å long with a knoblike structure at the distal end. The surface of the virus particle is arranged in hexagons forming a honeycomb. Preparations of extracellular rabies virus, obtained by concentration and purification of infective tissue culture fluid, contained particles exhibiting essentially the same morphological features (Sokol et al., unpublished data). Naturally, an appreciable proportion of the particles was partially decomposed by the manipulation of the virus in the course of the purification. The only variable in the morphology of rabies virus appears to be the length of the bullet-shaped particles. The bizarre forms occasionally observed, which show some characteristics of rabies virus particles, may represent either artefacts arising during purification and preparation of samples for electron microscopy, or abortive forms synthesized during late stages of infection, when the synthesizing ability of the host cells had already been damaged.

Chemical composition

Almost nothing is known about the chemical structure of rabies virus. As discussed earlier, substantial although indirect evidence suggests that it contains RNA. This suggestion was recently confirmed in our laboratory by isolation of viral RNA from highly purified rabies virus preparations labelled with tritiated uridine. The molecular weight of the viral RNA was found to be about 6×10^6. Its sensitivity to the degrading action of ribonuclease and its buoyant density of 1.64 in Cs_2SO_4 solution indicated that it is single-stranded (Sokol et al., unpublished data). Localization of the RNA within the virus particle is unknown, but most likely it is included in the core. The fact that the infectivity of a rabies virus preparation is destroyed or markedly reduced by treatment with lipid solvents or agents capable of emulsifying lipids suggests that the particles contain lipids as a structural component (Kissling & Reese, 1963; Kaplan, Wiktor & Koprowski, 1966). The protein moiety of the virus

probably consists of several components, as would be expected from the complex structure of the particle. One of the coat proteins is capable of agglutinating various erythrocyte species. However, rabies virus haemagglutinin does not seem to be associated with an enzyme capable of destroying receptors of the red blood cells (Halonen, personal communication).

Sedimentation and density

The average sedimentation constant (*S*) of infective rabies virus particles is 600 (Neurath, Wiktor & Koprowski, 1966). An appreciable proportion of the complement-fixing activity and almost all of the haemagglutinating activity contained in infective tissue culture fluid sediment together with the infective particles (Sokol et al., unpublished data). The results of these experiments revealed that the virus particles are heterogeneous with respect to sedimentation velocity. Similarly, preparations of rabies virus consist of particles differing in buoyant density in CsCl solution, the average density being 1.20 (Neurath, Wiktor & Koprowski, 1966).

Purification

Substantial progress in the study of the physical and chemical properties, as well as of the antigenic structure, of rabies virus will be made possible only when sufficient quantities of highly purified virus are available for analysis. Attempts have been made to purify rabies virus from suspensions of infective brains, but the procedures proposed were only partially efficient (Thomas et al., 1965; Muller, 1950; Sawai et al., 1954). Recently, rabies virus was purified from infective tissue culture fluid containing serum albumin instead of serum by the following procedure (Sokol et al., unpublished data). The virus was first precipitated by zinc acetate and the precipitate was then dissolved in a solution saturated with the disodium salt of ethylene diaminetetraacetate. The virus was then filtered through a Sephadex column, treated with ribonuclease and deoxyribonuclease, pelleted by high-speed centrifugation, and finally banded by centrifugation in a 10%–60% sucrose gradient. The visible band contained all the infective, haemagglutinating and complement-fixing activity. The final preparation had a ratio of infectivity to protein content 2600 times higher than the original tissue culture material.

Tasks ahead

The most urgent task is the correlation of the morphological characteristics of rabies virus with its chemical and antigenic structure. Degradation of the virus particles by various means (lipid solvents, surface active agents, proteolytic or lipolytic enzymes), separation of the degradation products, and determination of their chemical, physical and antigenic properties would be the most feasible approach to the elucidation of this problem. Such a study should include also the characterization of the isolated viral RNA (biological activity, size, base composition, secondary structure, etc.).

As stated earlier, the length of rabies virus particles is variable. We have obtained some evidence that there exists a shorter and a longer form of the virion along with particles of intermediate size. Attempts are being made to isolate in a pure form the short particles in order to determine whether they are capable of infecting susceptible cells, and to compare their chemical composition with that of long virus particles. This may reveal a possibly smaller RNA content or lack of some protein components in short particles. The possible interference by the short particles, should they be non-infectious, with the replication of complete virus may be an important factor in determining the pathogenicity of the virus.

VIRUS–HOST-CELL INTERACTION

Adsorption and penetration

There are considerable difficulties to be overcome in the initial serial propagation of rabies virus in tissue cultures. In general, initial infection of monolayers or dispersed cell cultures induces rabies-specific fluorescent antigen in a very small proportion of the cells, and serial transfer of either the supernatant medium or of cell extracts in a homologous tissue culture system results in a gradual decrease in infection. As a rule, after a few passages virus is completely lost. It remains to be determined whether this fact is due to the inability of the virus to be adsorbed on to, and/or to penetrate into, most of the cells, or whether only a small proportion of the cell population is able to support replication of the virus. It is also possible that interferon or other inhibitory substances are produced by infected cells and accumulated in tissue-culture medium making difficult, if not impossible, the serial transfer of an unadapted virus strain. The mechanism of this inhibition should be elucidated. Other factors worth mentioning in connexion with inhibition of rabies virus at the site of penetration relate to the presence of an inhibitor in bovine serum used in tissue culture medium (Halonen, personal communication). Re-

placement of the bovine serum by bovine albumin enabled us to obtain a higher yield of rabies virus in tissue culture. The mechanism of action of various rabies inhibitors present in animal sera should be investigated.

Polycations enhance adsorption and penetration of the virus (Wiktor, 1966; Kaplan et al., 1967). It has been found that the use of DEAE-dextran (25 to 50 μg/ml) at each transfer permits the indefinite propagation of the virus in any tissue-culture system. The enhancing effect of polycations may be conditioned by their positive charge, since infection by rabies virus is inhibited by polyanions. The polycations could act either by binding to the cell surface and creating new receptor sites for virus attachment, or they may form complexes with the virus particles which can be adsorbed easily on to the cell, or they may act intracellularly facilitating rabies penetration. The exact mechanism of the polycation action merits careful study.

Infection of cells in culture by rabies virus is enhanced by simultaneous exposure to LCM virus. This enhancing effect seems to be specific for rabies virus since no other virus was found to be enhanced by LCM virus (Wiktor, Kaplan & Koprowski, 1966; Koprowski, Wiktor & Kaplan, 1966). The " enhancing factor " is present in ultra-violet-irradiated preparations of LCM virus, but whether it is associated with the virus particle itself is not yet determined. Further investigations of the mechanism of this curious phenomenon and the nature of the enhancing factor are needed.

Replication

Highest yields of rabies virus are obtained from cells maintained at 31°C–33°C and lowest yields from those kept at 39°C (Kissling & Reese, 1963; Wiktor, unpublished data). Cells irradiated by ultra-violet light or treated with actinomycin D (Kaplan et al., 1967) support better growth of rabies virus than non-treated cells.

The enhancing effect of inhibition of macromolecular synthesis in the infected cells on growth of rabies virus should be further investigated.

Sites of virus assemblies

Concurrent with the appearance of fluorescent antigen within the cytoplasm of cells infected 8 to 9 hours earlier, amorphous matrices containing fibres which replace normal cytoplasmic structures are observed under the electron microscope (Hummeler, Koprowski & Wiktor, 1967). Mature virions are first seen at the edges of these matrices and, at a later stage of infection, within them (Hummeler, Koprowski & Wiktor, 1967). Virus also appears to be formed or released or both by a process of budding into intracytoplasmic vacuoles and, to a lesser degree, from the cell surface. Thus it seems that rabies virus in a unique fashion may be assembled either like myxovirus at the cell surface or like vaccinia virus at the intracellular matrix. Judging by the disproportionately larger ratio of virions present inside the infected cell to the amount of virus released, it seems possible that a large proportion of the synthesized virus may not be released from the infected cell. Whether only virus particles seen budding at the cell surface are infectious remains to be studied.

Formation of viral antigen at the cell surface does probably occur, since this may explain why rabies-infected cells lyse when exposed to antirabies serum containing complement (see above and Fernandes, Wiktor & Koprowski, 1964; Wiktor, 1967). Evidence has also been obtained by transplantation assays that rabies may confer upon its host cells new antigenic determinants (Defendi, Wiktor & Koprowski, 1967).

Whether, similarly to myxoviruses (Drzeniek, Saber & Rott, 1966; Rott et al., 1966), the rabies virus particle acquires host cell components is not known at present and the matter could usefully be studied.

Genetic studies

Until recently there has been no way to study the progeny of a single virus particle and to compare it with other genetically homogeneous material. With the development of a plaque assay system for rabies virus in agarose–cell-suspension medium (Sedwick & Wiktor, 1967) it is possible to undertake a systematic study of various strains of fixed virus of different origin, all of which can be plaqued. This plaque assay is now used routinely in the Wistar Institute laboratory for virus titration, the serum neutralization test, and cloning of the virus. Plaque-purified strains are now being used exclusively at the Wistar Institute for all investigations involving concentration, purification, and the study of their antigenic components.

Various strains of rabies virus are being characterized at present in order to find genetic markers associated with one or more of the following characteristics: temperature dependence, ara-C resistance, heat sensitivity, various plaque-size markers. If an adequate number of mutants is found, marker–rescue recombination and complementation experiments may lead ultimately to the mapping of the viral genome.

RÉSUMÉ

L'application des techniques virologiques modernes a permis aux recherches sur l'infection rabique de progrésser rapidement au cours des dernières années. Les résultats les plus marquants ont été obtenus grâce à l'utilisation des cultures cellulaires, de la microscopie électronique, de la méthode des anticorps fluorescents et de la technique des plages, ainsi que par l'étude des relations cellule-virus et l'étude physico-chimique de virus partiellement purifiés et concentrés. Les progrès seront sans nul doute encore plus décisifs lorsque l'on pourra exploiter pleinement les moyens de recherche actuellement disponibles.

Les nouvelles techniques permettent d'aborder les nombreux problèmes qui restent à résoudre. Le virus rabique est habituellement transmis par la morsure d'un animal enragé, mais on a signalé des cas d'infection à point de départ pulmonaire par inhalation d'aérosols. Bien qu'il soit amplement démontré que le virus se propage par l'intermédiaire des nerfs périphériques et non par voie sanguine, on a néanmoins constaté l'existence d'une virémie après infection expérimentale massive. On connaît très mal les phénomènes intracellulaires provoqués par l'infection du système nerveux central. La longue incubation de la rage et les infections abortives observées chez l'animal sont peut-être en rapport avec l'apparition d'immunoglobulines dans le tissu cérébral. Le rôle de l'interféron et des autres inhibiteurs du virus rabique doit être précisé. On a identifié au moins cinq espèces d'anticorps (neutralisants, fixant le complément, précipitants, inhibant l'hémagglutination, cytolytiques), mais on ne sait rien de leurs rapports et de leur rôle en ce qui concerne l'immunité.

Le virus rabique est un antigène puissant. Un vaccin expérimental préparé à partir d'un virus purifié et inactivé a fait preuve d'un pouvoir antigénique élevé chez la souris. Il serait nécessaire d'évaluer l'efficacité de préparations similaires chez l'homme et de mettre au point un vaccin actif suscitant une réponse immunitaire suffisante après 1, 2 ou 3 injections seulement. Ces recherches impliquent une amélioration des procédés actuels de purification et de concentration de l'antigène rabique.

L'étude de la dynamique de l'infection rabique et de la multiplication du virus dans divers systèmes de culture tissulaire doit être poursuivie. On possède déjà de nombreuses données sur la morphologie du virus, mais peu de connaissances sur sa composition chimique. On sait qu'il contient de l'ARN. L'étude de ses propriétés physiques et chimiques ne pourra progresser que lorsqu'on disposera de quantités suffisantes de virus très purifié. La technique des plages offre d'intéressantes perspectives pour l'étude génétique des diverses souches et l'on tente de découvrir des caractéristiques utilisables comme marqueurs génétiques.

Ces recherches permettent d'entrevoir la mise au point relativement prochaine d'un vaccin antirabique plus actif et moins coûteux que les préparations actuelles. Elles ont en outre l'avantage d'éclairer de nombreuses questions de virologie générale encore controversées.

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