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A peep into detection of rabies in 20th century

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Abstract : *The present review is an effort to preserve the techniques and approaches that were applied during the 20th century for detection of rabies in clinical pathology samples, by immunopathological approaches, patho-anatomical studies, ultra structural studies, histoenzymic analysis, biochemical studies as well as serological approaches.*
Keywords - *Diagnosis, Immunopathology, Rabies, Serology, Ultrastructure*

I. Introduction

Rabies is an extremely dangerous disease with a frequently long incubation period and highly distressing symptoms. Rabies is responsible for extensive mortality and morbidity in India. The prevalence of various myths and wrong notions amongst the general population complicates the problem in developing countries. Dogs are mainly responsible for maintaining the disease in urban areas while jackals, wolves and foxes etc. maintain the disease in sylvatic areas.

Melnick and McCombs first suggested the term *Rhabdovirus* in 1966 after it was found that the unusual bullet-shaped morphology of vesicular stomatitis virus was shared with other viruses such as rabies and sigma viruses and the International Committee on Nomenclature of viruses recommended it in 1970 after the term *Stomatovirus* was rejected as inappropriate [1].

In the universal taxonomic scheme of the International Committee on Taxonomy of Viruses, its usage has been formalized as the family *Rhabdoviridae* in the Order Mononegavirales [2].

II. Clinical Pathology

The characteristic increased total cell count in cerebro-spinal fluid (CSF) in viral infections exhibited increased values ranging from 10 to 100 while for rabies where it may be up to 500/ml [3]. Out of 57 rabid cases, glucose was detected in the urine of 38 herbivores (25 cattle, 10 goats, 1 horse, 1 sheep, 1 pig). Meanwhile, urinalysis was reported to be insignificant for diagnosis of rabies [4]. CSF examination of eight raccoons inoculated intra-muscularly with street rabies virus at 10 to 21 days after challenge revealed mean WBC count of 365 ± 316.6 with the values ranging from 27 to 860.0/ml [5]. CSF of rabid horses revealed slightly high total cell count with a predominance of lymphocytes [6].

Secretion of Virus

Some workers have isolated the virus from the saliva of the rabid animals. One of the earlier reports has recorded the titre of virus as high as 10^6 MLD_{50}/0.03 ml from skunk saliva [7]. In experimental rabies in sheep produced by street rabies virus of fox origin by intramuscular route, virus was demonstrated in the saliva of one of five infected sheep [8].

Dogs inoculated with (American and Ethiopian) canine street virus, excreted virus in their saliva upto 14 days [9] before appearance of signs. One dog that recorded intramuscular rabies excreted rabies virus in its saliva for 305 days.

Vaccine virus was isolated from saliva 1 hour after the liquid vaccine was placed directly into the mouth but not subsequently (tested up to 1 week after vaccination) [10]. In experimental inoculation of raccoons saliva samples were collected on 35, 63 & 92 days post inoculation (DPI) for virus isolation. Rabies virus was not detected in the saliva of any raccoon [11].

Nasal and Pharyngeal Swabs taken from various species and examined by immunofluorescence and culture in neuroblastoma cells gave promising results, but further research was needed [12].

III. Pathoanatomical Studies

No significant gross changes were reported in brain, ganglia and salivary glands in rabies [3,13,14]. Gross lesions in experimentally produced rabies in sheep by using street rabies virus were non-specific [8].

The lesions of rabies were reported to be typical of non-suppurative encephalomyelitis with ganglioneuritis and parotid adenitis. The inflammatory and degenerative changes were found most severe from the pons to hypothalamus and in the cervical spinal cord with relative sparing of the medulla. Ruminants showed little more than a few very small glial nodules (Babes nodules in this disease). There was typical peri-vascular cuffing and focal

gliosis. The cuffs were one or several cells thick and composed solely of lymphocytes. Ring haemorrhages confined to peri-vascular spaces were common about cuffed vessels. Babe's nodules were composed of microglia. These nodules varied from 6-7 cells to 100 or more. Diffuse/focal gliosis occurs in areas of gray matter such as pons and in spinal cord. Neuronal degeneration was found to be slight in herbivores [13].

Rabies positive cattle showed non-purulent encephalitis confined to the medulla oblongata [15]. Spongiosis was found in CNS of mice, dogs and cats experimentally infected by Denmark Bat Virus (DBV) strain of rabies virus [9]. Skunks inoculated intra muscularly with street rabies virus, revealed moderately extensive spongiform lesions that rarely affected basal ganglia or hippocampus. Spongiform lesions were characterized by fewer numbers of small vacuoles[14].

The major histopathological findings in rabid horses included diffuse peri-vascular cuffing with leucocytes (predominantly lymphocytes) in the meninges and neural parenchyma; neuronal degeneration, neuronophagia, gliosis and malacia of gray matter of the spinal cord [6]. Case of human rabies has been reported to have myocarditis in addition to encephalitis [16]. In naturally acquired rabies of raccoons, Negri bodies were detected by immuno-peroxidase and HE staining in cerebral cortex, hippocampus, brain stem, cerebellum, cervical spinal cord, and in the ganglia of the trigeminal nerves. The viral inclusions were also seen in ganglion cells in the tongue, parotid salivary gland, pancreas, intestines and adrenal glands [17].

Negri body was found to be round, oval, spheroid, amoeboid, elongate and triangular. The most characteristic feature of the Negri body reported was its internal structure. In the magenta-red staining of Negri body were contained small basophilic granules which were dark blue or black. Classically it formed a rosette pattern. But this pattern was an exception rather than a rule [18]. Intracytoplasmic position of the Negri body could be expected with reasonable consistency only in histological section of the brain. Pleomorphism of fine structure of rabies virus was revealed on comparison of morphological structure of Negri body in a human being and that in a brain of experimentally infected mice. The former consisted of a matrix of very fine material bearing larger granules or strands of higher electron density while latter showed better defined areas in granular matrix containing tubular; bullet shaped and elongated forms of viral structures [19]. Number of Negri bodies had little relation to the length of the incubation period though it was related to the duration of clinical disease. They were not found if the animal was killed instead of being allowed to die. In bat-transmitted rabies, Negri bodies were either absent or few and small [13].

Moreover, Negri bodies could not always be found in the brains of animals dying of rabies [18] and inclusion bodies, indistinguishable from rabies inclusion bodies were found in the brains of 8 non-rabid dogs [20]. This observation was supported in another study [21] wherein out of 187 brain tissue samples tested for rabies, FATdetected rabies antigen in 98% cases, while Negri bodies were detected in only 53% cases.

Further, in mice experimentally inoculated by CVS strain of fixed virus, by intracerebral and intra ocular routes, no Negri body was found in the CNS of mice although rabies antigen was detected in these mice by avidin-biotin peroxidase (ABC) technique [22].

In another study [6] Negri body was found in only 11 out of 21 rabid horses. Further, in a significant observation [13] it was recorded that Street rabies virus fails to produce Negri bodies in upto 30% of cases and fixed virus does not produce Negri bodies.

IV. Fluorescent Antibody Technique (Fat)

FAT was first adopted for use in rabies diagnosis [23]. FAT staining is most often performed by direct technique using acetone - fixed brain impression smears or cell culture preparations [24]. The predominant antigen detected in acetone - fixed cells is viral nucleocapsid.

Small laboratories should not attempt to prepare their own conjugated antirabies serum because of the difficulties involved, they should rather try to obtain the small supply usually required from a large central laboratory or from reliable commercial sources. All batches of conjugated sera should be carefully tested on known positive and negative specimens [25]. Good quality conjugates should be capable of being diluted as much as 1:20 or more to guard against the problem of non-specific fluorescence [26].

The "direct" FAT took the lead over all others for speed and accuracy combined [25]. Fluorescent - positive and mouse - negative specimens could be expected since the FA test detected inactivated as well as live antigen [26].However, all fluorescent - negative specimens have been recommended to be tested in mice to maintain a constant check on FAT [25].

FAT was employed to determine viral distribution in the body of the horse wherein the hind limb peripheral nerve specimens were found to be positive by FAT [27].The axonal transport of rabies virus in the central nervous system of rat was also monitored by specific fluorescence [28].Spread of rabies was likewise monitored by rabies immunofluorescence of areas of brain [29,30]. Rabies virus antigen was demonstrated in the optic nerve in 35 of 40 rabid animals by employing FAT [31], while submaxillary and parotid salivary glands were examined, naturally infected with rabies virus, by direct FAT on tissue smears of semi-ground glands [32]. With advancement of the disease, there was enhancement of the intensity of fluorescence in tissues [33].

The immunofluorescence test, using three different conjugates gave false - negative results in 144 of 296 rabies positive brains. This indicated the occurrence of variant strains of rabies virus for which a polyclonal conjugate was needed [34].

V. Histoenzymic Studies

The cerebral cortex showed diffuse activity of dehydrogenases [35,36]. Succinic dehydrogenase (SDH) was located more in the axons and dendrites of cortical neurons than in the perikaryon, whereas lactic dehydrogenase (LDH) was more active in the perikaryon than in the processes of the neuron. Purkinje cells were found to exhibit marked dehydrogenases activity while the granular cells revealed little SDH activity [37].

There has been considerable inter species variation in the distribution of acetyl cholinesterase (AChE) within the nervous system. However, AChE activity was taken as histochemical evidence of the cholinergic function of the neuron [38].

Alkaline phosphatase (AKP), in the nervous system has been found to be mainly concentrated in capillary and vascular endothelium though its physiological function at this site has been obscure. Strong activity of acid phosphatase (ACP) has been reported in the axons of peripheral nerves [36].Alkaline phosphatase activity was mainly concentrated in the blood vessels whereas the large venous sinuses were negative for AKP activity thereby indicating physiologic difference between arteriolar and venous blood [39].

MAO has been reported to be present in all components of the cerebellum [40].The majority of neurons of the cerebral cortex showed negligible AChE activity. MAO was present more in the gray matter than in the white matter [41].

In Ammon's horn, strong reactions for SDH & LDH have been observed in the molecular layer and the pyramidal cells [42]. Purkinje cells of cerebellum exhibited strong NSE, LDH, AC & SDH activity, moderate ATP & MAO activity while they were negative for AChE and AKP. The granule cells in cerebellum showed moderately strong LDH & SE activity, moderate SDH & MAO activity, mild ACP & ATP activity while they were negative for AChE and AKP activity [41].In spinal cord, the activity of LDH was generally stronger than that of SDH. There was more enzyme activity in the gray matter than in the white matter. Of the blood vessels, only those located in the grey matter gave moderately strong AChE reaction. The remaining grey matter showed very weak AChE activity [41].

VI. Mice Inoculation Test

Mice of any breeding strain may be considered generally suitable although preference should be given to the Swiss albino mice since it is very susceptible to rabies virus [43].

Extensive surveys of large number of rabies cases have shown that 10-15% of cases proved to be positive by mice inoculation testhad been missed by direct smear microscopic examination for Negri bodies [18].It is relatively immaterial which part of the brain tissue is chosen for the preparation of the suspension for inoculation, however, preference may be given to Ammon's horn, the cerebellum and parts of cortex [43].

In experimental rabies 10 sheep and 5 foxes produced by street rabies virus by i/m route, virus could be re-isolated from a single rabid sheep at very low titers from Ammon's horn only while the other sheep did not yield the virus. In case of foxes only one of the three rabid foxes yielded the virus [44].One out of five dogs survived intracerebral inoculation of three million MICLDs/0.03 ml of street rabies virus. Rabies virus was recovered from its saliva on three occasions[45].

VII. Ultra Structural Studies

An in-depth study showed that rabies virus had unique structural details [46,47].It was then established that all vertebrate Rhabdovirus particles are bullet shaped i.e. with one end hemispherical and other planar or concave [48]. Rabies virus particles were first described by thin-section electron microscopy [49]. In thin sections, Rhabdoviruses appeared to be bullet shaped only when sectioned exactly longitudinally. They had circular or elliptical profiles when sectioned transversely or obliquely [50] though individual spikes are not well resolved [51]. Depending upon the thickness of sections, the nucleocapsid had a varying appearance. In some longitudinally sectioned particles, the tissues of the nucleocapsid helix were seen clearly as cross striations.In very thin sections of particles cut through their centres, the nucleocapsid was seen as a dense beaded layer immediately beneath the envelope. In more commonly seen thicker section, the entire nucleocapsid appeared as a solid, dense form without further detail. The surface projection layer appeared as a thick and delicate zone entirely surrounding the virus particle [52].

Various workers have endeavoured to study the ultra structural details of Negri body. It has been demonstrated conclusively [53,54] by election microscopy that Negri bodies and nucleoprotein masses were identical.In street rabies virus infection, a close spatial relationship between virus particle budding and inclusion body formation has been reported but in case of fixed rabies virus strains, many inclusions were found without virus particle budding upon nearby membranes [55].

The matrix of inclusion bodies of fixed virus was homogenous consisting of thin filaments and that of street virus was heterogeneous including filaments (presumably the nucleoprotein)plus considerable condensed

material, membranous structures exhibiting transverse striations. Distinct double membranes without any relation to pre-existing cell membranes structures also emerged [56].Serial thick (light microscopy) and thin (electron microscopy) sectioning demonstrated conclusively that the mass of nucleoprotein (Negri body) need not have any precise margins and may as well contain entrapped host cell organelles and virus particles [52].

Most infected neurons of mice or hamsters that died after 20 to 30 days of street rabies virus infection were found to have normal organelle structure which was deformed only by the presence of inclusion bodies [57]. In infections caused by isolates directly from natural source, progressive involvement of more and more cells absence of cytopathic changes in infected cells; progressive accumulation of nucleoprotein and later dense inclusion body formation and focal occurrence of viral budding (rare on plasma membranes and more prominent on other membrane system) were observed [52].

The ultrastructural analysis of peripheral nerves of rat inoculated with rabies virus has been reported in detail [58]. Similarly, ultra structural quantitative analysis of lesions in the sciatic nerves has been reported [59], which included 40% degeneration of myelinated axons with only occasional degeneration of unmyelinated axons. However, electron microscopy showed no typical bullet shaped virus particles.Electron microscopy has been used to study the rabies virus transport and infection of human dorsal root ganglia neurons [60].

Matrix (Viral nucleocapsid), virions and anomalous viral products were found in various tissues of striped skunks observed electron microscopically though these were in varied proportions. Little accumulation of matrix and anomalous viral growth products were found in tissues that frequently produced high titres of virus whereas, replication with large amount of matrix and anomalous structures occurred in tissues that contained low or moderate titers of virus [61].The high yield from saliva was explained by electron microscopic studies showing that in contradistinction to patterns observed in nervous tissue, maturation of virus in salivary glands occurred selectively by a process of uniform budding of complete, standard, virions from acinar cell plasma membranes [62].

VIII. Antibody Analysis

Various techniques employed for detection of rabies virus antibodies include Enzyme Linked Immuno-Sorbent Assay (ELISA)[63-72]; Rapid Fluorescent Inhibition Test (RFFIT)[71, 72, 73-75]; Modified RFFIT[76,77]; Serum Neutralization Test (SNT)[67,69,78-83]; Haemgglutation Inhibition Test (HAI)[81,84-86]; Immunodiffusion[72]; Complement Fixation Test[72,87]; Rabies Agglutination Test (RAT)[84]; Indirect Immune Fluorescence (IFF)[11,89,90]; Counter Immuno Electrophoresis (CIE)[82]; Modified Counter Immuno Electrophoresis (MCIE)[36,81] and SDS - PAGE[68].

Various workers have assessed rabies antibodies in different species viz. Cattle[75,78,80,87]; dog[65,68,70,72,79,85,87,89,91]; sheep[71]; raccoons[11,65,77,93]; fox [65,67]; cat[65,67]; marten[67]; badger[67]; polecat[67]; skunk[65,93]; fruit bat[94] and rabbit[69].

IX. Biochemical Studies

High serum levels of Creative Kinase (CK) occurred in organic neurologic disease such as cerebral infection, meningitis and encephalitis occurring some days after the onset of symptoms and showing little relationship to prognosis [94,95]. Similar increases in total serum CK were observed in neurological disorders [96].

The presence of aspartate aminotransferase (AST) in tissues was used as a good marker of soft tissue damage [96]. Characteristic high values of AST have been found in viral infection wherein elevation was found to commence upto 10 days prior to onset of symptoms [94]. AST activity was found to exceed early in the genesis of the disease and following a crescendo-like rise, the activity was found to fall dramatically. Increased levels of AST were specifically found in cerebral necrosis.

Gamma glutamyl transferase (GGT) levels in the serum were found to increase in neurological disorder [95]. Increase in the level of blood urea nitrogen (BUN) was reported to be associated with dehydration, shock, fever, acute glomerulonephritis and virus relation [97].

X. Element Analysis

Though perusal of literature reveals little work on element analysis on samples from rabid animals, nevertheless, increase in the levels of calcium and magnesium has been reported in 8 dogs suffering from rabies[98].

Conclusion

Twentieth century effort was largely a pre-molecular diagnostic effort. Nevertheless, significant efforts were put in for effective rabies diagnosis. Clinico-pathological approach relied on the alterations in cerebrospinal fluid. Detection of rabies in various secretions/excretions of rabid animal was largely accomplished by immunofluorescence. While gold standard fluorescent antibody test (FAT) was the main stay for authentic diagnosis of rabies in most of rabies diagnostic laboratories, patho-anatomical detection of Negri bodies pitched in for all institutes where FAT facilities did not exist. In absence of molecular confirmation, the FAT negative cases were

confirmed by biological testing involving albino mice. Various techniques have been standardized for estimation of anti-rabies antibodies that was largely conducted only for establishment of post-vaccination immune status.

References

[1]. P. Wildy, Classification and nomenclature of viruses,First report of the International Committee on Nomenclature of Viruses, Monograph. *Virology,5,*1971, 1.

[2]. C. R. Pringle, The order mononegavirales, *Archieves of Virology.117*,1991, 137-140.

[3]. C. K. Singh and G.S. Grewal, Clinico-pathological and patho-morphological changes of induced rabies in buffalo calves,*Indian Journal of Animal Sciences,69 (12),*1999,1014-1017.

[4]. T.S.S. Rajan andV.D Padamanaban, Diagnosis of rabies with glycosuria in herbivores *Indian Veterinary Journal,69 (4)*,1987, 274-279.

[5]. C. A. Hanlon, L.Z. Ellen, A.N. Hamir andC.E. Rupprecht, Cerebrospinal fluid analysis of rabid and vaccinia-rabies glycoprotein recombinant, orally vaccinated raccoons (Procyon lotor),*American Journal of Veterinary Research,50 (3),*1989, 364 - 367.

[6]. S.L. Green, L.L. Smith, W.Vernauand S.M. Beacock, Rabies in horses: 21 cases (1970 - 1990),*Journal of the American Veterinary Medical Association,200 (8)*, 1992, 1133 - 1137.

[7]. R.K. Sikes, 1962. Pathogenesis of rabies in wild life I. Comparative effect of varying doses of rabies virus inoculated into foxes and skunks,*American Journal of Veterinary Research,23,* 1041.

[8]. R.S. Baltazar, M.F..A. Aubert, J. Blancou, J. Ambert, M.J. Barrat, E. Cain, A. Dardaine,C. Patron, M. Selve andR.S. Baltazar, Experimental rabies in sheep : susceptibility, symptoms, humoral immune response, lesions and virus excretion,*Revue de Medicine Veterinaire,137 (12),*1986,847 - 856.

[9]. M. Fekadu, Pathogenesis of rabies virus infection in dogs,*Reviews of Infections Diseases,10(4)*,1988, S679 - S683.

[10]. E.H. Follman, D.G. Ritter andG.M. Baer, 1992. Oral rabies vaccination of arctic foxes (*Alopex lagopus)* with an attenuated vaccine,*Vaccine,10 (5)*, 305-308.

[11]. R.E Hill,G.W. Beran andW.R. Clark, Demonstration of rabies virus - specific antibody in the sera of free-ranging Iowa raccoons (Procyon lotor),*Journal of Wildlife Diseases,28 (3),*1992, 377 - 385.

[12]. P. Steinhagen, Cultural and immunofluorescence diagnosis of rabies in living and dead animals from samples other than nervous system tissue: the mucous membrane test,*Tierarztliche Umschau,47 (11),* 1992, 829 - 832.

[13]. N.D. Sullivan, The nervous system. In (ed.), K.V.F. Jubb, P.C. Kennedy and N.Palmer*Pathology of domestic animals,* 3(Academic Press Inc. London1985).

[14]. A. Bundza and K.M. Charlton, Comparison of spongiform lesions in experimental scrapie and rabies in skunks,*Acta Neuropathologica,76 (3)*1988, 275-280.

[15]. W.Schulz, Rabies in cattle, its occurrance and laboratory diagnosis.*Manatshefte fur Veterinarmedizin41 (10),*1986, 327-329.

[16]. G.V Raman, A.Prosser, P.L. Spreadbury,P.M. Cockcroft, O.A. Okubadejo andG.VenkatRaman, Rabies presenting with myocarditis & encephalitis,*Journal of Infection,17 (2),*1988.155-158.

[17]. A.N. Hamir, G.Moser andC.E. Rupprecht, Morphologic and Immunoperoxidase study of neurologic lesions in naturally acquired rabies in raccoons,*Journal of Veterinary Diagnostic Investigation,4 (4),*1992.369 -373.

[18]. E.S. Tierkel, Rapid microscopic examination for Negri bodies and preparation of specimens for biological tests, In M.M.Kaplan and H.Koprowski(Ed.), *Laboratory Techniques in Rabies,* 3 (World Health Organization, Geneva. 1973).

[19]. D.K.Manghani, D.K.Dastur, A.N. Nanavaty andR. Patel, Pleomorphism of the structure of rabies virus in human and experimental brain,*Journal of the Neurological Sciences,75 (2)*, 1986, 181 - 193.

[20]. K. Zimmer, D.Wiegand, D.Manz, J.W. Frost, M. Reinacher andK. Frese,Evaluation of five different methods for routine diagnosis of rabies,*Journal of Veterinary MedicineSeries B37 (5),*1990,392 - 400.

[21]. T.A. Sinchaisri, T. Nagata, Y. Yoshikawa, C. Kai andK.Yamanouchi, Immunohistochemical and histopathological study of experimental rabies infection in mice,*Journal of Veterinary Medical Science,54 (3)*,1992,409 - 416.

[22]. R.A. Goldwaseer andR.E. Kissling, Fluorescent antibody staining of street and fixed rabies virus antigen,*Proceedings of Society of Experimental Biology and Medicine,*98,1958,219-223.

[23]. R.E. Kissling, The fluorescent antibody test in rabies, in G.M. Baer (Ed.),*The Natural History of Rabies,*I (Academic Press, New York,1975) 401-416.

[24]. M.M. Kaplan andH. Koprowski, in (Ed.),*Laboratory Techniques in Rabies,* 3 (World Health Organization, Geneva, 1973).

[25]. D.J. Dean andM.K, Abelseth, Fluorescent Antibody Test,in M.M. Kaplan and H.Koprowski(Ed.), *Laboratory Techniques in Rabies*, 3(World Health Organization, Geneva, 1973).

[26]. E.E. Meyer, P.G. Morris, L.H. Elcock andJ. Well, Hind limb hyperesthesia associated with rabies in the horses,*Journal of the American Veterinary Medical Association,188 (6),*1986, 629 - 632.

[27]. J.P. Gillet, P. Derer andH. Tsiang,Axonal transport of rabies virus in the central nervous system of the rat,*Journal of Neuropathology and Experimental Neurolgoy,45 (6),*1986, 619 - 634.

[28]. P.E. Ceccaldi, J.P. Gillet and H. Tsiang, Inhibition of the transport of rabies virus in the central nervous system,*Journal of Neuropathology and Experimental Neurology,48 (6),*1989, 620-630.

[29]. C.K.Singh and G.S.Grewal, Progressive spread of rabies viral antigen in central nervous system of infected buffalo calves,*Indian Journal of Animal Sciences, 68*,1998,1255-1257.

[30]. A.S. Shashenko, V.T. Sakovich andV.A. Letetskii,Diagnosis of rabies and Aujyeszky's disease by immunofluorescence applied to optic nerve smears, *Veterinarnaya Nauka Proizvodstvu,23*,1985, 8 - 9.

[31]. V. de A. Cortes, F.H. Ito, S.A. Vasconcellos andJ. de A. Cortes, *Revista da Faculdade de Medicina Veterinaria e Zootecnia da Universidade de Sao Paul,o 24 (2)*,1987, 181-186.

[32]. R. Hati andP. Sarkar, Dissemination of rabies street virus in experimentally Infected mice, *Indian Journal of Virology,3 (1-2),*1987, 18 - 25.

[33]. P. Stohr, K. Stohr, H. Kiupel andE. Karge, Immunofluorescence microscopy applied to field isolates of rabies virus from regions of eastern Germany using several fluorescein-labelled conjugates, *Tierarztliche Umschau,47 (11),*1992,813-814.

[34]. A. Meyer, in: J.G. Green field (Ed.), *Neuropathology,* 3 (Elservier Publishing Company, London,1963) 235.

[35]. C.K.Singh, G.S.Grewal and M. Bhardwaj, Modified counter immuno-electrophoresis for quantitative analysis of antibodies to street rabies virus,*Indian Journal of Animal Sciences*, 68,1998, 925-927

[36]. C.W.M. Adams, in*Neurohistochemistry*(Elsevier Publishing Company,New York ,1965).

[37]. J.B. Cavanagh andP. Hollan,Cholinesterase in the chicken nervous system, *Nature,190*,1961,735-736.

[38]. W.L. Hard andR.K. Hawkins,Histochemical studies on the area postrema. *Anatomical Record,108*,1950, 577.

[39]. S.L. Manocha andG.H. Bourne, Histochemical mapping of lactate dehydrogenase and monamine oxidase in the medulla oblongata and cerebellum of squirrel monkey *(Saimiri sciuireus),Journal of Neurochemistry,15,*1968,1033-1040.
[40]. S.L. Manocha andT.R. Shantha, In: Macaca mulatta *Enzyme Histochemistry of the Nervous system,*(The Academic Press (London) Ltd, London, 1970).
[41]. R.L. Friede andL.M. Fleming, A Mapping of the distribution of lactic dehydrogenase in the brain of the rhesus monkey,*American Journal of Anatomy,113*,1963, 215 -234.
[42]. H. Koprowski,The mouse inoculation test,in M.M. Kaplan and H. Koprowski (Ed.),*Laboratory techniques in Rabies,*3(World Health Organization,1973) 85-93.
[43]. R. Soria Baltazar, J. Blancou andM. Artois, Study of rabies virus isolated from a European bat, *Eptesicus serotinus,* pathogenicity for sheep and red fox,*Revue de Medicine Veterinaire,139 (6)*,1988, 615 - 621.
[44]. S.V.Gribencha, L.Ya. Gribanova, G.B. Mal'kov andI.F. Barinsky, Abortive and recurrent rabies in dogs inoculated intracerebrally with street rabies virus,*Voprosy Virusologi,34 (2),*1989, 217 - 221.
[45]. J.O. Almeida, A.F.Howatson, L. Pinteric andP. Fenje, Electron microscope observations on rabies virus by negative staining,*Virology*,*18*,1962,147.
[46]. L.Pinteric, P. Fenje andJ.D. Almeida, The visualization of rabies virus in mouse brain,*Virology ,20*,1963,208.
[47]. A.M. Frederick andA.K. Harrison, Electron microscopy of the rhabdoviruses of animals in D.H.L. Bishop (Ed.),*Rhaddoviruses,*I (CRC Press Inc. Florida ,1979) 43-64.
[48]. S. Matsumoto, Electron Microscopic studies on street rabies virus infected mouse,*Annual Report of Institute of Virus Research, Kyoto University*(Kyoto Daigaku Virusu Ken Kyusho Neu Kan Kiyo),1963,57.
[49]. G.H. Bergold andK.Munz,Ultrastructure of Local Indiana and New Jersey serotypes of Vesicular Stomatitis virus,*Journal of Ultra-structural Research,17*,1967, 233.
[50]. J.E. Coward, D.H. Harter, K.C. Hsu andC. Morgan, Electron microscopic study of development of vesicular stomatitis virus using ferritin - labelled antibodies,*Journal of General Virology*,*13*,1971, 27.
[51]. D.H.L. Bishop, in (Ed.),*Rhabdoviruses,*I(CRC Press Inc. Florida,1979).
[52]. K. Miyamoto,Electron microscopic studies on the Negri body,*Annual Report of Institute of Virus Research, Kyoto University* (Kyoto Daigaku Virusu Ken Kyusho Neu Kan Kiyo) *8(10)* 1965.
[53]. K. Miyamoto andS. Matsumoto,The nature of the Negri body,*Journal of Cell Biology,27,*1965, 677.
[54]. K. Miyamoto andS. Matsumoto, Comparative studies between pathogenesis of street and fixed rabies infection,*Journal of Experimental Medicine,125*,1967,447.
[55]. K. Hummeler, H. Koprowski andT.J. Wiktor, Structure and development of rabies virus in tissue culture,*Journal of Virology,1*,1967,152.
[56]. F.A. Murphy, A.K. Harrison, W.C. Winn andS.P. Bauer, Comparative pathogenesis of rabies & rabies like viruses, Infection of the central nervous system & centrifugal spread of virus to peripheral tissues,*Laboratory Investigation*,*29 (1)*,1973.
[57]. G.Minguetti, M.M. Negrao, Y.Hayashi, M. Hirose, O.T. De Freitas andO.T. De Freitas, Ultrastructural analysis of peripheral nerves of rats inoculated with rabies virus,*Arquivos de Biologia e technologia,28 (2),*1985, 257 - 264.
[58]. F.Teixeira, F.J. Arnanda, S. Castillo, M. Perez, L.Pern, O. Hernandez andM. Peon, Experimental rabies: Ultrastructural quantitative analysis of the changes in the sciatic nerve,*Experimental and Molecular Pathology,45 (3),*1986, 287 - 293.
[59]. H. Tsiang, P.E.Ceccaldi andE. Lycke, Rabies virus infection and transport in human sensory dorsal root ganglia neurons,*Journal of General Virology,72 (5)*,1991, 1191 - 1194.

[60]. A. Balachandran andK. Charlton, Experimental rabies infection of non-nervous tissues in skunks (*Mephitis mephitis*)and foxes (*Vulpes vulpes*),*Veterinary Pathology,31 (1),*1994, 93-102.
[61]. R.E. Dierks, F.A. Murphy andA.K. Harrison, Extraneural rabies virus infection - Virus development in fox salivary gland, American *Journal of Pathology*, 54*,* 1969,251.
[62]. P. Perrin, P.E. Rollin andP. Sureau, A rapid rabies enzyme immuno-diagnosis (RREID) : a useful and simple technique for the routine diagnosis of rabies,*Journal of Biological Standardization*,*14 (3),*1986, 217-222.
[63]. K. Mannen, K. Mifune, F.L. Reid Sanden, J.S. Smith, P.A. Yager, J.W. Sumner, D.B. Fishbein, T.C. Tong andG.M. Baer,Microneutralization test for rabies virus based on an enzyme immunoassay,*Journal of Clinical Microbiology,25 (12),*1987, 2440-2442.
[64]. L.D. Barton and J.B. Campbell, Measurement ofrabies - specific antibodies in carnivores by an enzyme-linked immunosorbent assay,*Journal of Wildlife Diseases,24 (2),*1988, 246-258.
[65]. R.M. Karamany, J. Kazar, S.A. Malik, S. Al-Mufti andM. Badriya, Rapid quantitative assay of rabies post-vaccination antibody by ELISA,*Acta-Pathologica, Microbiologica et Immunologica Scandinavica,96(3)*,1988,40-43.
[66]. T. Mebatsion, J.H. Cox andJ.W. Frost, Isolation and Characterization of 115 street rabies virus isolates from Ethopia by using monoclonal antibodies: identification of 2 isolates as Mokola and Lagos kat viruses,*Journal of Infectious Diseases, 166 (5),*1989,972-977.
[67]. C.E. Pixley, An evaluation of an indirect enzyme-linked immunosorbent assay and SDS - PAGE, western blot transfer techniques to detect rabies virus antibodies in canine sera, *Dissertation Abstracts International, B. Sciences and Engineering*,*49 (4),*1988, 962-963.
[68]. S. Nandi, A.C. Goel andK.D. Pandey, Immunogenic response to partially purified rabies virus as measured by ELISA,*Indian Journal of Virology*, *6 (1/2),*1990, 89-92.
[69]. R. Jayakumar andP. Ramadass, Immunoglobulin response to rabies virus immunization in dogs,*Vaccine,9 (9),*1991, 611 - 612.
[70]. I. Samina, J. Brenner, D. Katz and B.A. Peleg, Intradermal vaccination of sheep with an inactivated rabies vaccine,*Israel Journal of Veterinary Medicine*,*46 (3)*,1991,92-94.
[71]. E.Ya. Sazananova, S.V. Kuznetsova, E.V. Maslov, A.A. Boika, V.S. Ivanov andP.P. Kuznetsov, *Veterinariya Moskva, 8*,1991,62-64.
[72]. T. Mebatsion, C. Sillero Zubiri, D. Gottelli andJ.H. Cox, Detection of rabies antibody by ELISA and RFFIT in unvaccinated dogs and in the endangered simian jackal *(Canis simensis)* of Ethiopia,*Journal of Veterinary Medicine, Series B*, *39 (3)*,1992, 233-235.
[73]. R.E. Jr. Hill andG.W. Beran, Experimental inoculation of raccoons (Procyon lotor) with rabies virus of skunk origin,*Journal of Wildlife Diseases*, 28 *(1)*,1992, 51-56.
[74]. B.C. Ramanna andV.A. Srinivasan, Serological response in cattle to tissue culture rabies vaccine,*Indian Veterinary Journal,69 (1),*1992, 8 - 10.
[75]. H.O. Aghomo, O.O Oduye andO. Tomori, Experimental infection of dogs and mice with rabies virus isolated from the saliva of unvaccinated clinically healthy dogs,*Bulletin of Animal Health and Production in Africa,38 (3),*1990, 297-300.
[76]. C.L. Brown andC.E. Rupprecht, Vaccination of free ranging Pennsylvania raccoons *(Procyon lotor)* with inactivated rabies vaccine,*Journal of Wild Life Diseases,26 (2),*1990, 253-257.

[77]. P.M. Roeche, A.C. Cunha andA. King, Levels of neutralizing antibodies in cattle vaccinated against rabies,*Pesquisa Vetrinaria Brasileira,7 (3)*,1987,63-65.
[78]. V. Vrzal, S. Cupera, J.Chumela andL. Zatloukal, Antibody response of dogs to inactivated and live tissue culture vaccines against rabies,*Veterinarni Medicina,33 (8)*,1988,485-494.
[79]. T. Gugiu, E. Stirbu, K. Stirbu, I. Chiciudean and M. Gugiu, Immune repsonse of cattle vaccinated against foot and mouth disease and rabies,*Archiva Veterinaria Bucuresti*,*19,*1990,13-19.
[80]. A. Chauhan, D.K. Sood, S.M. Saha, M. Kapoor, R.K. Aggarwal andS.N. Saxena,The quantitation of rabies specific antibodies III. A comparative evaluation of modified Counter Immunoelectrophoresis, haemagglutination inhibition and serum neutralization titres of human sera,*Biologicals*,*19 (2),*1991,103-106.
[81]. P.M.Sanchez andE.L. Rublo, Titration of rabies antibodies in sheep serum by counter immuno electrophoresis and neutralization,*Tecnica Pecuaria en Mexico*,*29 (3),*1991,165-168.
[82]. F.Y. Aoki, M.E. Rubin andM.V. Fast, Rabies neutralizing antibody in serum of children compared to adults following post-exposure prophylaxis,*Biologicals,20 (4)*,1992, 283-287.
[83]. K. Mifune, K. Mannen, N. Miamoto andY.T. Arai, Further studies on an improved haemagglutination inhibition test with higher sensitivity for rabies virus antibody,*Bulletin of the World Health Organization*,*64 (1),*1986, 133-137.
[84]. C.N. Bota, R.K. Anderson, S.M. Goyal, L.J. Charamella, D.R. Howard andD.J. Briggs, Comparative prevalence of rabies antibodies among household and unclaimed/ stray dogs as determined by the immune adherence haemagglutination assay,*International Journal of Epidemiology,16 (3)*, 1987,472-476.
[85]. L.O. Wosu andH.N.S. Anyanwu, Seroepidemiological survey of rabies virus antibodies in non-vaccinated dogs in Nsukka environs, Nigeria, *Journal of Communicable Diseases,22 (2)*,1990,124 - 128.
[86]. C.U. Iroegbu andE. Uhuegbu, Incidence of rabies Virus complement fixing antibodies in unvaccinated dogs, humans and livestock in Anambra State of Nigeria,*Microbiologica,15 (2)*,1992,213 - 218.
[87]. P. Perrin, P. Vermisse andP. Sureau, A rabies agglutination test (RAT) for rabies antibody detection,*Journal of Biological Standardization*,*16 (4)*,1988,281 - 286.
[88]. S.K. Das, P. Sarkar ansdG.P. Sen,Application of indirect fluorescent antibody test in determining the post-vaccinal immune response against rabies in canines,*Indian Journal of Animal Health,25 (2)*,1986,151-153.
[89]. D. Seroka, L. Szkudlarek, E. Labunska andA. Jackowska, Indirect immunofluorescence technique for serololgical diagnosis of rabies,*Medycyna Doswiadozalna Mikrobiologica,38 (3)*,1986,176 - 181.
[90]. L. Buckner, M. Ackermann, H.K. Muller andU. Kihm, Serological studies on rabies vaccine of dogs in Switzerland,*Schweizer Archiv fur Tierheilkunde,128 (4)* ,1986,195-200.
[91]. W. Tepsumethanon, C.Polsuwan, B.Lumlertdaecha, P.Khawplod, T. Hemachudha, S Chutivongse, H. Wilde, M. Chiewbamrungkiat andP. Phanuphak, Immune response to rabies vaccination in Thai dogs: a preliminary report,*Vaccine*,*9 (9),*1991,627-630.
[92]. R.C. Rosatte, D.R. Howard, J.B. Campbell andC.D. MacInnes, Intramuscular vaccination of skunks and raccoons against rabies,*Journal of Wildlife Diseases,26 (2)*,1990, 225-230.
[93]. H.O. Aghomo, A.K. Ako-Nai, O.O. Odouye, O. Tomori andC.E. Rupprecht, Detection of rabies virus antibodies in fruit bats *(Eldolon helvum*) from Nigeria,*Journal of Wildlife Diseases,26,*1990, 258-261.
[94]. C.K.Singh and R. Singh, Experimental Rabies in buffalo calves: Biochemical and Element Analysis. *Indian Journal of Animal Sciences, 67,*1997,958-959.
[95]. J.W. Karamer andA. Meyer, Clinical Enzymology in J.G. Greenfield (Ed.),*Neuropathology,* 3(Elsevier Publishing Co. London ,1963), 235.
[96]. F.K. Widman, in: *Good dale's Clinical Interpretation of Laboratory Tests*, 7 (Davis Co. Philadelphia 1973) 170.
[97]. K.Sripad, M.T. Rai andS.Yathiraj, A note on biochemical changes in nervous disorders of dogs,*Indian Journal of Veterinary Medicine*,*7 (2),*1987,142 - 143.