CHAPTER II

REVIEW OF LITERATURE

Part I

EPIDEMIOLOGY

(1) RABIES: GENERAL HISTORICAL
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Rabies: General Historical:

The first recorded description of rabies in dogs and domestic animals was made by Democritus in 500 B.C. (Steele, 1975).

Aristotle in 322 B.C. and Celsius (A.D. 100) recognised the relationship of hydrophobia in man to rabies in animals and recommended cauterisation of wounds produced by rabid dogs. Galen (200 A.D.) favoured surgical resection of wound area (Johnson, 1965).

Kaplan (1977) reported that there are statements by early authors which suggest that rabies was prevalent in middle ages, not only in Europe generally but also in Britain. From about 1500 onwards rabies was widespread and frequent, largely among dogs but with many references to madness among wild beasts of many kinds. Foxes, badgers, and even bears were involved in epizootics, but without doubt wolves were the most feared of rabid wild animals, since it was well appreciated that people savaged by rabid wolves were at much greater risk of hydrophobia than those bitten by mad dogs.

The disease is mentioned in ancient times by Xenophon in Anabasis, Epimarchus, Virgil, Horace, and vivid. Lukian, a Roman writer, was of the belief that not only was the disease spread by biting dogs but that persons who became rabid could spread the disease by biting other persons and could affect a whole group of people (Smithers, 1958a).
The first large outbreak is described in Franconia in 1271 (Steele, 1975).


Zinke (1804) first demonstrated that rabies could be transmitted by the saliva.

Alt Graf zu Salm-Reifferschied (1813) was able to transmit rabies among dogs by saliva from a rabid animal which was inoculated. He also claimed that food smeared with contaminated saliva caused rabies. He was convinced that the saliva contained the infectious agent.

Krugelstein (1826) reported on every phase of rabies. He thought that the casual agent was in the saliva but believed that agent appeared de novo under various conditions. He stated that the rabies is without doubt a disease of nervous system.

Until the middle ages the epizootics were rare. In 1500, Spain was said to be ravaged by canine rabies. In 1586, there were epizootics among dogs in Flanders, Austria, Hungary and Turkey. It appeared in England in
1934-35, and in France, Italy and Spain in 1763. The first major epizootic of rabies in North America was reported in 1850, and Russia had an increase of rabies during 1860-1870 (Steele, 1975).

Moodley (1859) recorded a case in 1858 in a man died of rabies in Tanjore, India after he had been attacked and bitten by a mongroae. There was a case of rabies in a soldier in India in the same year following the bite of a jackal (Wrench, 1874).

Winkler (1975) reported that in 1880’s dogs were by far the most important vectors of the disease of human rabies in Europe and it was estimated that per cent cases caused by various species was: dogs 90%, cat 4%, wolves 4% and foxes 2%. Fox rabies has persisted in Europe and today foxes are considered primary reservoir of the disease.

By 1980 rabies had spread all over America and was attributed to the large number of idle dogs everywhere which were free to prawl around (Smithoors, 1958b).

Billings (1884) reported that there is no mention of skunks and foxes during this period.

Galtier (1879) reported that it was possible to transmit rabies to rabbits and from rabbits to rabbits. He has not stated the method of transmission.

The first recorded epidemic among domestic dogs in urban centres in Italy occurred in 1703 (Johnson, 1965).
Pasteur (1881) published his first report on rabies and the symptoms and histologic changes led him to the conclusion that the central nervous system and especially the bulb which joins the spinal cord to brain are particularly concerned and active in the development of the disease. He then reported success in producing rabies by injection of central nervous system material and spinal fluid.

He made further observation on rabies and concluded that entire nervous system is susceptible and a microbe of infinite smallness having neither the form of a bacillus nor a micrococcus is the cause of disease (Pasteur, 1884).

DeVesta and Zagari (1889) brought forward clinical and experimental evidence that rabies virus was spread from the bite to the central nervous system by passage along nerves.

The first outbreak of rabies in Africa was recorded in 1893 in a dog imported from England at Port Elizabeth (Usoh and Belino, 1973).

Negri (1903a) discovered the bodies in the horn of Ammon of the brain which bears his name. He used the presence of these bodies as a practical diagnostic test for rabies (Negri, 1903b).

Bartarelli (1904) showed that virus reached the salivary gland in rabid dogs by peripheral travel along the nerves.

Fermi (1907) reviewed the literature available at that time and concluded that it was difficult to demonstrate rabies virus in saliva.
Valladares (1909) observed during dog catching and stray dog destruction programme of Madras city, that during six month period in 1909, out of 4129 dogs brought for destruction, roughly over 36 were suspected rabies cases during four month period during which a special care was taken to identify such dogs.

Harvey et al. (1911) observed that an infection with B. pyocyaneus infection in dog simulate symptoms like rabies.

Nicolas (1906) demonstrated salivary virus in one dog on the fifth day prior to appearance of symptoms.

Konradi (1908) claimed to have demonstrated rabies virus in the fetuses of pregnant rabid bitches.

Webb (1913) observed that in ordinary cases of actual bites - superficial wounds on the remote parts of the body - the disease develops in about 16% of the bitten cases, and if the injuries inflicted are serious or on the muzzle, face and ears, about 80% of the attacked animals may develop the disease.

Magendie and Brechet (1921) were able to infect dogs with saliva from a human patient. This was the first demonstration that the agent of rabies in man and dog was same.

Rabies has been known since centuries B.C. In India it has existed for centuries. Graphic account of the disease may be found in early Sanskrit literature (Day, 1925).
Iyer (1925) stated that it is possible to stamp out the disease by stringent suppressive measures and prevent its reintroduction by adequate quarantine regulations as has been done in Great Britain by a Rabies Order of 1899.

Narayanan (1928) recorded the first incidence in Malay in middle of the nineteenth century and observed that the largest number of positive cases were found in the month of January, February and October. He also recorded the frequency of human infection from animals and dogs were highest.

Pillai (1934) reported sporadic outbreak of rabies in Penang, a little island close to Malay Peninsula which started in the month of January 1931 and lasted for two years. It was controlled by simple means like pamphlets distribution in prevalent languages, muzzling order of dogs, fine to non-cooperative people, distraction of stray dogs, isolation and observation.

The cases of rabies were reported from various species in India. Kulkarni (1939) reported an outbreak of rabies in military horses, which occurred after a year of dog bite. Ramiah (1932) reported paralytic rabies in elephants.

Ganpathy (1950) carried out an analysis of incidence in Madras city from the number of positive cases admitted at Madras Veterinary College Hospital. He concluded that the disease is prevalent throughout the year and not confined to any month or season, whereas in France it is March, April
and May when the bitches come in heat and subsequent dog fighting. The incidence was higher in the year 1936-1937, fall in the next few years with a rise to a peak in 1944-1945 and is still maintained. Restrictive measures are not slackened but may be the canine population is on increase or cases are being brought to notice due to public awareness.

Dogra (1950) stated that the reliable statistics are not available regarding incidence of rabies, among canine population of rural and urban area.

Ahuja and Brooks (1951) reported their observations on incubation periods, the shortest was 11 days reported at Kasauli and the longest incubation period of three years, two months and 21 days (1176 days totally) was recorded by Iyenger (1935), in India.

Bell et al. (1957) stated that attempts to transmit rabies infection by blood sucking arthropods have not been successful.

Faulkner (1959) reported that in Nyasaland there was a sharp increase in rabies and it figured most in the public eye. Therefore the most important step of the year was the formation of Rabies Control Unit in the country.

The average incubation period on leg bites was 60 days, forearm bites 40 days and for head bites 30 days. The incubation period varies also with the amount of virus introduced and the shorter incubation period after head
wounds may be in part attributable to the fact that these are usually severe and a considerable amount of virus introduced; also the distance to be travelled to the brain is short. Periods as short as six days and as long as 12 months have been reported (Rhodes and Von Rooyan, 1962).

Khera (1968) reported that out of the 1428 deaths due to rabies among the indoor human patients during the year 1959-1961, 393 were in Uttar Pradesh, 328 in Madras, 223 in West Bengal, 170 in Mysore, 68 in Madhya Pradesh, 65 in Rajasthan, 61 in Punjab, 59 in Kerala, 45 in Orissa, and 16 in Tripura.

Chalmers and Scott (1969) regarded certain geographical regions of Africa, West, Central and East Africa as the primary loci of rabies-canid climaxes. The first report originated in French speaking West Africa where a disease of dogs known locally as "Oulon fato" was identified as rabies by Bonffard in 1912.

Many epidemiologists have pondered on how rabies survives in nature so effectively when hosts are regularly killed by virus. An animal reservoir where the host is only mildly affected has been looked for and insect eating bats have been considered a possibility. Other authorities have suggested that the conventional difficulty in long term survival of a lethal pathogen is hardly applicable to rabies. They feel that there is no need to look for a reservoir of the usual type. The unusual length of
incubation period which may run into months, and of salivary
excretion of virus before the animal becomes paralysed may
be long enough to ensure its effective transfer and indefinite
persistance in the ecosystem (Burnet and White, 1972).

Rabies is enzootic on every continent except Australia
and has been reported from 63 countries. It was not present
in 30 countries, territories or Islands. Throughout the
world, dogs are the most important vectors of the disease,
although in almost every country various species of wild
life are important reservoirs or vectors (W.H.O., 1967).

Gamet et al. (1972) reported rabies in France from
1968 to 1971, since rabies appeared in East France. In 1971,
the number of confirmed cases were greater than previous
three years, cattle were most affected (40.4%) followed by
foxes (29.8%), cats (16.5%), dogs (9.9%) and sheep (1.5%).

Similarly from the Pasteur Institute of Southern
India, 14 species were found to be the source of infection
of rabies. Dog accounted for more than 95% of cases, next
in the order is cat (Veeraraghavan, 1972).

At Central Research Institute, Kasauli (India) during
the year 1971, 23 species were found to be the source
of exposure to the patients, chiefly dogs accounted for
92.5%, cattle 1.39%, jackal 1.32%, humans 1.3%, buffalo 1.23%.
Rest of the animals included are, bear, bijju, camel, cat,
donkeys, ass and mules, fox, sheep and goats, horses,
hyena, jarakh, monkey, mongoose, pig, rat, tiger, wolf and vultures (Thomas, 1973).

Arambulo (1974) stated that rabies is the most important viral zoonoses in Philippines. Significant reservoir is the dog, although rabies has been reported in cattle, pigs and cats, all followed by exposure to a rabid dog. Rabies virus has not been found in sylvatic hosts, rats and bats, shrews and monkeys.

Rweyemamu et al. (1974) reported that the early history of rabies in Tanzania is obscure. However, it appears to have been known before the Europeans arrived in the area. Dogs are comprising 90% of cases.

Abdussalam and Botton (1974) reported that in 1971, 53 countries claimed that they were free from rabies. They observed that in countries in Africa, the disease is endemic and the principal foci are in towns, where stray dogs form the principal link in the chain of infection. In Canada wild life rabies covered the whole country. In Haiti, British Honduras, Columbia, Venezuela, Guyana, Peru, Bolivia, Brazil, Chile the rabies is endemic. Stray dogs are chiefly responsible for the spread of rabies in Afghanistan and India. During the months of March, April, September and October the disease is at its peak and prevalent throughout Pakistan, Bangladesh reported an increase in the distribution of rabies in 1971. A wild life survey of Shri Lanka showed
little evidence, although the serum of one mongoose out of 150 examined showed a neutralising titre of 1/5. Malaysia was reported free from rabies in 1966. However rabies occurred in 1970, and is again rabies free. No cases were reported in 1971. Japan has had no confirmed case since 1956. Guam reported rabies for the first time in 1967 and by vigorous campaign of vaccination and elimination of stray dogs the disease has disappeared and not observed in recent years.

Rabies is endemic in countries of Asia.

In Europe, rabies is sporadic and wild life are the main reservoirs of the disease.

Tierkel (1975) observed that the identification over the years as rabies as a disease of dogs; to the exclusion of its importance in wild carnivoras - can be best explained by man's close relationship to the dogs than wild animals. The dog is still the principle vector animal in transmission of the disease to man and other animals in most countries of Asia, Africa and South America. There is still much to be done to undertake effective canine rabies control programme for reducing the hazard of the disease to man and domestic animals in the large areas of the world.

Steele et al. (1975) stated that rabies is unknown disease in cyprus. The earliest case was recorded before about 20 years.
Steele (1977) reported that rabies is not known in Samoa and South Pacific except on the continents surrounding Pacific ocean, North and South America, Asia and especially the Phillipines.

Andersons (1977) reported that during past 20 years rabies had been endemic in certain districts of Lativia (U.S.S.R.) among wild carnivora.
THE RABIES VIRUS
THE VIRUS:

Acton and Harvey (1911) stated that Negri bodies are not parasite in nature but are formed by the action of cytoplasm on extruded nucleolar spherules.

Manouelian and Viala (1924) described the Negri bodies and named them *Encephalitozoan rabiei*. They observed that great majority of bodies and corpuscles are the expressions of the intracellular growth of the specific organisms of rabies and that they multiply in cell cytoplasm as well as increase in size. They failed to find bodies in salivary glands and stated that the mode of transmission remain a mystery.

Levinson et al. (1945) stated that inactivation of rabies virus by ultraviolet rays have been considered in connection with vaccine production.

Lepine (1948) observed that rabies virus was inactivated by papain.
Veeraraghavan et al. (1954) stated that receptor destroying enzyme (RDE) of *Vibrio cholerae* given subcutaneously, intraperitoneally or intravenously was of no value in preventing onset of rabies in animals challenged with the virus intracerebrally or subcutaneously.

Veeraraghavan and Balasubramanian (1954) observed that ACTH does not appear to be of any value in animals which were given challenge virus intracerebrally. It does not alter the course of disease in animals which had started showing signs of rabies. It appeared to be of value when the virus was given peripherally in animals already under the influence of ACTH.

Kuwert and Liepenow (1959) stated that rabies virus was inactivated at pH 3 and 3.5 within 30 minutes, but between pH 5 and 10 was active after 24 hours. At pH 9 at 4°C after 34 days it was inactive. The virus was inactivated after 5 hours at 35°C, and at 2 hours in all dilutions of formaldehyde.

Johnson (1959) isolated rabies virus from the lactating mammary glands of a spotted skunk. In past, isolation of rabies virus from milk has been reported by Nocard et al. (1887) and by Remlinger and Bailly (1932).

Alba (1959) found that hyaluronidase added to rabies street virus suspensions appeared to have no effect on the course of subsequent infections in dogs.
Rabies related virus have been isolated from frugivorous bat brain on Lagos Island (Boulger and Porterfield, 1958), termed as Lagos Bat Virus (LBV).

Porterfield et al. (1958) isolated Nigerian horse virus from brain of a horse which had died of "staggers" a rabies like disease of horses.


Kotonkan virus (KOT) from culicoids spp. in Nigeria and Obodhiang (OBO) were isolated on several occasions from mosquitoes (Manacalia uniformis) in Sudan (Shope, 1971).

Kaplan et al. (1960) applied living virus to chick cell monolayer and challenged the cultures 24 hours later by the application of Western Equine Encephalomyelitis virus. They found that a large dose of rabies virus completely suppressed the formation of plaques of Western Equine Encephalomyelitis virus, whereas smaller dose of rabies virus was ineffective. This effect could be abolished by applying a mixture of rabies virus mixed with a suitable dilution of rabies immune serum, under these conditions WEE plaques appeared in reduced numbers. This is an example of interference.

Almeida et al. (1962) used negative contrast microscopy to study cultured hamster kidney cells infected with a fixed rabies virus. In infected cells they found irregularly shaped particles upto 400 nm in diameter.
Andrews (1962) stated that rabies seems to stand alone in classification of viruses, yet apart from its larger size, it is not far removed from arboviruses and one can imagine it as an aberrant member of that family, as it has been isolated from insectivorous bats. It may however, lie nearer to myxoviruses.

Bullet shaped virus particles were first described in rabies virus infected chicken embryo cell cultures by Davis et al. (1963).

Atanasiu et al. (1963) suggested that rabies virus may belong to Rhabdovirus group.

Harms (1963) observed that the resistance of rabies virus to disinfection is considerable. The most powerful virucidal preparation were 3 per cent caustic soda, 3 per cent formalin and 113 chlorinated lime solution. Exposure to ultraviolet light killed the virus on the surface of materials within two hours.

Infection of chick embryo cells with Flurry HEP strain of rabies virus induced interference with the multiplication of challenging Western Equine Encephalitis, pseudorabies, New castle disease, Vaccinia and Parainfluenza I viruses. Rabies virus inactivated by treatment with antirabies gamma globulin, heating and u.v. irradiation respectively lost its ability to induce interference. The interference phenomenon was used for titration of rabies virus in tissue culture (Selimov et al., 1965).
Turner and Caplan (1967) reported that the Pasteur strain of fixed rabies virus was inactivated by most common organic solvents but survived a single treatment with trifluorochloroethane. The virus was stable at pH values five and ten. Inactivation by heat was similar to that of other viruses. The virus was destroyed by trypsin and damaged by phospholipase C but not by ribonuclease. Enzyme inhibitors did not preserve the infectivity of mouse brain suspensions. Viral infectivity was diminished only after repeated freezing and thawing or long exposure to ultrasonic vibration. Equilibrium centrifugation in sucrose solution gave two peaks of infectivity corresponding to densities $1.13 \text{ g/cm}^3$ and $1.19 \text{ g/cm}^3$. Chromatographic purification on ECTEOLA-cellulose was unsuccessful. Partially purified virus was readily inactivated by the photodynamic effect of methylene blue. Potent suspensions of virus did not agglutinate red blood cells of several mammalian and avian species under different conditions of pH and temperature.

Reda et al. (1965) stated that the Pasteur strain of rabies virus in homogenised brain tissue were readily inactivated when equal volumes of 20 per cent viral suspension and two per cent of Bayer A 139 were mixed at pH 6.7 and 24-25°C without loss of complement fixing activity.

Tierkel (1963) stated that rabies virus was easily destroyed by sunlight and heat, and its infectivity becomes
negligible when exposed to ordinary environmental conditions of light, heat and air. This property has a practical application in that fomites have not been known to play a role in transmission of this virus.

Johnson (1965) observed that capacity of the virus to invade and multiply in the salivary glands, lungs, kidneys, pancreas and mammary glands makes it possible for the virus to maintain a cycle of infection not associated with encephalitis.

Pinteric and Fenje (1966) elucidated the surface and internal detail of bullet shaped rabies virus particles; they found mean dimensions of 140 by 100 nm, surface projections 6 nm long, a vesicular appendage from the planner end of particles, an axial channel, and the release of helical ribbon like internal components from intact bullet shaped particles. In addition a pyramidal or bell shaped variation of some particles was described.

Kaplan (1973) stated that rabies virus belongs to Rhabdoviruses, enveloped, bullet shaped, lipid containing and single stranded RNA. It is sensitive to lipid solvents, 45-70% ethanol, iodine preparations and quarternary ammonium compounds. Other relevant properties are resistance to drying, to repeated freezing and thawing, relative stability at pH values between 5 and 10, sensitivity to pasteurization temperatures and ultraviolet light. The nucleic acid is readily inactivated by B-propiolactone.
Shope et al. (1970) observed that Lagos Bat Virus and an isolate from shrews (IsAn 27377) both from Nigeria were found to be bullet shaped and to mature intracytoplasmically in association with a distinct matrix. They were related to, but readily distinguishable from rabies virus and each other by complement fixation and neutralization tests. The three viruses, including rabies form a subgroup within the Rhabdoviruses.

Fenje and Postic (1970) reported that only six of the 64 rabbits died when they were given intravenous injection of one mg of polyinosinic-polyisidyllic acid (poly I-poly C) within 24 hours before or after intramuscular challenge with 25-30 rabbit LD 50 of street virus from the salivary glands of an infected fox. All seven untreated controls died after this challenge. Assays of four serum samples taken two hours after uninfected rabbits were injected with the compound showed that the antiviral effect was due to high interferon levels, since it was species specific and was destroyed by trypsin but was not affected by ribonuclease. All 58 treated rabbits that had survived challenge had high neutralizing antibody titres 35 days after challenge and successfully resisted a second challenge which indicated that the development of active immunity had not been inhibited.

Janis and Habel (1972) studied the protective effect of interferon inducer, polyriboinosinic-polyribocytidylic acid (poly I:C) in rabbits and mice experimentally infected
with street and fixed strains of rabies virus. Six daily intravenous inoculation of 1.0 mg or 0.5 mg of poly I:C, beginning 3-24 hours before viral challenge protected all rabbits infected with fixed virus. Three daily intramuscular injections of 0.5 mg of poly I:C beginning three hours after viral challenge protected rabbits infected with street virus. Intramuscular injection of poly I:C produced a local interferon response and gave better protection in rabbits of infected into same muscle site as the virus. Street virus infected mice were protected by as little as 1.3 μg of poly I:C, the intramuscular route of injection gave better protection than intraperitoneal route. Mice inoculated with poly I:C were protected as long as 67 hours after injection of street virus.

Chlorite oxidised amylase (COAM), polyinosinic-polycytidylic acid (poly I:C) and combination of two drugs were evaluated for their interferon inducing properties and their ability to protect mice against rabies infection. Post exposure administration of one or two doses (100 μg each) of poly I:C significantly protected mice against rabies infection. Pre-treatment of mice with COAM three hours before poly I:C stimulation resulted in an enhancement of interferon response. However, the increased interferon titres were not reflected by increased protection against rabies infection and over that achieved with poly I:C therapy alone. Therapy with COAM alone did not protect
mice against rabies, but rather was associated with increased mortality (Harmon et al., 1974).

Mathew and Rao (1974) stated that Statolon, Cyclohexamide and Banakadali (wild banana) seed extract when tested for interferon production, did not protect mice against rabies infection. Vitamin A and DEAE - Dextran used as enhancers along with Statolon produced only a marginal fall in mortality rate. UV inactivated suspension of challenge virus standard halved the mortality rate when given intracranially 18 hours before rabies infection. Intracranially injection of LaSota strain of Newcastle Disease virus 48 hours before rabies inoculation of mice also halved the mortality rate. These agents appear to inhibit the multiplication of rabies virus probably by way of the interferon phenomenon.

Schneider and Schoop (1974) reported about 40 virus isolates from small rodents of the subfamilies Murinae and Microtinae obtained by Czechoslovakian, German and Swiss laboratories, which are serologically undistinguishable from rabies virus.

One more virus Duvenhage, was found in South Africa in 1970 in the brain of a man who had died, apparently from rabies, after being bitten by a bat, (Brown and Crick, 1977).

Presently rabies virus particles are described as cylindrical with one round or conical end and one planar or concave end. From the surface inward they consist of;
surface projections, membrane envelope and helical ribonucleoprotein capsid. The fine fringe of surface spikes or projections is 6 to 8 nm thick; this layer usually does not cover the planar end of particles. Individual projections, each with a knob like distal end, are placed on the virus surface at 4 to 5 nm intervals (Murphy, 1975).

Alvarado et al. (1976) isolated rabies virus from kidney, liver, spleen, heart, lung, parotid gland and aqueous humour of a dog that died of rabies. Samples of these organs were inoculated into the brain of adult and suckling mice, and the findings were confirmed by the observation of Negri bodies and immunofluorescence.

Saghl and Flamand (1979) found that 70 mutant strains of rabies virus formed plaques at 33°C but not at 38.6°C. A study of their biochemical properties showed that sensitivity to temperature involved transcription/replication of virus. Most of the mutants induced a fluorescence fainter than that of the wild type of virus.

Zaides et al. (1979) observed that rabies virus (strain Vnukovo-32) was composed of four major proteins (mol. wt. 65, 54, 37 and 21 thousand), one minor protein (mol. wt. 200,000) and an intermediate component (mol. wt. 43,000). These were the types of subviral particles (nucleocapsids) corresponding to another Rhabdovirus, VSV.
(111) VACCINE
Vaccines:

Pasteur *et al.* (1884) were the first to modify the pathogenicity of a virus by about 100 passages in rabbits. This was fixed virus. He used this strain, and prepared first vaccine against rabies which was used in dogs with success. In 1885, this kind of vaccine was used on a boy who had been severely bitten by a rabid dog. The boy remained well.

In 1886 Pasteur reported the results of treatment in 350 cases. Therefore he concluded that the prophylaxis of rabies is established. It is time to create a centre for vaccination against rabies (Steele, 1975).

Roux (1887) introduced the use of glycerol as a preservative for maintaining the viability of virus in infected tissue. This was applied to the production of vaccine by Chalmette (1891).

Babes and Lapp (1889) showed that it was possible to prevent the development of rabies in animals by injecting a specific antiserum simultaneously with or shortly after a dose of active virus.

Fermi (1908) pointed out various defects in Pasteur's vaccination programme, and introduced a new method in which the vaccine was treated with carbolic acid.

Fixed rabies virus inactivated with phenol, as a vaccine was used for immunization against rabies in man in British Army in India by Semple (1911).
Semple (1919), by modification of Fermi's method showed that a phenol treated tissue virus suspension could be rendered completely non infectious and still retain its immunizing capacity, as determined by clinical trials in man.

Umano and Doi (1920) immunised large number of dogs in Japan by using phenol inactivated fixed virus for preventing rabies.

Bichhorn and Lyon (1922) introduced into United States phenol inactivated vaccine for dogs to be given in single treatment.

Kerbler (1933) made some experiments to produce antirabic vaccine from sheep brains and the method is still being used. A vaccine prepared in similar manner from buffalo calf brain was prepared in India by Singh (1960).

Leach and Johnson (1940) isolated a strain of rabies virus from a child whose name was Flury. It was adopted to a day old chick. The pathogenicity for dogs was greatly reduced by 135 passages.

Koprowski and Cox (1948) used the same strain and carried it through serial passages in chick embryos. This attenuated strain was used in vaccine for dogs and subsequently in man.

Wong and Cox (1948) referred to experimental studies in which aureomycin failed to show therapeutic activity
against several important viral infections in animals such as canine distemper, rabies, newcastle disease and venezuelan equine encephalomyelitis.

Koprowski and Black, (1950) used attenuated rabies virus grown in chick embryos for immunisation in dogs.

A vaccine prepared by using fixed rabies virus attenuated by a 0.15% solution of silver nitrate proved as effective in mice and rabbits as attenuated virus (Nicolau et al., 1959).

Piskareva et al. (1959) prepared a vaccine from fixed live virus in a five per cent suspension of rabbit brain plus 10 per cent sucrose. It was inactivated by ultraviolet light. Tests on rabbits and mice indicated that the vaccine was better than that prepared by Fermi's method. It retained its immunogenic properties after storage for a year at 18° to 25°C.

Rabies vaccine prepared by treating rabbit brain containing fixed rabies virus with 15 per cent silver nitrate was more active than Fermi's vaccine in mice and rabbits and retained its activity for at least three months (Nicolau, 1961).

Veeraraghavan and Subramanyan (1961) reported that in experiments at the Pasteur Institute, Coonoor lyophilized and liquid phenolized rabies vaccines were assessed for antigenicity in relation to the NIH (United States National Institute of Health) Reference vaccine.
(the proposed International References preparation of rabies vaccine). They claim that phenolized antirabies vaccines can be lyophilized without loss of potency was confirmed. Lyophilized vaccines possessed high antigenic values and retained their antigenicity better than liquid vaccines.

Nazarov and Shishkov (1962) stated that experience with 5 per cent phenolized vaccine prepared from infected sheep brain showed that dogs required two annual inoculations for two or three years before they were immune to infection by biting; two inoculations alone were inadequate.

Formalin-inactivated vaccines of sufficient potency to satisfy the NIH standards were prepared by concentration of rabies virus grown in hamster kidney tissue cultures. The cytopathic effect produced by virus adapted to tissue culture permitted neutralization test. Rabies virus possesses essential lipid and is probably a virus of the ribonucleic acid type (Kissling and Reese, 1963).

Veeraraghavan and Subramanyan (1963) reported that guinea pigs which had survived a moderate challenge as a result of treatment with serum and vaccine, nearly 70 per cent resisted subsequent severe challenges with about 100 LD-50 of homologus and heterologus strains of rabies street virus from three to 15 months after the first treatment. There was no advantage in giving two booster doses of vaccine during this period.
Wiktore et al. (1964) observed that after adaptation to WI-38 cells, the HEP strain seemed to lose its lethal properties for monkeys injected intracerebrally, while acquiring a high immunising capacity. Inactivated vaccines prepared from two other strains of fixed virus (CVS and PM) were highly antigenic after adaptation to WI-38 cells. This fact and the availability of the live HEP virus adapted to WI-38 cells indicated that production of effective and safe anti-rabies vaccine for man is now feasible.

Brown et al. (1973) introduced a modified live virus (MLV) rabies vaccine prepared by growing high passage (HEP) Flurry strain on an established canine kidney cell line, and was tested in dogs to determine its immunogenicity. Intramuscular route produced better immune response.

Polyvalent vaccine consisting of freeze dried living vaccine (distemper and hepatitis) suspended in inactivated liquid vaccines (Leptospira and rabies) was tested in 54 Beagle pups aged 3-4 months. The innocuity of the vaccine and the immunity conferred was similar to those of the component vaccines. No immunological interference between the components was demonstrated. There was no adverse effect on the growth of vaccinated animals (Chappuis et al., 1973).

The ERA vaccine strain of rabies virus propagated on continuous porcine kidney tissue culture cells (FK 15)
was inoculated into the yolk sac of chick embryos. Two inoculated eggs were subsequently fed to each of 12 serologically rabies negative foxes. Six (50%) of the foxes developed demonstrable rabies serum neutralizing antibody within 4 week of vaccination and resisted challenge to street virus 14 weeks after vaccination. Testing of inoculated eggs for virus stability in the presence and absence of a casein hydrolysate sucrose stabilizer at 6, 22 and 37°C for up to 15 days revealed no advantage to the stabilizer vaccine mix in eggs (Debbie, 1974).

Hattwick and Gregg (1975) stated that the latest in human vaccine is Human Diploid Tissue Culture (HDTC) inactivated with B-propiolactone or by tri-n-butyl phosphate. It conferred better immunity with a good neutralization titre, when inoculated in four doses at zero, three, seven and 14 days.

Vaccine prepared from ERA strain (Embryo readopted) is effective in bovines, and the immunity is for two to three years. If pregnant cows are vaccinated, calf is having antibody titre up to six months. If the dam is unimmunized, calf can be vaccinated at four to six months age with a booster at one year (Abelseth, 1975).

Barth et al. (1975) reported that "Medivak" an inactivated vaccine prepared from rabies tissue-culture
virus was used in horses for immunization against rabies. A single dose of 2 ml produced serologically detectable antibody that persisted for a year.

Mature and immature foxes (Vulpes vulpes) and striped skunks (Mephitis mephitis) were fed mice infected with street isolates and a fixed strain of rabies virus. Deaths from rabies occurred only among immature foxes and skunks. Some of the animals that did not die developed neutralizing antibodies and were resistant to challenge with rabies virus (Ramsden and Johnston, 1975).

Winkler and Baer (1976) vaccinated foxes against rabies with two modified live vaccine viruses ERA/BHK-21 and PRI strain, in sausage baits. Antibody profiles in vaccinated foxes and intramuscular challenge with street virus showed ERA/BHK-21 vaccine to be more effective than PRI vaccine. The addition of a stabiliser helped maintain virus titres when the baits were subjected to high (35°C) temperatures.

HDCV - Human diploid Cell Strain vaccine when available plus HRIG (Human rabies immune globulin) is safe and effective and recommended to replace present treatment (Anderson et al., 1980).
Latent, abortive, carrier, inapparent rabies.

Pasteur (1882) considered resistance to reinoculation a strong indication of previous abortive infection and others have used the same technique for confirmation.

Konradi (1916) stated that a dog bite may be infectious as long as 14 days before appearance of signs of illness.

Jonnesco and Teodasiu (1928) isolated virus from saliva seven days before appearance of signs of illness.

Recovery from paralytic disease following an experimental infection via intramuscular or intracerebral routes with a bovine strain of virus was noted in dogs by Johnson (1948). He also infected six dogs with a bat strain of virus, one died 14 days later and the remainder remained healthy for three months. Rabies virus was isolated from fatal but not from the surviving cases.

Any adjuvant conditions such as central nervous disease, trauma, or toxic action may convert a latent case of rabies into an active one or may appreciably shorten the incubation period (Lepine, 1951).

D'Silva (1952) recorded instances of "fatal neuro sterilisation" and of recovery of rabies. Three cases of experimentally induced rabies of prolonged duration are described and in one instance virus was recovered from guinea pig after 38 days of illness. He observed that
there is a definite relationship between inoculation period of rabies and the duration of illness.

Remlinger and Bailly (1946) regarded the carrier state as rare, of relatively minor importance in epidemiology and a phenomenon that should not enter into consideration in public health practices.

Starr et al. (1952) stated that there are few reported cases in which dogs have recovered from the disease after signs of rabies developed.

Johnson (1952) reported that 21 out of 28 dogs in which virus was demonstrated in brain, showed virus in their salivary glands (75 per cent). In rabid foxes, salivary virus was demonstrated in 130 of 150 animals (87 per cent), in rabid cattle the ratio was 16.34 (53 per cent).

Perhaps the most commonly used criterion of non-fatal infection is the isolation of virus from saliva, brain or other tissues of animals that appear normal, yet it should be emphasised that virus may be present in the CNS and salivary glands well before onset of illness (Wright, 1956).

Andral and Serrie (1957) stated that there are at least two reports of rabies virus being isolated from the saliva of asymptomatic dogs.
Andrews (1957) observed that a fatal infection of rabies helps neither parasite nor host may at times be fallacious. It is the severe encephalitis causing the victim to salivate, run wild and bite which is the means by which the infection spreads.

Latency and abortion are not always clearly distinguishable from masked, inapparent, chronic and recrudescent infection, eclipse, long incubation periods, and the carrier state (Walker et al., 1958).

McDermott (1959) stated that latent infection is reserved for situations in which the presence of the microbes cannot be demonstrated by any method now available, and the fact that infection is present can only be demonstrated retrospect by the emergence of overt disease.

A serological survey of wild animals carried out by the centre for Disease Control of U.S.A. revealed evidence of subclinical infection (Tierkel, 1959).

Yurkovsky (1962) reported rabies infection transmitted by normal dogs, but unsupported by laboratory evidence.

Soave et al. (1961) reported reactivation of infection by repeated inoculations of ACTH in one of six guinea pigs that had survived inoculation of street virus five months earlier.

Soave (1964) further reported reactivation of infection in one of 10 guinea pigs eight and a half months after inoculation, attributed to stress of crowding.
Serle and Andrei (1953) demonstrated specific rabies antibodies in the sera of five stray dogs. Twenty six months later, examinations of sera before and 10 days after a single injection of rabies vaccine revealed a decrease in neutralizing antibodies and a difference between the LD-50 of the challenge virus standard before vaccination, and that after vaccination. It was concluded by the authors that the antibodies were the result of spontaneous recovery from inapparent infection.

Bell (1964) stated that despite the evidence to the contrary, rabies is still commonly considered to be uniformly fatal in man and in the common host vectors. In an experiment to investigate a high incidence of survival in experimentally infected mice, he found that after intraperitoneal injection of several strains of rabies virus a varying proportion of obviously infected mice survived despite persistent severe sequelae. The identity of illness was established by identification of virus recovered from mice, by correspondence of signs of illness to those of rabies, and by demonstration of a high degree of acquired immunity to challenge with rabies virus. The experimental reproducibility of this abortive infection may afford a basis for further inquiry into the phenomenon.

Rabies may be the only viral disease in which uniform lethality is necessary for survival of the virus in nature, simply because only a very extensive infection of brain
cells can produce the necessarily gross change in temperament and habit required. Indiscriminate aggressiveness against other animals including carnivores is quite abnormal in any free living mammal but it is only such behaviour that will allow the sequence of infections to be maintained, once that degree of infection is reached, recovery becomes physiologically impossible (Burnet, 1966).

A survey carried out in Madras and Delhi to investigate the carrier state among dogs, revealed that five dogs had presence of neutralizing antibody out of 131 serum samples examined (Veeraraghavan, 1966).

Vaughn et al. (1965) measured the titre of virus in salivary glands. It varied from a trace to as high as \(10^{-7.9}\) MLD 50/g. He carried out a study to measure the salivary excretion periods for rabies virus in relation to onset of illness in dogs. In 26 rabid dogs excreting virus in saliva, the earliest initiation of viral excretion observed was three days before onset of illness.

Veeraraghavan et al. (1966) reported the occurrence of human rabies following bites from an apparently healthy dog. The boy developed clinical symptoms and died of rabies 44 days after being bitten by the dog. The dog which appeared healthy was kept for four years following the incidence and never showed clinical signs of disease. Rabies virus was recovered from the saliva of the dog 13 times during the first four months soon after the incident, and
once, a year later following a course of prednisolone treatment, but no neutralizing antibodies were demonstrated in the serum (Veeraraghavan et al., 1970).

Johnson (1966) recorded the isolation of rabies virus from a long-tailed weasel in Alaska, and sporadic occurrence of rabies in spotted skunks in California and elsewhere, and believed that Mustelidae and Viverridae constitute the "true carriers" in their respective environment. However, Lessman (1971) failed to find the support for that concept in intensive studies of wildlife in Germany.

Held et al. (1967) studied the possible role for the carrier state in the epidemiology of human rabies that occurred in United States between 1946 and 1965. He observed that source of infection was known only in 149 (63%) cases.

Veeraraghavan and Gajanana (1971) observed an apparently healthy dog whose bite had resulted in a case of hydrophobia, for 13 months. During this period, 75 saliva samples were examined by FAT and animal inoculation, and were found negative. Rabies virus could not be isolated from saliva of an animal after cortisone or carbachol treatment. Serum showed absence of neutralizing antibodies. On post mortem, all specimens were negative by biological test and FAT.
Ereegovae and Pima-Kostoglou (1968) while examining serum samples from human beings, a horse, a ox, a sheep, a pig, a dog, a rabbit, a guinea pig and a rat found naturally occurring antibodies to rabies which neutralized upto 250,000 LD-50 of Rabies virus in vitro and upto 31.6 LD-50 in rats.

Schmidt (1969) stated that the complement fixation test (CFT) is one of the most valuable serological procedures available. Although generally less sensitive than neutralization, haemagglutination inhibition or fluorescent antibody techniques, it possesses the advantage of greater simplicity.

The hyperimmune antirabies serum produced by injection of guinea pigs either with 10 ml of brain suspension from rabid guinea pigs or with six ml of rabies vaccine grown in cell culture and inactivated with beta-propiolactone was shown to have a serum Neutralization (SN) titre of 4.5 but a complement fixation (CF) titre of 80, and had no anti-complementary activity. This hyperimmune serum was used in determining the complement fixation (CF) titre of antigen. The results showed that the complement fixation titre could be used to estimate the infectivity of same kind of antigen (Soulebot et al., 1969).

Nilsson et al. (1975) carried out complement fixation test on sera of 25 dogs inoculated with HEP Flurry vaccine.
Titres ranged from 0 to 256, at 15 or 90 days after vaccination. They did not correspond quantitatively with neutralization titres but 18 of the 26 samples produced a similar qualitative reaction in two tests.

Bogel and Kaplan (1968) have suggested that serological evidence must be carefully interpreted, as according to them antibody can be detected before the onset of symptoms. They have advised simultaneous test for detection of rabies virus itself.

Bell et al. (1971) carried out serologic, cytologic, and virologic studies on 120 dogs collected in a rabies enzootic area. Evidence of chronic, carrier or abortive rabies was not found. Adequacy of the techniques for isolation of virus and for demonstration of antigen by the fluorescent antibody test was demonstrated in concurrent studies on clinically rabid dogs and on dogs that apparently had abortive infection as a result of experimental inoculation of attenuated virus.

Stewart and Sulkin (1966) reported that rabies virus evokes interferon in the CNS as well as somatic organs.

A dog inoculated intracerebrally with fixed mouse adopted rabies virus recovered spontaneously from the disease without sequelae. Serum and brain neutralization titres were respectively 1:4200 and 1:3200 against 468 LD-50 of the virus. Electrophoretic analysis of the serum on the 14th day after
inoculation when the dog was still showing the symptoms and on 26th day, when it had fully recovered showed quantitative variation of the protein fractions. The fluorescent antibody and mouse inoculation tests did not reveal any rabies antigen in different organs (Markus et al., 1969).

Fenje and Postic (1970) found that protection from rabies by interferon has been inferred from the increased survival of rabbits inoculated with either poly I - poly C at suitable intervals before or after inoculation of rabies virus. They entertained the possibility that potentiation of the immune response may have accounted for the results with poly I poly C.

Wiktor et al. (1972) indicated that interferon elicited by intracranial inoculation of attenuated rabies virus can cause abortion of infection by virulent street virus at the stage of ascent of the nerve.

A healthy carrier state in dogs seems to have been documented in Ethiopia, where during 1965 - 1971, five out of 1083 healthy unvaccinated dogs tested were found to excrete rabies virus intermittently with their saliva for periods up to three years. One dog died of bronchopneumonia, and three dogs were still alive and well at the time of report (Fakadu, 1972).

Bell et al. (1972) stated that a high neutralizing titre in cerebrospinal fluid apparently indicates recovery from rabies. Recovery seems very rare but there had been no
method of investigation previously. The possibility of a healthy carrier seems even more remote. Twelve to 20 weeks old pups were given LEP vaccine intracerebrally along with vaccine or street virus intraperitoneally. Most showed no sign of illness and developed no titres. Five became sick and two died on day 10, after onset of paralysis on day 9. They also had no titres. The other three had mild to severe nervous signs from day 11 to 14 but recovered by day 18 to 21. At five months they had serum titres of 717 to 1920; CSF titers of 122 to 400. Vaccinates did not have high CSF titers even when serum titers were high. In a larger experiment, they could not find a carrier form of infection in more than 500 intensively studied dogs in an enzootic area in Argentina where large number of dogs were vaccinated and prevalence of disease was declining.

Kathuria (1972) reported that out of the 109 serum samples collected from horses and examined for the presence of rabies neutralizing antibody, 13 were found positive.

Baer and Cleary (1972) have demonstrated that passive antibody can mask the virus or cause temporary latency with later spontaneous activation similar to that occurring in canine hepatitis.

Afshar et al. (1972) reported results of 125 sera obtained from apparently healthy stray dogs, 91 human sera and 26 camel sera. These sera were tested for the presence
of rabies virus precipitating antibody. Thirty two per cent of stray dog sera were positive, whereas human and camel sera were negative. When 101 salivary gland samples collected from 51 dogs and saliva samples from 58 dogs were tested for presence of rabies precipitating antigens, six salivary glands and one saliva sample were found positive. The results suggested that inapparent or abortive infection with rabies virus may occur in dogs.

Arko et al. (1973) reported that a Beagle vaccinated against rabies, showed signs of rabies 20 days after it was given a challenge inoculum of street rabies virus. The Beagle recovered and the virus could not be isolated. The level of neutralising antibodies were higher in serum and CSF of the recovered dog. In experiment with other Beagles, high CSF antibody titer occurred only in the Beagle that had recovered.

Evidence from laboratory and field studies in mouse, rat, guinea pigs, opposum, skunk, bat, bobcat, raccoon, fox, pig, dog, donkey and man suggests that infection by rabies virus may lead to various outcomes, i.e. inapparent infection survival with residual signs, or death. Wild animals from rabies endemic areas in the U.S.A. demonstrated evidence of previous contact with rabies virus, as did dogs in Ethiopia and Thailand and rodents in Thailand and Central Europe. Such evidence suggests that exposure to rabies
Knowledge about mechanisms underlying the different host responses may improve the chances of human survival after exposure to rabies virus (Doege and Northrop, 1974).

deDiaz et al. (1975) used brain homogenates from 1015 normal dogs and found neutralizing antibodies in two brain samples.

Nilsson and Cortis (1975) reported that a five year old female dog developed clinical signs of rabies 16 days after being inoculated intramuscularly with rabies virus of bat origin. The diagnosis was supported by isolating virus from saliva and by demonstrating it in corneal cells. Recovery from clinical illness was complete 31 days after inoculation with no demonstrable sequels. Serum neutralization test performed 36 days post inoculation revealed a titer of 1:625. No treatment was given to animal.

Fakadu (1975) has reported the findings in five healthy unvaccinated dogs brought for rabies observation after biting humans. One of these dogs died of bronchopneumonia after nine months of observation. Another dog died with furious form of rabies after nineteen months of observation. Rabies virus was repeatedly isolated from the saliva of both these animals. The three remaining dogs are still alive and asymptomatic 72, 50 and 62 months after the first isolation of rabies virus from their saliva. Furthermore, rabies virus can still be isolated from the saliva of
these dogs by mouse brain inoculation, eventhough the dogs were found to have rabies neutralizing antibody.

While reviewing the literature, Gribencha (1976) concluded that there was evidence that various forms of rabies virus infection occurred under natural conditions, and that further research on abortive and symptomless infections would be desirable.

Afsher and Bahmanyar (1978) reported the results of the surveys using the serum neutralization tests, in countries where natural rabies occurs. From 2.0 to 18.3 per cent of serum samples from apparently healthy dogs without a clinical history of disease had antibodies, suggesting previous exposure to rabies or antigenically related virus. These dogs may have been asymptomatic or recovered clinical cases of rabies in nature.

Summary of the results of the serological surveys on the occurrence of rabies virus antibodies in dogs.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of serum samples</th>
<th>Numbers in which antibodies present</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionescu (1944)</td>
<td>29</td>
<td>2</td>
<td>6.8</td>
</tr>
<tr>
<td>Serie &amp; Andral (1963)</td>
<td>N.R.</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Bell et al. (1972)</td>
<td>405</td>
<td>57</td>
<td>13.9</td>
</tr>
<tr>
<td>Afshar et al. (1972)</td>
<td>125</td>
<td>4</td>
<td>3.2</td>
</tr>
<tr>
<td>Nanavati (1973)</td>
<td>100</td>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td>Yasmuth et al. (1974)</td>
<td>49</td>
<td>9</td>
<td>18.3</td>
</tr>
<tr>
<td>Farro et al. (1974)</td>
<td>102</td>
<td>5</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Fakadu and Baer (1980) reported that two dogs inoculated with a strain of rabies virus from Ethiopia, showed typical signs of rabies eight days after inoculation. After three or four days with a deterioration in physical condition, both animals began to recover. Both animals recovered completely, although one than died of pneumonia. An increase in serum neutralizing antibody and in CSF neutralizing antibody was noted. Such concentrations have been noted only in persons and animals that recovered from rabies.

A dog inoculated with a rabies virus isolate from the saliva of an apparently Ethiopian healthy dog was monitored for more than nine months. Saliva and serum samples were collected three times a week and cerebrospinal fluid weekly. Saliva samples collected on days 42 and 169 after the dogs recovery produced fatal infections in mice inoculated intracerebrally (Fakadu et al., 1981).
(v) RABIES IN DOMESTIC ANIMALS
RABIES IN DOMESTIC ANIMALS

Henderson (1942) recorded an outbreak of rabies in sheep in India in which about 1300 animals were exposed.

Ramanarayanan (1942) reported a strange case in a dog which was clinically normal and showed frenzy once, was put to sleep after observing for 12 days was positive for rabies.

Cases of rabies in a she-buffalo were recorded by Reddy (1953) and Kishan Rao (1953).

Dogra (1950) observed that reliable statistics are not available regarding the incidence of the disease amongst canine population of rural and urban India.

Bhatia (1951) observed temperature rise and glycosuria in rabies fixed virus infections. (Bhatia and Mehta, 1951).

Azizuddin and Ganapathy (1953) reported glycosuria in five cases examined. They observed that glycosuria as a feature in cases of rabies in cattle cannot be brushed aside, and that examination of urine from suspected cases of rabies will greatly help in antemortem diagnosis of cattle, where symptoms are atypical.

Kehouk et al. (1961) observed that the blood of sheep inoculated with fixed rabies virus showed increases in protein, glucose, nitrogen and cholesterol content in the terminal stage of the disease. The chloride and fibrinogen content was not altered. Anaemia with progressive
thrombocytosis was observed. Electrophoresis revealed an increase in beta 1 and beta 2 globulins.

Kauker (1961) could not find Negribodies nor rabies virus in two calves at postmortem born of rabid cow. Despite the negative evidence he suggested that such animals must remain suspect.

Cholesterol transfer of rabies antibodies was demonstrated in eight of 12 calves born of vaccinated dams even when last vaccination had taken place 10 to 13 months before parturition. The antibodies persisted for 12 to 14 weeks after birth. Calves with cholesterol antibodies did not respond to rabies vaccination but those without antibodies did, as shown by a marked increase in neutralization antibodies which persisted for about six months (Williams, 1961).

Schneider and McGroarty (1933) recorded oral transmission of rabies in a lamb which was nursed for four days by its experimentally infected dam. Both the sheep and its lamb developed rabies and died six and 14 days, respectively, after maternal infection. The fixed strain of virus was recovered from the brains of both animals.

Ziolo (1961) observed lower levels of cholesterol and vitamin C in adrenal cortex of sheep infected with the fixed virus (Pasteur strain) than in healthy animals. In the infected sheep the fall in cholesterol values was most marked in zona fasciculata though the deeper parts of the zona glomerulosa and reticularis were also affected.
The vitamin C content was low in zona fasiculata and reticularis but not in zona granulosa. Staining with Sudan III and IV showed no appreciable difference between the cortex of the infected and healthy sheep.

Ewes, three to five months old were injected intracerebrally with fixed rabies virus for vaccine production. The ewes were bled out six to seven days later and their foetuses (20) were removed. Brain and spinal cord from these foetuses and from five lambs born before bleeding were examined by animal inoculation, histologically, and by fluorescent antibody test. No rabies virus could be detected in the foetuses or lambs, and it was concluded that virus did not cross placenta (Roslkowski et al., 1972).

Wachendorfer (1962) reported that in West Germany the incidence of rabies is now higher in cats than in dogs. Seasonal variation follows the pattern of infection in foxes. It was not possible to demonstrate latent infection in any of the 395 wild mice caught in endemic areas. In a part of the country, Hesse, cats were the main source of human infection but were of less importance as source of infection for domestic animals.

Characteristically, incubation periods of rabies are extremely variable with reliable periods of more than seven months in a naturally infected bat (Moore and Raymond, 1970), 611 days in an experimentally infected cow (Abelseth and Lawson, 1972), minimum periods of more than four months in
seven of 30 quarantine dogs (Hole, 1969), and as long as 120 days in experimentally infected mice (Baer and Cleary, 1972).

Vaughn et al. (1963) inoculated 86 cats intramuscularly with rabies virus. Twenty six developed fatal rabies. Virus was recovered from brain and identified by the serum virus neutralization test. Rabies virus was also isolated from salivary glands of 23 of 26 cats. Negri bodies were identified in 21 including three with negative salivary glands. The incubation period was nine - 51 days (average-18) while the period from first symptoms to death was one-eight days (average-five). Three cats with negative salivary glands had dumb rabies and these three were the only ones found not to excrete virus. Initiation of virus secretion ranged from day one before the onset of symptoms (five cats) to three days after onset (two cats) and it continued until cat died. They conclude that a true exposure to rabies would not have occurred for a person in direct contact with any of the virus excreting cats before the day preceding the appearance of clinical illness.

The period of salivary excretion of rabies virus in relation to the onset of illness was studied in 54 rabid dogs. Of these 26 excreted virus in the saliva. Excretion began between three days before onset of illness to two days after onset (Vaughn et al., 1965).
Beer and Olson (1972) reported recovery from rabies in pigs. Six pigs were bitten by a rabid skunk. After 30 days of bite, two pigs developed symptoms of central nervous system and a paralysis of forelimbs and progressed backwards. After two months other pig developed similar signs. After 10 days pigs appeared normal and sera were tested for serum neutralization antibodies. The titres were: 1:95; 1:160; 1:230 and 1:480. They all were slaughtered after one month of the test but their brains were not examined.

Martell et al. (1973) recorded a first case of transplacental transmission of rabies in cattle. A five month pregnant cow was bitten by a rabid dog and developed rabies after 21 days. Rabies virus was isolated from the tissues of the cow as well as from the fetal calf. The titre of the virus in brain of cow was $10^{-4.8}$ and in calf brain it was $10^{-4.2}$.

Apparent reactivation of rabies virus occurred in a cat that suffered paresis and partial paralysis in the hind legs after experimental infection with low passage virus of bat origin. About 10 weeks after onset of illness the neutralizing antibody in serum and (CSF) cerebrospinal fluid were 1920 and 256 respectively, and the disability seemed stabilized. Subsequently, the serum and CSF dropped as low as 1024 and 140, but the decrease was followed by gradual increase in both titres to maximum of 8960 and 1120 respectively. Because of the steadily deteriorating
condition the cat was killed 126 weeks after inoculation. Postmortem studies were not reported (Bell, 1975).

Instances of infections from dogs due to other means such an indirect exposure via fomites are so rare as to be negligible (Tierkel, 1975).

Verma et al. (1976) analysed the data for one year in South Delhi area where 68 clinically diagnosed cases of rabies were recorded. Out of the 32 brains examined, Negri bodies were demonstrated in 23 cases, and in three animals the diagnosis was confirmed by biological test. The incidence of rabies seems to be more in puppies in the age group of zero-three months. The sex and season has no appreciable effect on the occurrence of the disease. In one dog, the infection could not be diagnosed clinically even after 20 days of close observation, but when put to sleep after 21 days, Negri bodies were seen in impression smears of the brain.

In Meghalaya, due to preventive measures like eradication of stray dogs and registration and vaccination of pet dogs and issue of ration card, the incidence has gone down to 11 per cent and 13 per cent in the year 1975-76 as compared to that of 1973-74. The population of stray dogs almost being nil, the disease is now transmitted mostly by wild animals (Megha Singh, 1976).

Minor (1977) observed clinical signs in 19 dogs until death in Kenya. He concluded that dumb form is more frequent
and intense conjunctival congestion was an important
diagnostic feature.

It is evident that during the pathogenesis of rabies
virus infection, a viraemia may occur, and it is therefore
possible that the virus crosses the placenta and reaches
the foetus during pregnancy (Afshar, 1979). Presence of
rabies virus was recorded in foetuses of pregnant rabid
dogs (Konradi, 1905). However, Johnson (1979) reported
that there is no evidence to indicate that virus will be
present in the blood or blood forming organs of rabid animals.

Afshar (1979) confined domestic and wild carnivores in
cages designed to exclude bats and arthropods and left them
in a bat cave. Dogs, cats, raccoons, and skunk failed to
develop rabies after 30 days of confinement.

Between 1944 and 1977 the distribution of confirmed
cases of rabies in farm livestock in Nigeria were 15 in
cattle, eight in goats, four each in sheep, pigs and horses
and two in donkeys respectively. The least cared for pastoral
sheep dogs as well as stray dogs have so far been the sole
propagating animal species of rabies to farm livestock
although wild fauna have been suspected. The fact that cases
are sporadic and lower incidence of livestock rabies is
generally reported than the number that actually occurs
makes the formulation of a definite control measures
difficult. The need to educate nomadic herdsman to be able
to recognise rabies in their farm livestock is indicated by
seemingly increasing cases in recent times, so as to minimise
public health hazard (Okoh, 1981).
(v1) RABIES IN WILD LIFE
AND BIRDS
Rabies in Wildlife and Birds:

The natural history of rabies indicates that the permanent hosts of the virus are found in the families Mustelidae and viverridae. Epidemics of rabies in domestic dogs, foxes, coyotes, jackels, and wolves are the result of the development of large populations of such animals in regions where rabies is present as an inapparent infection in other animals (Johnson, 1965).

Rabies was present in Europe in wolves in France as early as 1271 (Sikes, 1970).

Epizootics of fox rabies in Massachusetts in first decade of nineteenth century was reported by Thacker (1912). Fox rabies epizootics occurred in Albana in 1890 (Wilkinson, 1894).

Winkler (1975) stated that at least seven major epizootics of fox rabies were reported in Europe between 1803 and 1925, followed closely by reports of human beings attributed to fox exposure. In one area of France, between 1851 and 1857, 770 human rabies deaths were attributed to the following animal exposures; dog (707), wolves (38), cat (23), fox (1), cow (1).


Greval (1932) reported cases of rabies occurring from mongoose bite. In a study from 1922 to 1931,
he described 32 cases of mongoose bites out of which two mongoose were negative. He discussed the possibility of rabies coming into domestic carnivora from wild carnivora and concluded that mongoose bite should be treated as serious bite.

Greval and Nicholas (1941) have advised antirabic treatment for tortoise bite. Same authors have drawn the attention to bats having proved harbouring rabies virus.

Pandit (1951) quoted two instances of proved rabies in a tiger, one in 1943 and the other in 1950. He discussed the possibility of rabies emitting into domestic carnivora from wild carnivora.

Almejew (1959) described rabies in camel.

Dunscombe (1960) stated that 11 cases in camel were recorded in Sudan between 1944-53. Silberstein (1959) recorded 29 cases in Israel, and Chevrier (1959) recorded 14 cases in Morocco.

Villa et al. (1959) demonstrated rabies virus in two foxes by inoculation and neutralization tests in mice and guinea pigs in Argentina out of 42 foxes and six weasels.

Snyman (1937) stated that in South Africa the yellow mongoose is regarded as the chief wild life vector.

Remlinger and Bailly (1939) fed dog ticks (Rhipicephalus sanguineous) on two inoculated dogs and later were able to demonstrate rabies virus in ticks.
They did not determine whether or not these ticks could transmit the disease naturally.

Rabies virus could not be isolated from the brains and salivary glands of 1913 rats and 220 shrews trapped or found dead in Lagos in 1959 (Boulger and Hardy, 1960).

The rabies virus has been isolated from brain, salivary glands, kidneys and lactating mammary glands of a naturally infected skunk (Johnson, 1959).

Mao Millan and Boulger (1960) found that in Nigeria, ground squirrel is very common and is widely hunted for food with the aid of dogs. It is suggested that hunters' dogs may infect the ground squirrel and that transmission of the infection from this source to man has taken place in the past. Because of a general fear, as per the belief in parts of Northern Nigeria that a fatal madness follows the bite of the ground squirrel in one to six months of the bite of the ground squirrel, its danger to man is now more potential than actual, but the animal must be considered a source of rabies infection if a rodent host of rabies in West Africa is postulated.

Rhodes and Van Rooyen (1962) stated that fox is the chief vector in North America and Germany. Virus is excreted in saliva for long periods but symptomless carriers have not been recognised. The wolf is the common vector in Central Europe, Russia, Iran and India, Jackels are important sources of infection in North Africa and
Middle East, skunk in North America, mongoose in Puerto Rico and South Africa. Other species are badgers, coyotes, muskrats, opposums, raccoons, rats, squirrels and weasels. They further stated that relative importance of wild life and dogs as reservoirs of infection is difficult to assess, in view of the large number of dogs.

Pitzschke (1963) observed Negribodies in the brains of 20 out of 13,683 roe deer intended for human consumption. Eight cases were confirmed by mouse inoculation.

Burnet (1960) stated that in South Africa the great reservoir of infection are common yellow mongoose. There is no evidence of subclinical infection of rabies in the mongoose and developed infection is always fatal.

Certain species of carnivora susceptible to infection in bat caves by non bite route may spread rabies to other species by bite route (Constantine, 1966a). Intramuscular inoculation of rabies virus from Mexican free tailed bats in bats, dogs, cats, foxes, coyotes, ring tails, raccoons, striped skunks and opposums caused deaths in all species except in cats and opposums, suggesting that free tailed bats may transmit rabies by bite among themselves and to certain species of carnivora (Constantine, 1966b).

Freeman and Wilcox (1973) and Hickman and Hutcheson (1973) observed rabies in raccoon kittens which were kept as family pets, and Cole et al. (1973) observed rabies in a bobcat. These reports indicate that wild animals even
if they are very young are potentially dangerous, and that wild animals when caught should not be handled by untrained personnel.

Abdussalam and Botton (1974) while reviewing the global situation reported that in Rhodesia after a steady decrease since 1966, there was a sharp increase in rabies cases due to sharp rise in the incidence in jackals and mongoose. In Africa wild life rabies is generally on increase. In Canada wild life rabies covers the whole country. A wild life survey carried out in Shri Lanka showed little evidence of the disease, although the serum of one mongoose out of 150 animals examined showed a neutralizing titre of 1/5, virus could not be isolated.

Bell and Moore (1975) reported apparent reactivation of rabies in a cat that suffered paresis and partial paralysis in the hind legs after experimental infection with a low passage virus of bat origin.

McDiarmid (1975) observed that by 1960 raccoons were recognised as potent new hosts for the virus and that despite the occurrence of the disease in a wide range of wild life, the main orders involved are Carnivora and Chiroptera. In Europe, the red fox is the main transmitter of rabies, and from Poland during world War II, the disease was spread to entire Europe. He further observed that if ever a solution is found to the problem of wild life rabies,
it will be probably come from the work of ecologists rather than from the veterinary or medical profession.

In 1975, wild life accounted for 84 per cent of rabies cases in United States. Skunks were predominant with 46 per cent, next in order were bats 19%, foxes 10%, raccoons 7%, cattle 6%, dogs 5% and cats 5%. World wide, the animals most commonly found positive for rabies are dogs and vampire bats in Latin America, foxes in Europe, Stray dogs in Asia, dogs, jackals and mongooses in Africa, foxes and skunks in Canada. Few countries were free from rabies, most were islands surrounded by protective ocean barriers (MMWR, 1976).

Shah and Jaswal (1976) reported an episode of an attack of rabid wolf. They reported that a dog that ate the carcass of a pig which had died of rabies itself developed rabies 12 days later. This raises the possibility of infection by oral route.

Toma and Andral (1977) stated that main vector is the fox, 68-85 per cent of rabid animals in European countries are foxes. They concluded that the intensive measures necessary for control of fox populations, and thus for control of the virus, are unlikely to be constantly applied over wide areas, which means that enzootic rabies will continue to exist, with successive waves every three to seven years.

Baer (1975) stated that for the control of wildlife rabies, specially in foxes and wolves, oral vaccination was
started in 1970. The (ERA) Embryo: Re-adopted vaccine used in this study was grown on BHK-21 cell lines and had MICLD-50 $10^{-6.5}$. A very high titre was required.

Vaughn (1975) stated that the disease in cats most often occur as "spillover" infections from underlying foci in domestic animals and wild life species, and there is no evidence to suggest that cat rabies is a problem in areas of the world where the disease has been controlled effectively in other species of animals.

Wachendorffer et al. (1978) reported that during 1967-72, samples of serum or coagulated blood were obtained from 483 wild animals of 17 species of carnivora, Artiodactyla, Lagomorpha, Insectivora and Rodentia, 21 feral cats and a feral dog in rabies infected regions of Bavaria, Austria, Hessen and Czechoslovakia. None of the animals were suspected of rabies and no neutralizing antibodies against rabies virus could be demonstrated.

Matouch (1978) used mice inoculation to estimate quantitatively the amount of rabies virus present in various parts of the central nervous system and the salivary glands of 21 naturally infected foxes. The highest titres were found in the salivary glands, with decreasing amounts in the hippocampus, the olfactory lobe, spinal cord, cortex, medulla, oblongata, and cerebellum.

Kahl et al. (1978) carried out a survey of all costs involved in wild life rabies and its control. They found
that administrative costs at the level of the State Government and the provinces are considered relatively constant, whereas all other cost components appear to depend largely on the local epidemiological conditions in countries and communities.

Rao et al. (1980) recorded three cases of rabies in lions in a zoo. Eosinophilic cytoplasmic inclusion bodies similar to Negribodies were found in one brain. It is thought that the disease may have originated in mongoose.

Qdergaard and Krogsrud (1981) reported for the first time rabies in 12 arctic foxes, three reindeer and one ringed seal, on Spitsbergen island of Svalbard Islands. These islands are located between Arctic ocean and Barents sea, and most of the year islands are surrounded by ice.

In Iowa State of United States of America, it was seen that the number of cases of cat bite is on increase during the period 1977-1981. Reports of documented rabies in animals have doubled in USA in the last three years. In 1981, for the first time, the number of rabid cats out numbered the number of rabid dogs, by approximately 20%. No cases of human rabies occured in Iowa in 1981 (Sharrar et al., 1982).

In United States, 5,150 confirmed cases of rabies were reported for 1979, the largest number since 1956. Out of these 88% (4,509) occurred in wild animals, 12% (636) in domestic animals and 5 humans. Canada recorded 1,655 cases of rabies, 1,231 (74%) in wild animals and 424 (26%) in
domestic animals. No human cases were reported. In Europe, Finland, Norway, Sweden and the United Kingdom continued to be rabies free. Bulgaria and Portugal reported no cases for 1979. One case was reported by the Netherlands in a dog imported from India, and one case was reported in Spain in a cat imported from North Africa. No data was available from Romania, and other 133 countries reported rabies (GDC-MMWR, 1981).

Rabies in birds:

Remingler and Bailly (1929, 1930) reported the recovery of domestic fowls from rabies virus infection following clinical disease.

Asymptomatic rabies in two eagle owls has been reported by Von Lote (1940).

Schneider and Eissner (1966) inoculated a week old 26 cockrels intracerebrally with street virus that had been isolated from a fox and passaged in mice. Eleven developed after an incubation period of 20-37 days symptoms of encephalitis, with loud, excited cheeping and fluttering followed by drowsiness and depression. Two others developed at 144 and 177 days a staggering gait followed by apathy and torticollis, and died three days later. Rabies virus was isolated only from the brain of one moribund bird killed 31 days after infection. Another bird with encephalitis symptoms killed at 31 days was positive to
the fluorescent antibody test of the brain as were two birds that died at 37 and 41 days and four of six killed at 60 days. All birds that died or were killed after this time were negative. In a second experiment of 66 chicks infected intracerebrally, one or two were killed daily up to the 26th day after infection and then at irregular intervals up to 109th day. Histological examination revealed slight glial activation at the 10th day after infection, developing into acute, non purulent inflammation of grey matter at 30-50 days. Virus was isolated regularly from the brain from the sixth to 15 days after infection (the highest titre was $10^{3.5}$) and again on 33 day. The FA antibody test was positive from the sixth to 45th day. No virus was isolated from saliva and cloacal contents nor from any organs, other than brain. It was concluded that poultry need not be included in police measures against rabies.

Schneider and Burtscher (1967) reported clinical disease and subsequent recovery from rabies in fowls by using street and fixed virus strains and inoculating intracerebrally. They concluded that proliferation of rabies virus in the brain leads to a rapid production of CNS-bound antibodies which stops the spread of virus within the brain, and hence recovery from disease in some hosts. They also presumed that in most of the cases this antigen-antibody reaction could cause further histopathological changes leading to death.
Gough and Jorgenson (1976) carried out a serological survey on 343 birds representing six orders and 22 species. They found rabies neutralizing antibodies in 23 samples, with predatory birds (owls, eagles and hawks) representing the highest rate (23 per cent) and non predatory birds (starlings, crows and ravens) the lowest rate of positive samples. They concluded that the high proportion of positive samples recorded for the birds of prey is an indication of previous exposure to rabies, possibly mediated by their eating habits in nature.
Rabies in Laboratory Animals
Rabies in Laboratory Animals

Remlinger (1908) reported successful transmission of rabies to mice and rats using a fixed strain of virus given orally.

Webster (1937) while conducting epidemiologic and immunologic experiments with rabies noted that some mice remained healthy and survived rabies virus infection, but when several months later the brains of the recovered mice were tested for rabies virus or characteristic lesions, they were found to be positive.

Remlinger and Bailly (1938) described airborne transmission of rabies in laboratory rodents exposed to mechanically generated 'mists' of rabies virus suspensions.

Prakash Rao (1942) recorded cases occurring in dogs due to fight and subsequent bites by bandicoots. He stated that the Director of Pasteur Institute, Coonoor, Southern India has written to him that mysterious deaths in livestock were reported from nearby areas and one of the rat sent for examination gave definite evidence of rabies. Still there were no recorded evidence of rats being carrier of the disease. The brains of the bandicoots sent from the area also did not reveal presence of rabies virus.

Jacotot and Nguyen-Dinh-Lam (1947) recorded non fatal rabies in vaccinated and challenged guinea pigs.

Schneider et al. (1957) succeeded in transmitting a strain of virus to unweaned hamsters by inoculating their lactating mothers.
The susceptibility of rats to oral infection has been reported by Shen and Shumneikina (1959).

Soave et al. (1961) reported that guinea pig inoculated intramuscularly with rabies street virus survived for five months, but developed paralysis in the inoculated limb and died of rabies 13 days after initiation of a course of ACTH inoculated subcutaneously every 48 hours in ten unit doses.

Soave (1962) further reported that 12 out of 23 guinea pigs remained healthy up to seven and half months after the intramuscular inoculation of rabies virus. The administration of adrenocorticotropic (ACTH) hormone to the surviving guinea pigs reactivated the infection causing disease and death. It was concluded that latent, rabies infection could lead to death under stress conditions.

Colusi (1961) observed that in 10 mice given a daily increasing dose of adrenocorticotropic hormone (ACTH) subcutaneously (4 doses of 0.2 to 0.5 i.u.) from the time of intracerebral injection of street rabies virus, symptoms first appeared a day earlier than in 10 mice not given ACTH, but the interval before death prolonged by several days.

Removal of lymph nodes draining the sites of intramuscular injection of street virus did not lower the resistance of rats to rabies, it delayed the appearance of virus neutralizing antibodies by 10 days (Gorshunova, 1962).

Soave (1964) observed that a guinea pig inoculated intramuscularly with rabies virus survived more than eight
months but developed clinical signs and died from rabies after being subjected to the stress of overcrowding with other guinea pigs that had also been inoculated with the virus.

Robertson and Beauragard (1964) exposed 69 hamsters from 18 to 33 days old to rabies positive suspensions by rectal instillation. None of the animals became infected during the 60 day observation period.

Rabies virus was recovered from blood and brain after eight and 50 hours respectively after rectal infection (Reagan and Bruackner, 1953).

Bell (1964) in a well designed study observed that survival of mice after clinical rabies was found to be a common, reproducible and expected occurrence.

Soave (1964) reported that in natural conditions of stress such as crowding might reactivate rabies virus infection in guinea pigs inoculated with rabies virus several months prior to the application of the environmental challenge.

Veeraraghavan (1964) examined 330 bandicoots and found bodies resembling Negribodies in 36 specimens. The pooled brain material of these 35 bandicoots when inoculated intracranially in mice did not develop rabies. However, bandicoots were susceptible to experimental rabies infection and average incubation period was over 52 days.
Soave (1966) recorded the development of rabies in 16 and eight per cent, respectively, of mice with mouth lesions or intact mucosa, after ingestion of rabies infected mouse brain.

Phungsab et al. (1967) recorded the occurrence of rabies virus antigen in the brain tissues of 15 per cent of 221 healthy rats trapped in Thailand.

Smith et al. (1968) isolated rabies virus from the brains of 37 (3.6 per cent) of 1037 trapped rodents and other small mammals in Thailand. The highest positive rates (7.9 per cent) was found among Bandicota indica.

Fischman and Ward (1968) succeeded in transmitting rabies by feeding the virus to mice, hamsters, guinea pigs and rabbits using a stomach tube and thus bypassing oral cavity. They concluded that for the establishment of such infection large doses of virus were required and that younger animals were more susceptible. They further reported that adult mice readily developed rabies and died after eating infected infants.

In a detailed comparative study on rabies infection in mice it was shown that the survival rate is related to the route of infection, with the intracranial route having the lowest and the intraperitoneal the highest rate of survival. Of 530 mice infected intraperitoneally with rabies virus originally isolated from bats, 145 (27 per cent) developed
no apparent illness during 30 days of observation (Lodmell et al., 1969).

Enright (1970a) observed that corticosteroid treatment of mice, started within 24 hours after rabies virus inoculation, increased mortality up to 20 per cent above that in untreated mice. In contrast, steroid treatment started 48, 72, 96 hours after rabies virus inoculation did not significantly increase mortality. Upto 50 per cent higher mortality occurred in the corticosteroid treated mice than in the controls after small doses of rabies virus were injected. In addition to consistently higher mortality, corticosteroid used in conjunction with rabies virus resulted in two changes in the mortality pattern, shortening of the mean incubation period of rabies in mice receiving LD-50 dose and an aggravation of sublethal infections causing deaths with abnormally or comparatively long incubation periods in mice that otherwise might have survived. These two actions increase the variability in time of onset and time of death, with an increase in early as well as in late (delayed) onset times.

Correa-Giron et al. (1970) showed that mice developed rabies with varying mortality rates following the oral administration of various strains of rabies virus, some of which originated from the rabid brains of humans and bovines. The virus was recovered from the buccal mucosa upto nine days after administration indicating the local proliferation of virus. In addition, the virus was recovered from the
brain, tongue, lung, stomach, salivary gland, oesophagus-trachea, heart, mesentery, kidney, intestine and spleen but not the liver. The virus was demonstrated in all these tissues by fluorescent antibody technique. They recovered the virus from the stomach at six hours after ingestion, and to seven days thereafter, suggesting resistance of the virus to the effect of gastric secretion, and its replication in this organ.

Mortality in rats, hamsters and guinea pigs inoculated with bat or vaccine strains of rabies virus was usually slightly higher in corticoosteroid treated groups. Prolonged treatment of dogs with high doses of prednisolone after low egg passage (LEP) rabies vaccination did not induce signs of rabies nor prevent production of measurable amounts of neutralizing antiserum to rabies virus (Enright et al., 1970b).

Sodja et al. (1971) isolated some strains of rabies like viruses in rodents in Czechoslovakia. There were 121 strains of virus isolated from 928 small wild animals. Ten of these strains were identified as rabies virus. These viruses were obtained from four different species Microtus arvalis, Mus musculus, Apodemus flavicollis and Clethrionomys glareolus. The problems of identification were low titre, weak or atypical fluorescent antibody staining and low immunogenicity.

Winkler et al. (1972) stated that the ease with which wild rodents can be experimentally infected with rabies
and the potential which some rodents have for transmitting rabies, are not supported by surveillance or epidemiological data from the field. It would appear that other factors may operate in nature to prevent rodents from serving as effective rabies reservoirs. One possible explanation is that rodents bitten by rabid carnivorous may seldom survive long enough to succumb to rabies infection.

Mathew and DeSouza (1973) reported that out of the 345 bandicoots collected from Madras city, seven bandicoots showed bodies resembling Negribodies, which on mouse inoculation were negative for rabies. They found incubation period in experimental intracerebral inoculation to range from 7 to 32 days with an average of 12.4 days. Experimentally infected bandicoots, sacrificed at the height of hyperexcitement stage, were found negative for rabies when their brain, salivary gland, blood and subscapular fat were examined.

Fischman and Strandberg (1973) studied inapparent rabies virus infection in mice. They reported 50 per cent survival rate using a fixed virus strain at $10^{-1}$ dilution intraperitoneally. The survivors and those that were infected with the $10^{-2}$ dilution of the virus were found to be immune when challenged two weeks later with a large dose of rabies virus inoculated in their foot pads. They failed to produce rabies in weanling mice after intracerebral inoculation. They noticed that as neutralizing antibody
developed in the brain tissue from four days after inoculation, virus growth in the brain diminished and had completely disappeared by the seventh day after infection.

Gribencha and Salimov (1974) reported that only 213 (50 per cent) of 427 mice developed disease after intraperitoneal inoculation with a strain of "street" virus originally isolated from a rabid human brain. Of the 213 mice that developed disease, nine (4.2 per cent) recovered and the remainder died. The recovered mice had high titres of serum and brain tissue neutralizing antibodies that were correlated with the severity of the clinical manifestation of the disease. No antibody could be demonstrated in the blood or brain tissues of five mice that remained healthy. Of the 214 mice that survived infection, only 178 died when challenged with a "fixed" strain of virus via the intracerebral routes and the remainder (36) recovered following the development of paralytic rabies. Again, exceptionally high virus neutralizing antibodies were found in the brain tissue and serum of these recovered mice.

Titov (1975) reported rabies carrier state among wild strains of mice in the field and assumed that these could be a source of infection for the carnivores animals that prey on them, thus perpetuating the infection in nature.

Nonfatal rabies was successfully reproduced in rabbits infected intracerebrally with a high pathogenic
strain of street virus isolated from a man who had died after a dog bite and in rats infected intracerebrally with the CVS strain of fixed virus. All the animals were pretreated with a sublethal intraperitoneal dose of live rabies virus. The surviving animals showed residual neurological symptoms (except one rat) in the form of paresis and high titres of virus neutralizing antibody in the brain comparable to the level of serum antibody. Successful reproduction of abortive rabies in rabbits infected intracerebrally with the classical strain of street virus suggests that different forms of rabies infection probably exists in nature (Gribencha, 1975).

Kaplan et al. (1975) reported non fatal rabies in adult mice after intracerebral inoculation of the HEP or the temperature sensitive (ts 2) rabies virus. The authors found that these latently infected mice showed clinical disease when they were given immunosuppressive agents, i.e. cyclophosphamide or antithymocytic serum.

Preble and Youngner (1975) have suggested that the selection of temperature sensitive mutants may play a role in the establishment of maintenance of persistent virus infections.

Perl et al. (1975) observed cytoplasmic inclusions with microstructure compatible with Negribodies in the brains of paralysed mice one and half years after abortive infections.
Tierkel (1975) stated that experimental oral transmission of rabies by feeding infected tissues to laboratory animals and to skunks is yet to be demonstrated in dogs.

Doses of one ml of 10 per cent rabbit brain emulsion containing LD-50 - LD-90 of infective fixed virus were given intramuscularly to 98 guinea pigs in groups of 20 and six hours after inoculation, half the animals received twice daily intramuscularly doses of 100 mg/kg Vitamin C. Thirty-five of the 50 untreated guinea pigs developed fatal rabies but only 17 of the 48 animals treated with Vitamin C died. Controls remained healthy (Banic, 1975).

Gribencha and Ovsyannikova (1976) showed that in the absence of disease in mice and hamsters the intensity of virus multiplication in the brain is relatively less than that found among the fatal cases.

Haldar et al. (1977) studied the resistance of nonspecific immunogen, a trypsinized Mycobacterium phlei preparation in inducing resistance against rabies virus in the rabbit at single multiple inoculation and compared with that of specific immunogen, heat inactivated infected rabbit brain. Treatment of rabbits experimentally infected with rat intramuscular challenge virus strain with nonspecific immunogen was studied and compared with that of commercial five per cent Semple's vaccine. Treatment with nonspecific immunogen showed more than 57 per cent survival.
Nair et al. (1978) reported no evidence of rabies in 500 bandicoots (Bandicota bengalensis) examined. However, these animals were highly susceptible to infection with rabies virus by intracerebral and intramuscular routes and virus was present in the salivary glands. They developed a dumb form of disease.

In three districts of State of Punjab in India, a study was carried out during a five year period (1972-76) of rabies exposure due to animal bites and it was found that about 0.86 to 1.5 per cent of bites were mongoose bites. In a three month intensive study in Amritsar city alone, it was observed that 4.9 per cent bites were from mongoose only (Chugh et al., 1978).
(viii) B A T    R A B I E S
Carini (1911) found Negri bodies in the brain of cattle dying of an undiagnosed disease of epizootic proportion that killed thousands of animals in Santa Catarina, South Brazil. The disease was recognised there by 1908.

Haupt and Rehaag (1921) conducted extensive investigations and the actual transmission of rabies by bats was established during an outbreak of paralytic disease of cattle in Brazil.

Since 1921, there had been recognised epidemics of paralysis in South American cattle. It became more frequent and in 1928, there was a very extensive outbreak which caused the loss of something like 30 per cent of total cattle in Paraguay. In 1930 in Trinidad outbreak occurred by vampire bats (Burnet and White, 1972).

During the period 1931-34 there was an outbreak of paralytic rabies in Brazil. Rabies virus was isolated from naturally infected vampire bats, Desmodus rotundus, and this bat was shown to be able to transmit rabies for a period of five months as a symptomless carrier (Quairoz Lima, 1934).

Pawan (1936) reported first human deaths due to rabies transmitted by bats which occurred in Trinidad in 1929. He also recorded an asymptomatic rabies virus infection of salivary glands in vampire bats. He further showed that bat could transmit rabies by bite and that they sometimes recovered from furious form of the disease and than while asymptomatic, continued to transmit the virus for long periods of time (Pawan, 1948).
Malaga-Alba (1958) stated that vampire bat rabies is the most frequent cause of death in South American native cattle, and estimated the losses during 1956 to be about eighty million dollars.

Veeraraghavan (1955) mentioned that an unidentified bat was purported to have bitten a man who later died of rabies in India, but the bat was never tested for evidence of the disease.

Boulger and Porterfield (1958) isolated rabies like virus from frugivorous bat brain on Lagos Island. The virus is now known as Lagos Bat Virus (LBV).

Munoz (1959) mentioned that paralytic rabies in cattle was first reported in Ecuador in 1947 and vampire bats are thought to be the source of infection.

Sulkin and Greve (1954) reported in 1951, a human rabies death in Texas in which the case history contained a reference to the patient's having been bitten by a bat.

In United States, since 1953 bat rabies is on increase, and by 1967 most of the States had reported bat rabies. While tabulating the data on bat rabies in United States, Sulkin and Allen (1974) observed that bat rabies is on increase, from 0.5 per cent as compared to wild life rabies in 1953 to 13.7 per cent in 1972. Only Alaska and Hawaii have not reported the isolation of rabies virus from bats.
Bell (1959) reported that an insectivorous bat that attacked a man in Western Montana was induced to bite unweaned mice. Subsequently, the bat died and brain and salivary gland suspension were inoculated in other mice. Rabies virus was isolated from all the three groups of mice.

Bell and Moore (1960) isolated rabies virus from brown fat of naturally infected Eptesicus and Myotis bats. In each case virus was present in very low infective concentration. The bats exhibited no apparent signs of rabies at the time of sacrifice.

Sulkin et al. (1959) induced infections with rabies virus in bats Tadarida maximana and Myotis lucifugus by intramuscular route or by injection in the intrascapular brown fat. The virus was recovered from brown fat in 23, the salivary gland in 37 and the brain in 91 of 104 T. maximana and from brown fat in 18, salivary gland in 10 and the brain in 55 of 59 M. lucifugus. It was also recovered from the brown fat in 27 of 44 hamsters. The titres indicated that virus multiplies in brown fat. Since the brown fat yielded rabies virus upto 75 days after infection and was the only site in a few bats, it appears to be a natural reservoir for this agent.

Avery and Tailour (1960) reported a detection of bat rabies in Canada in 1957 when the virus was isolated from E. fuscus a big brown bat taken from Vancouver area in British Columbia.
Sulkin et al. (1957) stated that brown fat has been incriminated as a storage site for rabies virus in bats and it was theorized that because gravid bats are more susceptible to infection than non-gravid animals, activation of virus from a latent state in brown fat may occur during pregnancy.

Sulkin (1962) demonstrated bite transmission of rabies virus from a Myotis showing signs of irritability and aggressiveness to a suckling mouse.

Sulkin et al. (1960) indicated that environmental temperature has a profound effect on the course of experimental rabies. When Mexican free-tailed bats were inoculated with canine rabies virus and placed immediately in temperatures between 5° and 10°C both virus and bat remained inactive during the period of simulated hibernation. When these same animals were exposed to 29°C, however, the virus in "cold storage" began to multiply, reaching detectable levels in about the same length of time of inoculation. This suggests that suppression of virus activity in animals kept at low temperatures was due to reduced metabolic activity of the host.

Goodwin and Greenhall (1961) reported the feeding habits of vampire bats, in order of decreasing importance by preference as those preferred by vampires are, cattle, horses, goats, pigs, poultry, sheep, dogs and man.

It should also be mentioned that bites of mammals by infected vampire bats are mostly associated with feeding,
and the transmission of rabies is incidental (Afshar and Bahmanyar, 1978).

When rabies virus was inoculated in bats it invaded the brown fat where it multiplied and persisted for a long time. Occasionally it was demonstrable in this tissue only. During experimentally induced hibernation there was little or no multiplication but after hibernation the virus multiplied reaching detectable levels in various tissues. Isolation of virus from the brown fat of naturally infected bats and demonstration of its neurotropic properties indicate that brown fat in bats may provide a substrate for maintenance or multiplication of the virus (Sulkin, 1962).

Allen et al. (1964) supported the hypothesis that the interscapular brown adipose tissue of the bat is capable of sustaining rabies infection and so contributing to the ability of bats to act as reservoir of rabies.

Constantine (1962) reported that two persons who entered Frio cave in Texas, U.S.A., a bat cave harbouring large number of bats, subsequently died of rabies without any evidence that they had been bitten by bats. The author explained this to be an aerosal infection.

Bats may be latently infected and serve as carriers excreting virus in saliva for months. More than 20 species of bats have been found infected in North America, and infected insectivorous bats have been found in Yugoslavia and Turkey (Rhodes and Van Rooyan, 1962).
Beaurgard and Stewart (1964) reported in a course of study involving 72 bats from 24 countries of the province of Ontario, (Canada) rabies infection was detected in five big brown bats through mouse inoculation test.

Baer and Bales (1967) found that in addition to brain tissue, rabies virus was demonstrated in submaxillary salivary glands, brown fat, kidney and lung tissues of infected bats. However, dissemination of virus in bats infected by intramuscular and subcutaneous inoculation was less widespread than in animals infected by intracerebral inoculation. Inapparent infection was detected in only one bat in this study.

Smith et al. (1967) reported rabies virus infection in two of the 79 dog faced fruit bats *Cynopterus brachyotis* in Thailand naturally infected.

Constantine (1967) conducted experiments by holding coyotes (*Canis laterans*) and foxes in a cave for 24 to 30 days in cages which precluded contact with the cave environment except air filters through 26 mesh/inch screen.
Elaborate steps were taken to protect them from exposure to bats, carnivorous, arthropods, or other possible source of infection. All 12 of 12 foxes and coyotes died of rabies. He then concluded that it might be possible for some cave dwelling carnivorous to become infected with rabies as a result of exposure to virus aerosols in bat caves.

Baer and Bales (1967) were able to show neutralizing antibodies against rabies virus in serum samples from six out of 52 bats which survived observation period of 181 days after intramuscular inoculation, and no signs of rabies infection was observed.

Winkler (1968) demonstrated the presence of rabies virus in air samples taken inside the bat caves, airborne virus apparently originated from infected nasal secretion of bats.

Moore and Raymond (1970) noted an incubation period of at least 209 days in a naturally infected bat kept under uniform conditions.

In the survey covering years 1963-1967, it was noted that the rabies virus infection rate was highest in migrating bats which winter in United States, presumably in contact with bats of the same species known to be the carriers of rabies virus (Sulkin and Allen, 1974).
Sikes (1970) reported that rabies virus has been isolated from 26 of 39 species of insectivorous bats of the United States. Although six persons have been reported to have died from exposure to rabid bats, the importance of bats in the total ecology of rabies has not been determined.

Sulkin and Allen (1974) stated that bats are more widely distributed throughout the world than any other mammal except man. They are believed to be primarily tropical animals, and are second only to rodents in numbers of living genera and species and are the largest order of mammals in overall abundance.

Winkler (1975) stated that the exact source of the airborne virus in bat caves has not been established but it seems probable that aerosols of infectious saliva are generated as bats emit their high pitched sounds, urine droplets which continuously rain from bat covered ceilings may also contaminate the atmosphere. Salivary glands and urinary tracts have been found to contain virus both in naturally and experimentally infected bats but the amount of virus in the urinary tract appears to be low.

Bigler et al. (1975) while analysing the data of Chiropteran rabies in Florida stated that the examination of 6447 bats between 1954 and 1961 yielded 55 (0.85 per cent) positive animals. However, out of 2293 bats 263 (10.3 per cent) were positive from 1962 to 1973.
The yellow bat, *Lasiurus intermedius floridanus* accounted for 183 (63 per cent) of all cases of Chiropteran rabies. Cases were recorded throughout the year, peak being in the months of July and August.

Nilsson and Nagata (1977) isolated rabies virus from the brain of an insectivorous bat *Molossus obscurus* found semi paralysed in day time. They further reported an isolation of rabies virus from brain, salivary gland and intrascapular gland, heart, lungs and testis of bat *Desmodus rotundus* in Brazil.

Trimarchi and Debbie (1977) examined *Eptesicus fuscus*, the big brown bats and *Myotis lucifungus*, the little brown bats in New York State, for presence of rabies virus and virus neutralizing antibody. Eight of the 278 *E. fuscus* were found to have virus, while 18 of 187 had antibody titres around 1:8. One of the 33 *M. lucifungus* yielded virus, while three of 127 had antibody.

Stouraitis and Salvatierra (1978) identified the presence of rabies virus by immunofluorescence, biological and sero-neutralization tests in apparently normal blood sucking (*Desmodus rotundus*) and insectivorous (*Artibeus planirostris*) bats in Bolivia.
IX. RABIES IN MAN
Rabies in Man:

In man the disease called rabies is a severe inflammation of the brain and spinal cord (encephalomyelitis) associated with invasion of these tissues by rabies virus. It is virtually always fatal (Warrell, 1977).

In man one of the manifestations of the infection - an inability to swallow liquids - has given the name hydrophobia, and since the development of symptoms in man is sufficiently different from that in other animals, hydrophobia is the term used only to describe the disease in human beings (Kaplan, 1977).

Sulman (1950) recorded two cases of abortive hydrophobia, one of which occurred in 1921 and the other in 1948. The second case was in a boy, nine years of age, who developed hydrophobia six weeks after he was bitten by a rabid cat. The diagnosis was based on characteristic symptoms but not on laboratory evidence.

Starr et al. (1952) stated that there have been no proved cases of human recoveries but there is evidence that animals may sometimes recover.

Irons et al. (1957) reported a case of a scientist occasionally working in rabies laboratory and no bite history got contact in bat caves.

Humphrey et al. (1960) reported a similar case in a Mining Engineer who had to go to bat caves due to his occupation, and contacted rabies.
In group studies involving the serological examination of 236 veterinary personnel in U.S.A. there were 15 serum samples with neutralizing antibody activities indicative of previous active stimulus; seven and eight of these serum samples protected two-four of five and five of five mice respectively, against 10C-300 LD-50 doses of CVS fixed virus (Rueggsgger et al., 1961).

In another study, 30 of 200 sera samples from cave explorers in America showed neutralizing activity (Brodsky, personal communication to Doege and Northrop, 1974).

Tierkel (1963) observed that rabies is fatal. No man has recovered from the disease after clinical symptoms have begun. Rabies is more infective to younger than older animals, which is probably also true in man. In 15 year period 1946-1959, more than half the total human rabies deaths in the United States were in children under 15 years of age.

Vaughn et al. (1965) studied the period of salivary excretion of rabies virus in relation to the onset of illness and observed that the recommendation of WHO Expert Committee on Rabies that vaccine should be administered to a person bitten by an animal which showed symptoms of rabies upto ten days later should be reappraised.

A case of a woman in early stages of rabies who apparently transmitted the disease to her nursing baby has been reported (Tierkel, 1963).
Robertson and Beauregard (1964) reported transmission by rectal instillation, Atanasiu (1965) reported transmission by inhalation, and Soave (1966) reported transmission by ingestion.

Winkler (1975) observed that among the abbarant routes of transmission, the airborne one is in many ways the most and potentially important.

Babes (1912) in his "Treatise on Rabies" reported two human fatalities because of hot breath felt to them by rabid wolf on their faces. But the evidence is lacking (Winkler, 1975).

Nocard et al. (1887) reported isolation of rabies virus from milk, and Remlinger and Bailly (1932), found virus in milk on two occasions.

Johnson (1959) isolated virus from lactating mammary glands of spotted skunks.

Schneider (1975) stated that little is known of the excretion of rabies virus through milk, although the lactating mammary gland, if found infected, may represent a potential source of oral infection.

The rabies virus has been isolated from the urine of a child that had rabies (Bhatt et al., 1974).

Martell and Delvalle (1971) observed in one study in Mexico that 40 out of 1000 (4 per cent) apparently healthy cattle selected at random at Mexico City Slaughter House of
Ferrera were shown to be positive for rabies by fluorescent antibody technique and mouse inoculation test.

Warrell (1977) suggested that laboratory animals can be infected by eating food containing the virus but this route has never been confirmed in man. There have been several anecdotal reports including one from Charles Darwin in 1837.

Baer (1975) observed that people often consume meat from rabid cattle, even clinically normal cattle may have infective brains.

Infections of domestic animals that are transmitted to man could obviously be eliminated by the detection, than cure or elimination of all infected animals. Rabies is one of the exception in which there is now no substitute for strict supervision and control of domestic canines through liscensure and rabies immunization (Kilbourne and Smillie, 1969).

Iyer (1925) suggested that it is possible to stamp out the disease by stringent suppressive measures and prevent its reintroduction by adequate quarantine measures as has been done in Great Britain by a Rabies Order of 1899.

Fanner and White (1970) stated that man is irrelawant to the ecology of rabies. To the virus, man constitutes a blind alley, for humans cannot transmit infection. The fact that rabies is lethal for all the species of these animals raises two questions, the first is how have such vulnerable species survived a highly lethal virus that perpetuates
itself by inducing the animal to run around in a mad frenzy biting everything in sight. Is there a more important reservoir for which the virus is relatively harmless? Recent evidence suggests that the bat may be such a reservoir. The virus has been isolated regularly from salivary glands and brown fat pads of symptomless bats, and indeed transmitted to other animals via aerosols created by bat secretions.

The global rabies report of WHO for the year 1958 stated that rabies was present in 52 countries and absent in 23 countries. A total of 947 human rabies deaths were reported from sixty five countries. The greatest toll of human lives was reported from India, the Philippines, Mexico, Thailand and Egypt (WHO, 1960).

Sikes (1970) observed that rabies has a public health as well as agricultural-economic significance. In 1966-67 there were 1,336 human deaths due to rabies reported to world health organisation.

Jhala (1971) stated the futility of saving the patient as the treatment has to be palliative. He observed in 24 consecutive deaths that 21 had no treatment, two had incomplete treatment, and one died before treatment can have any effect. Thus except in four per cent the deaths could have been prevented.

Kundu (1976) reported that in India, every year about three million people receive post-exposure antirabies therapy and 15,000 deaths are due to rabies. The economic
loss is estimated to be 142 million rupees. In India, dogs are mainly responsible for the spread of disease to man and other domestic animals.

A fatal human infection with Mokola virus is recorded by Familusi et al., (1972).

Howard et al. (1972) reported a fatal case in a veterinarian who had handled rabies infected tissues for preparing an experimental lot of rabies vaccine. He had pipetted the aliquots of suspensions and had homogenised infected tissues in a type of blender known to produce aerosols. There was no indication of laboratory accident but the epidemiological evidence suggested possibility of aerosol infection as the patient was suffering from a chronic respiratory condition which might have increased his susceptibility to aerosol infection.

Dickerson and Sharpless (1972) made further observations on this case and reported that patient was serologically negative for the rabies antibody at the onset of this illness, he had received pre-exposure rabies vaccination before 13 years with a booster dose after three months. The serum antibody tests after both vaccinations were negative.

CDC (1974) reported that for the first year on record, no human rabies, either imported or acquired domestically, were reported in United States.
Hattwick et al. (1972) reported a recovery from rabies in a six year old boy. He was bitten on the left thumb by a bat and two days after he completed a 14 day course of Duck Embryo rabies vaccine he developed clinical rabies. Rabies virus was isolated from bat but not from the child. Serum, cerebrospinal fluid and brain tissue antibodies against rabies rose to levels compatible with clinical rabies rather than vaccination. Rabies serum neutralization titres peaked at 1:163000, three months after onset of disease. Recovery from clinical rabies was complete six months after onset. They stated that rabies serum neutralization titre after 14 day course of duck embryo vaccine ordinarily average 1:32, and rarely exceed 1:600 even transiently. They reported nine cases from literature which were supposed to have recovered from clinical rabies.

Winkler et al. (1973) reported a case of a 56 year old man died of rabies who was exposed to a fixed strain of rabies virus, the infection apparently resulted from inhalation of an aerosol generated in a laboratory during the manufacture of vaccine. The victim had received preexposure vaccination against rabies 13 years earlier but had not developed demonstrable serum antibodies.

Mans' best protection against rabies is the immunization of dogs and cats, which should than be liscenced, elimination of stray animals, and avoidance as far as possible of wild animals that may be exhibiting unusual behavioural patterns (Edwards and Smith, 1975).
Porras et al. (1975) reported a case of a 45 year old woman who was bitten by her dog in Argentina where rabies is endemic. The diagnosis was made from blood and cerebrospinal fluid antibody levels. Since she had been given vaccine, the illness could have been severe post vaccinal encephalitis. She recovered after 13 months.

Unfortunately, subsequent attempts to cure rabies with intensive care have failed in USA, Brazil, India and England. Especially in poorer countries, money should be used for prevention, including the emergency treatment of animal bites and immunization rather than for attempts at intensive care (Warrell, 1977).

In 1972-1976, a total of 594 patients with rabies were admitted to hospitals in Shri Lanka. Ninety-three per cent of persons, where case history was available were found to have been bitten by dogs. Out of the 19 persons receiving treatment, only three completed the full course (WHO, 1978).

Shah and Jaswal (1975) stated that in case of humans antibody titer is not a measure of protection against rabies. It is the only easily available measure of one type of response to treatment by antirabic vaccine.

Tierkel (1975) observed that in Asia, nationwide morbidity data are spotty and generally poorly reported. The rabies has continued to be a major communicable disease problem in most Asian countries. The two countries which stand out as having the largest rabies problems are India
and Philippines, both in terms of dog rabies cases and human mortality. Other Asian countries recognizing it as a sizable problem are Thailand, Burma, Pakistan, Ceylon, Indonesia, Bangladesh and Vietnam. In all these countries dogs are identified as the principal vector animal in transmission to man and other animals.

Warrell (1977) observed that in areas of the world where rabies is endemic, veterinary surgeons or other animal handlers, zoologists and cave explorers are particularly likely to be exposed to infection. For reasons which are unknown, rabies is up to seven times commoner in men than women, this also applies to animals. About half the cases of rabies are in children less than 15 years old.

A 55-year-old woman in Maryland was bitten by a rabid bat in May 1976. In June, while being treated with the currently recommended regimen of rabies post-exposure prophylaxis, she developed rabies and died. The regimen included 20 IU/kg of human rabies immunoglobulin (RIG) and 23 doses of Duck embryo vaccine (DEV), a primary series of 21 doses of DEV, with two boosters. Despite the wound care, RIG, and 21 doses of DEV, the patient developed her first symptoms of rabies eight days before her first scheduled booster injections of DEV (Barnhart et al., 1976).

In 1974, 412 human deaths were reported from rabies, most of these cases occurred in Latin America and Asia with the majority in Brazil and India. World wide, the animal
most commonly found positive for rabies are dogs and vampire bats in Latin America, foxes in Europe, stray dogs in Asia, dogs, jackels and mongooses in Africa and foxes and skunks in Canada (WHO, 1976).

Tillotson et al. (1977a) reported a case of rabies in a preimmunised laboratory technician, possibly infected with an attenuated strain of rabies virus by the inhalation route. The patient developed malaise and headache and later on fever, nausea, became lethargic and intermittently delirious. The diagnosis was made on the basis of antibody level rise. Serum antibody titers by tissue culture neutralization after a week of illness were 1:16, after next four days 1:32, and again after 10 days it went up to 1:64,000. One month after illness, the cerebrospinal fluid rabies antibody titer was 1:16,225. The most recent booster after preimmunization was given before about five months of illness, and two weeks after booster the antibody titer was 1:32. The patient survived. After four months his antibody remained unchanged at 1:175,000 since it peaked after one month of his illness (Tillitson et al., 1972b).

The city of Sao Paulo in Brazil has taken on a major project for the state by undertaking the control of rabies. The incidence of rabies in humans decreased from 12 cases in 1972 to no cases in 1976. In 1977, there were four cases and in 1978, three cases. The decrease in human cases paralleled a decrease in the incidence of the disease in dogs, from 573
cases in 1972 to 19 cases in 1976. In 1977, there were 292 cases and in 1978, 209 cases (VPH Notes, 1979).

Human to human transmission of rabies via corneal transplant has been reported from France. The donor of the cornea was a long time resident in Egypt, who was known to have had exposure while in Egypt, to a dog who died. The recipient had developed symptoms 41 days after corneal transplant. Retrospective studies of brain of a donor revealed presence of numerous Negribodies. Rabies was suspected during the course of the recipient's illness and confirmed at autopsy by fluorescent antibody staining, serology, and isolation of the virus from brain tissue. The recipient had no known contact with animals outside his home, and his dog was in good health (Galian et al., 1980).

Thougcharoen et al. (1981) reported a human to human transmission of rabies via corneal transplant in Thailand. Two recipients of corneal transplant from a same donor died of rabies, the first after 22 days and the second after 33 days of transplantation. Retrospective studies on the brain tissue of donor revealed Negribodies in cytoplasm of few nerve cells when stained with hematoxylin-eosin stain. In one recipient, brain material at autopsy was found positive for rabies by fluorescent microscopic examination. In second case the autopsy was not permitted.

Parrish et al. (1959) found bite rates of 194.6 bites per 100,000 persons per year among human males and
88.4 among human females in their study in Pittsburgh. About 62 per cent of those bitten being under 20 years of age. High risk group were school and pre school children, tradesman, routeman and veterinarians. The dog bites reported were 76 per cent on extremities, 16 per cent on the head, face or neck and eight per cent elsewhere on the body. Seventy-one per cent of the dog bites were received between noon and midnight, and one third of the bites were provoked by the person bitten. They concluded that the figure represents only a small percentage of those actually bitten.

Schawabe and Abou Daoud (1961) reported 12 bites per 100,000 per year in Lebanon. They observed a dog bite rate among Christian Lebanese of approximately twice that among Moslems. A grouping of the data by age showed that both Christians and Moslems 10 years of age or younger were bitten with approximately equal frequency. Among persons over 10 years of age, Christians were bitten 2.5 times as frequently as Moslems.

Rhodes and Van Rooyan (1962) stated that in United States, it has been observed that over half the cases are in children under the age of 15 years.

Statistics on reported animal bites compiled for 1966 by the Indiana State Department of Health reveal an overall dog bite rate of approximately 169 per 100,000 per year, and for Marion County, 612 per 100,000 per year. Of these bitten, 80 per cent were below 20 years of age. Of the dog bites
reported, 71 per cent were on the extremities, 17.9 per cent on the head, face or neck and 11.1 per cent elsewhere on the body. The dog bite rate was lowest in December and January rising to a peak in May (Schawabe, 1969).

Cochary and Davies (1960) reported bite rates of atleast 350 per 100,000 per year in Israel. Number of males bitten were higher than females. Eighty-five per cent of the bites were on the extremities, 9.5 per cent on the head, face and neck and 5.5 per cent elsewhere on the body. Forty-two per cent of the bites were provoked by the persons bitten.

Brobst et al. (1959) reported that in 1957, an estimated of 611,500 Americans were bitten by animals, and most of these bites were by dogs.

Shah and Jaswal (1976) reported analysis of 1910 cases attending antirabic treatment at Aurangabad Medical College, for a period of one year. Sixty-nine per cent were from city and 31 per cent were from rural area. But on considering the severity of bite of 478 cases, 17.3 per cent were from urban area and 42.2 per cent from rural area. The incidence of bite was not significantly altered by age, sex or season. About 67 per cent of bite occurred without provocation. Severe bite class III formed 6.3 per cent of the total. Out of the 478 cases advised antirabic treatment, 222 (42.2 per cent) took the complete treatment. Follow up of cases elicited information about 368 cases of which 126 had been bitten by proven rabid animals. In these 126 cases, deaths were
8.7 per cent among incompletely treated and 48.2 per cent among the untreated.

An epidemiological study of dog bites in Jaipur city was conducted by Maheshwari and Sharma (1976) at antirabic clinic of S.M. Hospital. Nearly 70 per cent cases came from urban area. The incidence of dog bite was more in males and school going age group of 5.14 years than in females. Community survey revealed that there was massive under reporting of cases of dog bite. Less than 50 per cent cases came for treatment. There was a high incidence of defaulter rate, and people took irregular or incomplete treatment.

Raichowdhuri et al. (1973) reported a fatal case of rabies in man. The individual had never come in contact with any rabid animal in last three years. However, he had two pet dogs aged eight and two years respectively. These dogs were observed, their saliva was tested for presence of rabies. One dog died after eight months of observation. Cortisone administration to reactivate the rabies virus was given. Rabies virus could not be detected from two dogs on any occasions. The authors concluded that these dogs were probably not responsible for causing hydrophobia.

Thomas (1973) reported that at Central Research Institute, Kasauli (H.P., India) a total of 57,675 patients attended the Institute for antirabic treatment. Out of these, 38,096 took full treatment, and 16,529 were partially
treated (23.66 per cent). There were 17 deaths among treated group out of which seven were fully treated.

Addo (1977) outlined the circumstances that could be considered provoked attacks and where human exposure to rabies likely. These situations include;

(i) Playing roughly with pets,
(ii) Stepping on their toes and tail,
(iii) Attempting to help injured animal in pain,
(iv) handling live but undomesticated animals like squirrels etc.,
(v) handling apparently healthy but unfriendly or unfamiliar domestic animals and pets and,
(vi) playing or interfering with babies of nursing animals.

Unprovoked bites and attacks from animals like cattle, sheep and goats is considered abnormal and such an animal should be regarded as suspect (WHO, 1973).

Addo (1977) explained that the probability that a biting animal was shedding the virus at the time of bite depends on the animal species and its degree of susceptibility to rabies, the local prevalence of rabies in the region and in that animal species, the clinical status of the animal at the time of biting and the animal's history of antirabies vaccination.

Oboegbulem (1979) stated that all the four serotypes of rabies group of viruses are found in Africa.
Being primarily rural communities and less enlightened populations, isolated cases of animal bites and individual exposures are hardly reported. Ignorance, mass illiteracy and lack of informative education account for concepts.

Maetz (1979) reported during the period 1973-1976, 8072 animal bites episodes in Jefferson country, Albana, USA. Eighty-eight per cent were attributed to dogs. Although rabies is no longer a major health problem, injuries to people from animal bites due in part to the size of pet dog population have become of increasing concern. During the four year study, the frequency of animal bites increased consistently in the spring and summer months. Sixty-two per cent of the dog bites were males. Both, males and females, in the age group of zero-nine had a significantly higher rate of bite injuries than other age groups.
REVIEW OF LITERATURE

PART - II

DIAGNOSTIC METHODS

(1) NEGRI BODIES
NEGRI BODIES:

Negri (1903) reported that he had detected microscopically an organism, which he considered the specific etiological agent of rabies. He described within the neuronal cytoplasm, round to oval inclusion bodies which stained most consistently with the methylene blue and eosin method of Mann. They measured 1-27 μm in diameter and frequently contained a central, small dark staining inner body. He further stated that there were privileged sites for encountering this bodies and that the large neurons of the Ammon's horn were particularly favoured.

Gowers (1877) described granules in the neurons in cases of rabies.

Babes (1892) described the encephalatic lesions in rabies. At different level of neuraxis, he had varified a marked hypermia followed by deapedesis and formation of perivascular nodules. This constitute "encephalitis focus" in which various types of mononuclear cells, lymphocytes, monocytes and plasmocytes can be seen. These nodules are found in neuronophagic areas.

Golgi (1894) found that in fixed virus infection, the nerve cells become thinner and the nucleus smaller, with accompanying karyolysis, vacuolisation of cytoplasm, complete disappearance of Nissl's body and hypertrophy, followed by degeneration and neurofibrils.
Intracellular formations in rabies infection were also observed by DiVestea (1894).

VanGehuchten and Nels (1900) have described histologic lesions which they consider specific, at least by their ganglionic localization. These lesions consist of an endothelial cell proliferation arising from the capsule of the spinal or sympathetic nerve ganglia. The normal nerve cells are gradually replaced by clusters of small round cells. The few remaining nerve cells undergo alterations in their structure and in the shape of their cytoplasm and nucleus. These lesions vary in intensity according to the species considered and from one animal to other as well. Significant lesions are found in the dog, but in rabbits and human beings they are of lesser intensity. The fact that they invariably occur led the authors to recommend that they be observed for a rapid diagnosis.

Specific inclusions of rabies infection were discovered independently by Bose (1903a, b) and he published his accounts almost simultaneously with Negri (1903). But the discovery was credited to Negri, and these bodies are known as Negri bodies. However, Volpinc (1904) stated that the Negri bodies were nothing but a reaction of infected cells.

Negri bodies are very small in the neurons of salivary glands and tongue (Manouelian, 1906).

Lentz (1909) first observed the extracellular structures found in nerve cells in the central nervous system of rabbits infected with the fixed virus.
Lentz bodies are similar to Negri bodies in that they are eosinophilic and contain basophilic inner structures, and their exact nature is still unknown although they are thought to be the degenerative nucleus of neurons. Light microscopic observations of fixed virus infection revealed that some degenerative nerve cells were reduced in volume and had intensively eosinophilic cytoplasm. These necrotic cells, some of which had pyknotic nuclei, were found among normal or slightly damaged cells. It therefore seems likely that the Lentz bodies are severely damaged nerve cells themselves (Matsumoto, 1975).

Negri (1909) considered these bodies to be a parasite which invaded the central nervous system and propagated within the neurons. But Remlinger (1903) had indicated that the infective agent was submicroscopic.

Volpino (1906) and Babes (1907) considered the basophilic kernel within the centre of the Negri body to be the etiological agent of rabies. They thought that the red staining mass around the organism was produced by the neuron in response to injury caused by the agent.

Lentz (1909) and D'Amato and Faggella (1910) thought that Negri body did not contain any etiological agent, but instead to represent a degenerative change within the neuron.

Cornwall and Kesava Pai (1910) observed that the largest neurons involved tend to have the most prominent inclusions
and they emphasised that Negri bodies tend to be found in regions of the brain in which there is little inflammatory reaction.

Action and Harvey (1911) noted the morphologic similarities between the Negri body and the nucleolus and postulated that the latter had been extruded from the nucleus into the cytoplasm.

There is a variation in the morphology of the cytoplasmic inclusions. Negri (1903) stressed the presence of a minute, basophilic internal granule within the inclusion.

The differences in the morphology of Negri body depended upon the species of animal infected (Acton and Harvey, 1911). The rabbits tend to form very small, yet multiple Negri bodies (upto 2 \( \mu m \)) in which inner bodies are difficult to see. Guinea pig may produce very prominent inclusions. In cow the Negri bodies are perhaps largest of all.

The dog has a tendency to produce rather large Negri bodies in which there is a prominent internal granule (Iwamori and Yamagiva, 1945). Skunk may produce very prominent inclusions (Moulton, 1954).

These differences appear to be related primarily to the species infected, rather than the clinical course or the viral strain. If a dog specimen is obtained containing large Negri bodies with a prominent inner granule, rabbit inoculation with the specimen will likely produce small, multiple
"rabbit like" inclusions with inconspicuous inner bodies (Acton and Harvey, 1911).

The diagnostic value of Negri bodies was shown by Negri himself. He examined 98 clinically rabid dogs and found inclusions in all of the brains except one as reported by (Babes, 1912b).

Levaditi et al. (1924, 1924a, 1924b, 1924c) put forward the theory that rabies virus was microsporidium which had a definite life cycle with two phases. The first one was a filterate phase in which the agent resembles spores. The spores passed along the nerves from the site of the wound and reached the central nervous system. There they underwent intracellular proliferation to form pansporoblasts or Negri bodies. The pansporoblast which represented the visible phase of the organism contained "plages" each of which was an aggregation of microsporidium spores. Round them the neuron secreted a hyaline substance which formed a capsule, isolating the colony from the rest of the yf cells. The development of pansporoblast depended on the virus and the host. If the virus was very virulent it might destroy the nerve cell before the pansporoblast could form. The absence of Negri bodies in "fixed" virus infections might be explained by the fact that the rate of multiplication of the ultra-microscopic forms is so rapid that the animal dies before the cystic forms are produced.

Good pasture (1925) recognised Negri like bodies containing no internal structure in the brains of rabbits
infected with a street virus and termed them "Lyssa bodies" for practical as well as theoretical reasons. He emphasized that Lyssa bodies were for the most part small. In general, a number of small inclusion bodies were intermingled with typical Negri bodies and small Negri bodies contained fewer inner bodies than large ones. The morphological appearance of inner bodies under the electron microscope is quite similar to that of the cytoplasmic area where virus assembly occurs, contiguous to the edges of the inclusion body. It therefore appears that Negri bodies are formed by the fusion of contiguous small inclusion bodies, and that the presence of the inner body is not essential; the term Negri body may thus be used in a broad sense to include lyssa body (Matsumoto, 1975).

Negri-Luzzani (1913) summarized ten years of experience in the use of Negri body examination for the diagnosis of rabies. Her studies indicated that about 90 per cent of cases of rabies showed Negri bodies. However, confirmatory animal inoculation of the suspected material was not done in all cases.

Thomas and Jackson (1930) reported the results of histologic examination of the hippocampus versus the oculomotor nucleus (as seen in cross section of the mid brain at the level of the posterior quadrigeminal bodies) on a series of 48 proven rabies cases in rabbits. They found that in 25 (51 per cent) cases Negri bodies were more frequent in the mid brain sections than in the hippocampus. In only one case were the Negri bodies more frequent in the hippocampus than in the mid brain. Further more, in 11 cases (22 per cent)
Negri bodies were absent in the hippocampus but present in the mid brain. Reverse situation was true in only one case.

Marinesco and Stroesco (1931) inoculated dogs with streetvirus and killed at daily intervals after onset of illness. They found an increasing percentage of cells containing Negri bodies as signs progressed. No Negri bodies were found 12 hours after the onset of illness. After one day of illness 30 to 40 per cent of cells examined contained Negri bodies, after five days of illness, 80 per cent were positive for Negri bodies. However, there is no indication as to how the inoculations were standardized, nor which anatomical structures were examined.

Szatmari and Salyi (1936) concluded that the distribution of the lesions in rabies was dependent on the location of the wound, but the specific data on which these conclusions were based is not stated.

Horgan and McKinnon (1937) studied 37 brains, mostly of dogs and found that 10 (27 per cent) had Negri bodies in both hippocampus and midbrain, 18 (48.6 per cent) in the hippocampus only, and in no case was the mid brain involved when the hippocampus was negative.

Veeraraghavan (1944) described a protozoan parasite found in rabies infected brains and suggested that this parasite might be the etiological agent in rabies, the spore forms representing the filterable phase. He disagreed with
the plausible theory put forward by Levaditi et al. (Loc. cit.) but accepted that the presence of Negribodies in brain is definite evidence of rabies infection and failure to demonstrate these bodies, while providing strong presumptive evidence against rabies, leaves the diagnosis in doubt and necessitates the issue of an inconclusive report.

Johnson (1942) reported that the Negribody could not be demonstrated in approximately 10 per cent of naturally infected animals and that under certain conditions this percentage might rise to 25.

In rabies neuropathology the distribution of Negribodies is described as being related to the site of the bite (Wolf, 1950).

Wolman and Behar (1952) studied by histochemical methods, the production of Negribodies in mice inoculated intracerebrally with street virus and sacrificed at daily intervals. One day after inoculation tiny basophilic granules were observed in the cytoplasm of the hippocampal neurons. Animals sacrificed three and five days after inoculation showed larger and increasing numbers of inclusion. By the sixth day following inoculation, the inclusions were even more numerous and many contained typical inner bodies, which were Feulgen positive. Yet eight days after inoculation many of the inclusions had lost their inner granules, thus having the appearance of lyssa bodies, and these inclusions were noted to be Feulgen negative. The authors concluded that
there was a progressive reduction in the number of viral particles in the late stages of disease, with a resultant loss of basophilia and Feulgen-positive material within the inclusion.

Occasional appearance of eosinophilic inclusions similar to Negri bodies in the neuronal nuclei of animals infected with Polar Madness - a type of rabies seen in northern Russia, was recorded by Kantorovich (1957). In the late stages of experimental rabies infection, RNA-containing intranuclear inclusions were found by acridine orange staining method (Sokolov and Vanag, 1962).

Crandell (1965) described the presence of intranuclear neuronal inclusions in animals inoculated with brain material from an outbreak of rabies among dogs and foxes in Greenland. The animals also showed typical intracytoplasmic Negri body inclusions.

Frye and Enright (1964) induced rabies in mice by inoculating 0.1 ml of mouse brain suspension of rabies virus (titer 10^{-1.75}) intramuscularly. In blood films stained by Shorr's modified techniques and Klieneberger-Hobel's method about one per cent of erythrocytes contained discrete intracytoplasmic inclusions. The highest incidence of these bodies appeared at the eighth day and most of the mice died on 13 day after inoculation. The specificity of these inclusions has not been established. While they are related to the development of rabies, it has not been demonstrated
conclusively by either fluorescent antibody microscopy or mouse inoculation that they are due to infection with rabies virus.

Fischer (1961) examined dogs, guinea pigs and mice at various intervals after intracerebral infection with fixed rabies virus strains. Pitman-Moore, Novi-Sad, Budapest and Flury. Negribodies were present in the cerebral cortex and the hippocampus in large numbers, and earlier than in other parts of the central nervous system. In dogs and sheep these Negribodies were smaller than those due to street virus, in mice and guinea pigs there was no difference in size.

Tustin and Smit (1962) observed on the basis of histologic examination of 1074 proven cases of rabies from a wide range of animal species, that at least for diagnostic purposes, examination of hippocampus (71 per cent of proved cases) was more frequently positive than examination of other regions of the brain (46 per cent of proved cases).

The number and character of Negribodies were recorded in sections of five parts of the brain of 90 rabid dogs, cattle, cats and meercats. In dogs the cerebellum, and in cattle the medulla oblongata were the sites where negribodies could be demonstrated most readily when they were absent from the hippocampus. In dogs the hippocampus and cerebellum and in cattle the medulla oblongata had the greatest number of Negribodies. The hippocampus in cats and meercats was the region where Negribodies were superior in numbers, size and inner structure. To ensure the greatest possible accuracy in
the histological diagnosis of rabies, the hippocampus and cerebellum should be examined in the dog, the hippocampus and medulla oblongata in cattle and the hippocampus alone in cats and meercats. As a second choice the medulla oblongata is recommended in each of these species, except for the cerebellum in cattle (du Plessis, 1965).

Miyamoto and Matsumoto (1967) suggested that fixed virus injures nerve cells so extensively that the full development of the typical Negri body as recognised by the light microscope cannot occur.

The Negri body which is pathognomonic for rabies is found in the cytoplasm of neurons. There may be several such inclusions present in one cell, and their sizes range from 2 to 12 μ in diameter. These inclusions are well demarcated to some extent upon their situation in the cell, for example, those in the first part of dendrite are usually elongated. The typical Negri body is not a uniform structure. Generally within the inner part of the structure small granules occur which are basophilic whereas the bulk of the inclusion is strongly acidophilic, but the specificity of structures that are completely acidophilic should be regarded with some doubt (Jennings, 1967).

Lee and Becker (1972) examined spinal cord in cases where it was not possible to examine brain due to mutilation or decomposition. In a study of 248 rabies suspected cases, brains and spinal cords were examined by fluorescent antibody
technique and mouse inoculation. There was a hundred per cent correlation between two procedures. It was concluded that where it is not possible to get brain, examination of spinal cord is equally reliable.

Atnasiu (1975) suggested that if the fluorescent antibody technique is not available for diagnosis and Seller's stain is the only means of rapid staining, the animals should not be killed since it is thought that the development of Negribodies is related to the period of illness before death.

Gorshunova et al. (1976) showed the presence of numerous Negribodies in the hippocampus of the brains of mice infected intracerebrally with a recently isolated strain of rabies virus. Bodies with similar staining properties were also visible inside erythrocytes in the cerebral blood vessels. Staining by Bracket method showed that they contained ribonucleic acid.

The validity of the reports suggesting that Lyssa and Negribodies are non specific in the light microscopic diagnosis of rabies was investigated with Seller's impression technique. A substantial proportion of specimen found to be non rabid with fluorescent antibody technique showed structures indistinguishable from Lyssa or Negri bodies. Neither histological examination nor inoculation of animals with non rabid Seller positive material explained the nature of these rabies like structures. The simplicity and reliability of fluorescent antibody technique and the occasional serious complications of prophylactic antirabies measures render Seller's method obsolete (Derakhshan et al., 1978).
(11) ANIMAL INOCULATION AND
HISTOLOGIC DIAGNOSIS
(ii) **ANIMAL INOCULATION AND HISTOLOGIC DIAGNOSIS:**

 Pasteur (1881) presented the first results on the transmission of the disease by the intracerebral inoculation in rabbits. He also reported that all the forms of rabies were due to the same organism, located in the brain and that submeningeal inoculation infallibly transmitted the disease (Pasteur, 1882).

 The histologic diagnosis of rabies evolved in three steps. The first was the histologic studies of the neuraxis in man and animals succumbing to rabies, and the description of specific lesions called Babes nodules (Babes, 1892).

 The second period began with the description of Negri (1903), of intracytoplasmic inclusion bodies, which was used as a practical diagnostic test for rabid animals.

 The third and the last period is characterised by the specific identification of the rabies antigen by specific immunologic techniques, immunofluorescence (Goldwasser and Kissling, 1958), the immunoperoxidase (Atanasiu et al., 1971a) and the ferritin technique by electron microscopy (Atanasiu et al., 1963).

 Negri (1903) described within the neuronal cytoplasm round to oval inclusion bodies which stained most consistently with the methylene blue and eosin method of Mann. Negribodies were also stained by Van Giesen stain (1906).

 Abstracts of (1909) in Indian Veterinary Journal contain a note on the diagnosis of rabies taken from German Journal.
of Hygiene. A Report from Pasteur Institute of Milan stated that 179 samples were examined by Negri's method using Mann's staining technique. The editorial comments stated that about a year ago such specimens were seen at Raymond Research Institute, Calcutta.

Pasteur (1881) had shown that rabbits can be inoculated with rabies virus. Galtier (1879) had already found rabbit as a good laboratory animal for study of rabies.

Hoyt and Jungleblut (1930) found mice as a good laboratory animal, who were quite susceptible to rabies.

Harvey and Acton (1923) used serial dilutions of rabies virus for intracerebral inoculations in rabbits in tests of the relative content of virus in grey and white matter of infected brains. This was the first quantitative approach in case of rabies virus.

Webster and Dawson (1935) developed a standard mouse inoculation test for the diagnosis of rabies, and it was found that specimens found negative by various staining techniques were found positive by mouse inoculation.

Gangulee and Gangulee (1944) summarised the various methods used at Raymond Research Laboratory and Bengal Veterinary College, Calcutta, and recommended a method evolved at that laboratory. In this method, the sections are brought to absolute alcohol level and double strength Gram's iodine is flooded on section for 10 minutes. Thereafter eosin is applied for one minute, and the alcohol is burned out.
Slide is then cooled, washed in distilled water and decolorised with 90 per cent alcohol. It is then counter-stained with methylene blue and differentiated with rectified spirit. They have stated that the colour differentiation of red and blue is very prominent with this method.

Lentz (1907) suggested a variation in Mann's method of staining Negribodies.

Sellers (1927) established a rapid staining technique for detection of Negribodies. It required two stock solutions, one of methylene blue, another one of basic fuchsin. These are stored without filtration in screw cap bottles under refrigeration and protected from light. Two parts of methylene blue are mixed with one part of basic fuchsin for staining. Impression smears while they are moist, are immersed for about 5-10 seconds, and rinsed with running water, dried at room temperature, and examined under microscope. Fresh well spread material gives best results. The cytoplasm of the nerve cell stains blue or purplish blue, the nuclei and nucleoli strong blue, interstitial tissue rose pink, nerve fibers deeper pink, erythrocytes copper red and Negribodies magenta red or cherry red.

Petragnani (1928) used indigocarmine - a blue dye of acid properties, which is sometimes used as a plasma stain in contrast to some red dye such as carmine or basic fuchsin sometimes haematoxylin - mixed with acid fuchsin as a stain for Negribodies.
Lepine (1935) used 0.5 per cent basic fuchsin in 50 per cent ethanol and 0.2 per cent aqueous solution of safranine in equal parts, for staining Negribodies. The stain was kept for 10 minutes on a slide, thereafter slide was washed with a mixture of ethanol and acetone to remove excess stain, stained for 15 seconds with Unna's polychrome methylene blue, and differentiated with ethanol-acetone for few seconds. Slide is washed under running water, dehydrated and mounted. Negribodies appear poppy red to mauve pink.

Seller's method may be used with rapid deoxan embedding as described by Schleifstein (1937).

A staining method used in United States was described by Stovell and Black (1940). In this method smears, impressions, are fixed in acetone and stained first with one per cent alcoholic solution of ethyl eosin (pH 3.0), rinsed and counterstained with 0.23 per cent methylene blue in 22 per cent alcohol (pH 5.5), differentiated in 0.38 per cent acetic acid until brownish red, washed, dehydrated, clarified and mounted. Negribodies appear brownish or pure red, nucleoli pale blue and other structures pink.

Negri-Luzzani (1913) indicated that Negribodies were present in about 90 per cent of cases of rabies. However, confirmatory animal inoculation of the suspect material was not performed.

Steele (1953) stated that since Negribodies cannot always be found in the brains of animals dying of rabies,
it is important that animal inoculation for demonstration of the virus be done on Negri negative specimens. Surveys of large numbers of rabies cases have shown that 10 to 15 per cent of these cases proved positive by mouse inoculation had been missed by direct smear microscopic examination for Negribodies. He further stated that mouse test is simple and inexpensive. Swiss White mice are uniformly susceptible, low in cost, and easily handled. The intracerebral inoculation of a specimen of infected brain material will produce typical and constant symptoms in five to 11 days with consistent production of Negribodies.

Emanniloff (1959) described a differential staining technique for Negribodies in brain material from horses. He used a stain first described by Gerlach in 1917 which consists of three parts of Ziehl Neelsen Carbol fuchsin, six parts of methylene blue in alcohol and 100 ml of a 1:10,000 solution of potassium hydroxide in distilled water. By this method ganglion cells are stained blue and Negribodies red to purple.

Deilles and Chevrier (1959) stained sections of rabies infected brains for 10 seconds with one per cent aqueous alcoholic solution of basic fuchsin, washed and stained with one per cent aqueous alcoholic solution of methylene blue for 10 seconds. It was then washed and treated with 1.5 per cent aqueous solution of chromic acid. With this method, Negribodies appear red or crimson and as a rule, the smaller the Negribodies, the more intense is the stain.
Linder (1960) used the method first described by Muromtsev in 1926, in which methylene blue and tannic acid were used to stain Negribodies. When examined by oil immersion lens, Negribodies appear purplish red with a deep blue to black inner zone. The author claimed that this method is said to be suitable for decomposing material.

Massignani and Malferrari (1961) observed that the component which was responsible for the better results obtained by the method of Massignani and Refinetti (1958) as adopted to Negribodies was an eosin solution combined with phosphotungstic acid.

During 1953-1960, rabies was diagnosed microscopically in 828 of 2,268 central nervous system samples either by methylene blue eosin (Lentz, 1907) or Rhodamine-B staining (Paarmann, 1954). Rhodamine-B was suitable for use with decomposing material and therefore superior to methylene blue eosin method, but it was sometimes difficult to distinguish Negribodies stained with rhodamine-B from nonspecific inclusions, particularly in deer and cats (Wachendorfer, 1961).

Glycerin damaged the rabies virus, the extent depending upon the temperature and period of storage. In infected brain tissue in glycerine, virulence was absent after eight days at 28°C or two to three days at 37°C. In hot climates therefore it is necessary that suspect material should be despatched to the laboratories by the quickest routes in
refrigerated packages in which the internal temperature does not exceed 22°C (Rampon and Barbesier, 1962).

Mitchell and Monlux (1962) examined 100 cats suspected of rabies by various laboratory methods to establish which would give an unequivocal diagnosis. Microscopic examination of impression smears for Negribodies was not reliable, but histological examination of the brain and animal inoculation tests gave consistent results.

Pilo Moron et al. (1967) stated that rabies can be diagnosed more quickly by inoculation of unweaned mice instead of adults.

Van der Marve (1962) described an acid fuchsin - methylene blue method based on formalin fixation. A paraffin sections (3 μ) could be cut and stained within 90 minutes and in urgent cases frozen sections (10 μ) within 45 minutes yielding comparable results. Though the definition was superior in former, Negribodies were easily recognizable even when the material was badly autolysed and poorly fixed.

In a comparison of different methods of diagnosing rabies in dogs, of 94 brains negative for Negribodies with Seller's stain 33 (35 per cent) were positive by mouse inoculation, while of 68 showing Negribodies with Seller's stain, 14 (20 per cent) were negative by mouse inoculation. Results of histological examination after staining according to Mann were comparable with those after staining according to Seller's. Only two of five brains positive by mouse
Inoculation were positive in the gel precipitation test. In the complement fixation test, some infected brains gave very low titers (Nguyen, 1962).

Krieg (1963) observed acidophile, round, homogeneous formations (Luzzani bodies) in Ammon's horn smears stained by Muromtsev's method in 11 out of 220 rabies free cats. In 27 of 50 cats there were also Loewenthal bodies in the medium sized ganglion cells of the thalamus. The differentiation between these inclusions, Negri bodies and Babes-Koch granules is difficult and is doubtful cases, rabies should always be confirmed by fluorescent microscopy and serology.

2,455 cases suspected for rabies were examined at Onderstepoort between 1952-1962. 1,074 were positive on intracerebral injection into three week old mice and/or presence of Negri bodies. The overall efficiency of the Negri body test was 66 per cent in cases where the mouse test was positive. This percentage varied considerably in different species, being higher in ruminants and wild animals than dogs and cats. Negri bodies were not found in five pigs and four equines which were biologically positive. There was no statistical difference in the accuracy of histological examinations of animals that were allowed to die and of those that were killed while suffering from rabies. Thus suspected animals showing clear symptoms of rabies need not be kept until they die naturally. There was greater correlation between biological and histological results with hippocampus
examination (average 70 per cent) than with other parts of the brain (average 46 per cent). The use of different staining techniques did not influence the efficiency of histological test, though the acid fuchsin methylene blue method described by Van Der Marve (1962), was preferred for its simplicity and constancy (Smit, 1963).

Lillie (1965) stated that Oxyphil inclusion bodies such as Negribodies and the like can well be shown by azure eosin and phloxine azure sequence methods. She has used safranin O eriocyanine A method and found best results. She has recommended the methods of Stoval and Black (1940), Mann's methylene blue eosin technique and Schleifstein's rapid method (1937). She has described a method of Zlotnik (1953) for Negribodies in fixed tissue which makes use of picric acid containing orange G after haematoxylin, and along with acid fuchsin, phosphotungstic acid is used. The Negribodies appear purplish red with blue inner granules, nerve cell, cytoplasm bluish, nucleoli dark purple, and erythrocytes yellow.

Seshadri and Chandrasekaran (1963) studied brain materials from 18 canine and nine bovine cases positive for rabies. They tried three methods of making impression smears viz., forceps method, cork or wooden spatula method and blotting paper method. Blotting paper method was recommended for routine use. Seller's method proved to be rapid, simple and reliable yielding uniformly satisfactory results.
The authors stressed the importance of study of histological sections of Ammon's horn in minimizing errors.

Burnet (1960) stated that rabies is a classical example where diagnosis is normally made on symptoms plus the histological demonstrations of Negribodies in the brain without recourse to serological methods.

Bosch (1966) recommended a method for staining Negribodies in paraffin sections of Massegnani and Malferrari (1961). The method gave elegant results, however, it has a minor disadvantage in rather complex procedure for making the eosin solution. In working with xethene dyes at low pH, the author observed that phloxene B (a dye closely related to eosin) could be applied quite simply to produce a durable stain of the rabies inclusion bodies. Mayer's acid hemalum was used for nuclear staining but other similar hematoxylin mixtures can be substituted.

Hartwich and Shouman (1967) observed that there were more doubtful cases of diagnosis of rabies by staining techniques than mouse inoculation.

Rabies virus was isolated from the brain of 22 cattle, nine dogs, two horses, two pigs, a mule, and a cat. Of the 37 brains, 27 were stored at -20°C and 10 at 4°C until use. On intracerebral inoculation of mice, all the strains except one produced higher titers in unweaned mice than in adult mice,
indicating that unweaned mice should be used for diagnosis of rabies (Nilsson et al., 1968).

Subrahmanyam and Pathak (1971) examined 37 brains of suspected rabid dogs and found that Negribodies were detected in only 15, whereas 23 were found positive by mouse inoculation and FAT.

Levaditi et al. (1971) stated that the comparison of three diagnostic methods, immunofluorescence, a rapid staining and an animal inoculation shows that the animal inoculation technique is most valuable, followed by the immunofluorescence, and then rapid staining.

Koprowski (1973) stated that the mouse inoculation test inspite of its simplicity, depends greatly on the accuracy of its performance for dependable results.

Atanasiu (1975) observed that animal inoculation gives a precise diagnosis, but the animals do not die until five to 10 days after inoculation and this does not permit rapid decision for human treatment. The rapid staining method is very rapid, yet the absence of Negribodies does not necessarily indicate absence of rabies. Nevertheless, it may indicate the existence of an extensive contigent encephalitis.
(111) TISSUE CULTURE AND SEROLOGY
Levaditi (1913, 1914) reported the first successful propagation of rabies virus in spinal ganglia maintained in a medium containing coagulated monkey plasma. A continuous release of infectious virus was noted two months after the initiation of the cultures. By this time the original nerve cells had completely disappeared and the cultures were composed of fibroblastic cells. It was not possible, however, to transfer the infection to new cultures established from infected animals.

Stoei (1930) infected cultures of embryonic chicken brains and hearts explanted in rabbit plasma clots with rabies virus of mammalian brain origin and the infection was maintained for five consecutive passages.

Successful serial cultivation of rabies virus in rabbit embryo brains suspended in Tyrode solution was reported by Kanazawa (1936).

Webster and Clow (1936, 1937) were the first to propagate virus in tissue culture. The medium consisted of Tyrode solution containing normal monkey serum plus a suspension of minced mouse embryo brain. The inoculum consisted of brain from a mouse prostrate on the seventh or eighth day following an intracerebral injection of rabies virus. Material passed through such culture medium, as often as 88 times, and the supernatant was still found to be virulent.

The growing of rabies virus in the developing chick embryo was a major breakthrough in 1940. Bernkopf and
Kligler (1940) and Dawson (1939) at the same time succeeded in growing rabies virus in chick embryo.

Dawson (1939) inoculated the brain of an embryo and Bernkopf and Kligler (1940) inoculated chorio-allantois. They pointed out that the chick embryo virus after many passages showed greatly reduced virulence for rabbits. The findings of Dawson (1939) were similar. This method was later adopted by Koprowski and Cox (1948) for production of a chick embryo origin vaccine.

Parker and Hollender (1945) demonstrated that rabies virus will multiply more efficiently in tissue cultures derived from embryonic mouse brain than in those established from the brains of new born or adult animals.

Plotz and Reagan (1942) were the first to isolate street rabies virus directly in cell culture. They infected tissue cultures with material taken directly from the brain of a human patient with rabies and from the brain of a rabid dog. These strains were maintained for 11 and nine passages respectively.

Kissling (1958) reported the serial passage of both fixed and street rabies viruses in primary hamster kidney tissue cultures. The potential usefulness of fixed virus cultivated in cell cultures for the preparation of a vaccine with demonstrable efficiency in experimental animals was shown by Kissling and Reese (1963).
Atanasiu et al. (1961) cultivated fixed strain of rabies virus in a strain of ependymal cells grown in a medium composed of casein hydrolysate, Earle's solution and inactivated horse serum. The virus multiplied in cultures and gave rise to intracytoplasmic inclusions demonstrable by Mann's staining method. Diagnosis could be confirmed by neutralization of virus with immune serum, and the whole procedure could be completed in two to five hours.

Kendo (1965) reported that after several passages in chicken embryo cell cultures, the HEP Flury strain of rabies virus produced a marked cytopathic effect which could be used as a means for the titration of viral infectivity.

Primary embryonic chicken fibroblasts are very susceptible to rabies virus and many strains of fixed and street virus have been propagated in these cells. However, the system suffers from the disadvantage that most chick embryos are infected with other viruses primarily of the lymphomatosis group, and mycoplasma. These contaminants can interfere with the growth of rabies virus and produce inconsistent results (Cohen et al., 1963).

Hayflick and Moorehead (1961) used human diploid cell strains and they are being extensively investigated for the production of rabies antigens and vaccine for human use. Various strains of rabies virus have been adopted to growth in this cell system by the serial subcultivation of infected cells and by cell mixing technique (Wiktor et al., 1964). A concentrated, purified and inactivated rabies vaccine
propagated in HDCS cells is now available for immunization of human beings (Clark et al., 1975).

Except chicken fibroblast, other primary cell lines used for rabies virus propagation have been derived from dog salivary glands (Depoux, 1963), dog kidneys (Hronovsky et al., 1966) and rhesus monkeys (Lang et al., 1969).

A strain of virus originally adopted to hamster kidney cell cultures after a series of passages on embryonated chicken eggs was propagated in pig kidney cell cultures (Abelseth, 1964a). This is now widely used as ERA vaccine for animal immunization (Abelseth, 1964b).

Aksenova et al. (1966) reported the successful propagation of fixed strains of rabies virus in several primary and serially propagated cell cultures including hamster kidney, dog kidney, sheep embryo kidney, the intestine, rabbit kidney, and human embryonic kidney, skin, muscle and lung.

Because of the extreme susceptibility of baby hamster kidney cell line BHK/21 (MacPherson and Stocker, 1962) to rabies virus, it had become most favoured medium for use in rabies virus investigations. The hamster cell line Nil-2 (Diamond, 1967), also supports rabies replication to high titer and is used as substrate for vaccine production.

Rabies virus has been successfully propagated in cells of several established cell lines of poikilothermic vertebrate origin derived from fish (Solis and Mora, 1970), snakes,
turtles and lizards (Clark, 1972). Fresh water fish cell line FHM and Russell's viper cell line, VSW, supported the replication of fixed rabies virus strains to high titer without adaptation. The infection was successfully maintained in serially subcultivated VSW cells for more than 100 passages, and in serially subcultivated cells of a lizard cell line, GL 1, for more than 60 passages (Wiktor and Clark, 1975).

In general, no specific cytopathic effect can be observed in monolayers of cell cultures infected with rabies virus (Wiktor and Clark, 1975). However, Yoshino et al. (1966) observed in monolayers of chick embryo cells that the viability of rabies infected cells was affected to a point that plaque formation occurred. Similar phenomenon was seen in agarose suspended cells of hamster origin (Sedwick and Wiktor, 1967).

Wiktor and Clark (1972) stated that infected cells can be maintained in an actively growing state by regular trypsinization and division for prolonged periods of time. Chronically infected cells, however, may demonstrate a lower cloning efficiency as well as a slower rate of multiplication.

Diaz et al. (1973) observed a cytopathic effect in fused hamster embryo cells infected with CVS strain of virus. The use of this system was recommended for virus titration and determination of virus neutralizing antibody.

Thomas (1975) stated that serologic tests for detection of rabies antibody provide valuable tools for epidemiologic
studies, including the determination of the immune status of man and animals, and the diagnosis of rabies infection by the presence of antibody in the specimens from live or dead animals. The mouse neutralization and indirect fluorescent antibody have been most frequently used for determining antibody levels in persons immunized against rabies.

Serologic tests in tissue culture reported include (1) inhibition of cytopathic effect in cell cultures (Kissling and Reese, 1963), (2) inhibition of fluorescent foci in cell culture (King et al., 1965; Lennette and Emmons, 1971), (3) inhibition of plaque formation in cell cultures (Sedwick and Wiktor, 1967).

The classical plaque test have not been found satisfactory for plaquing rabies virus using agar, agarose or methylcellulose. The agar was replaced by Sephadex G 200, and Sephadex Monolayer plaque test (SMP) was found convenient by Schneider (1973).

The indirect fluorescent rabies antibody (IFRA) test is another serologic test which is rapid, sensitive and reproducible method for measuring antibody. This test was used in Columbia where antibody levels in dogs from various areas of the country were correlated with their vaccination history to determine the possible occurrence of nonfatal rabies infection (Clammer, 1967 Personal Communication to Thomas, 1975).

Bebbie et al. (1972) developed fluorescent antibody technique for neutralization testing in cell cultures.
They used BHK-21 (135) cells grown in Lab-Tek TC chamber slides with ERA virus. A confluent monolayer of cells formed within 24 hours was inoculated with the serum virus mixture. After five days of incubation, the slides were harvested, stained by FA and examined. In this study, 29 of 30 sera gave titers within a single fivefold dilution of the mouse neutralization test.

Wiktor et al. (1973) reported that the envelope glycoprotein of rabies virus was shown to be the antigen responsible for the induction of virus neutralizing (VN) antibody formation and for the protection of animals against subsequent challenge with rabies virus. Preparation of two other envelope proteins and of nucleocapsid protein, derived from disrupted virions, induced the formation of only low levels of VN antibody and gave poor protection of animals against rabies, which could be explained by the amount of residual glycoprotein present in these preparations. Purified preparations of free viral nucleocapsid, isolated from infected cells, did not induce VN antibody formation, but elicited in immunised animals antibodies demonstrable by CF or FA tests.

Atanasiu (1973) described a method in which a constant dose of the previously titrated virus is neutralized against a series of different dilutions of the serum. The method is used mainly for the assay and potency testing of therapeutic antirabies serum and immunoglobulin but it is also applicable to any serum containing rabies antibody.
Smith et al. (1973) developed a rapid fluorescent focus inhibition test (RFFIT) that can be performed in 24 hours. In RFFIT, BHK-21/S 13 cells treated with DEAE-dextran are added to a mixture of test serum and tissue culture adapted rabies virus (incubated for one and half hours) and monolayers are allowed to form. After 24 hours of incubation, the cell monolayer are examined by FA staining for the presence of non-neutralized rabies virus.

Louie et al. (1975) concluded that RFFIT should be substituted for conventional mouse neutralization test (MNT), for measurement of rabies antibody. The RFFIT was more sensitive, reproducible, accurate and convenient of the two.

Rudd et al. (1980) described a tissue culture test for primary isolation of street rabies virus from the brains of suspect animals. It was reliable and comparable in sensitivity to the standard mouse inoculation technique. The test which yields final results in 48 hours was performed in BHK-21 cells on tissue culture chamber slides. The addition of diethylaminoethyl dextran to the cell suspension before seeding the slide promoted viral invasiveness. The method may be considered as a substitute for the mouse inoculation test currently used to confirm the immunofluorescence test.
(iv) SEROLOGIC TESTS
(iv) **SEROLOGIC TESTS:**

Rabies virus infected cells produce complement fixing soluble antigen (SA). This naturally occurring non-infectious antigen was first reported by Polsen and Wessels (1953). These workers used clarified tissue homogenates or cell culture fluids as a source of SA.

Oudin (1946) and Quishterlony (1949) described gel diffusion techniques which have led to important applications of immunoprecipitation reactions in the diagnosis of virus diseases.

Van den Ende et al. (1957) reported that soluble antigen (SA) was produced in suckling mouse brain in parallel with infectious virus and was partially purified by precipitation at pH 4.3. They characterized the antigen as insensitive to pH variation over range 6-10, stable to heating at 56°C and to treatment with dilute phenol or formaldehyde, but sensitive to trypsin digestion.

Kipps et al. (1957) determined that an increase in infectivity preceded the first appearance of SA in mice after intracerebral injection of rabies virus, and that SA continued to increase after the maximum level of infectivity had been reached. When mice were immunized with SA, high levels of CF antibody could be obtained but no (N) neutralizing antibody was induced.

Villemot and Provost (1958a,b) were the first to show the presence of two precipitable fractions in tissues.
containing rabies virus. Soluble antigen and precipitating antibody diffuse towards one another through the agar gel and react to give a precipitate, which forms two lines clearly visible to the naked eye.

Sharma (1959) obtained positive agar gel precipitation on glass slides and in tubes and plates by testing brain of rabid animals against antirabic serum and also with serum from clinically affected animals and known infected brain or vaccine.

Linsert et al. (1959) observed that complement fixation reaction was positive in all animals in which Negri bodies were demonstrable and also in about 23 per cent in which Negri bodies were not demonstrable.

Animals were infected intracerebrally with the fixed virus strains "Pitman Moore (FM-mice)", "Budapest (Sheep)", Novi-sad (Dogs) and the Flury strain (Guinea pigs). Complement fixation tests were performed and infective antigen was titrated in mice. Brain was examined from animals killed at 10 hour intervals between 10 and 120 hours after infection. Changes in CF titer and virus titer were described and compared. During the incubation period CF titer increased in parallel with virus titer. When the animals developed symptoms, the CF titer continued to rise while the virus titer fell. Between half hour and 30, 40 or 50 hours after intracerebral injection there was a latent phase during which neither method detected virus in the brain (Kuwert, 1960).
Thiery (1960) made a comparison between the method of Villemot and Provost (1953) and the classical gel diffusion methods. He concluded that when brain tissue is examined by gel diffusion method, the result will be positive if sufficient rabies antigen is present. When serum is examined, a positive result is obtained if there is an increase in $\gamma_2$ globulins, an increase that is normally associated with rabies but varies in degree from one animal to another.

Grasset and Atanasiu (1961) found that the immunoprecipitation reaction gave consistent results only if antigen and antibody were present in suitable proportions. They obtained negative results not only with tissue cultures or brain tissues having a low virus titer although giving a positive inoculation test, but also with sera of low neutralizing power.

Atanasiu et al. (1963) used the technique of Grasset and Atanasiu (1961) to identify rabies virus cultured on BHK-S 13 cells in the presence of hyperimmune horse serum and the results were excellent.

Grasset (1967) while presenting the causes of error and their remedies stated that the test has its uses as it was found of value when use could not be made of suspect brains for demonstration of Negribodies, for the fluorescent antibody test or for biological tests. It is considered that under present circumstances the test should be regarded as a complementary diagnostic aid.
Kolomankin et al. (1966) reported that the agar gel precipitation test was applied to brain suspension from 257 animals suspected of rabies and 96 that had died from other causes. A positive result was of diagnostic value but since a positive result was obtained in only 37 per cent of rabid animals, a negative result is valueless.

WHO (1966) reported that complement fixation and gel diffusion precipitin tests are not yet considered satisfactory for routine diagnosis. However, tissue culture methods are not yet sufficiently developed for routine diagnosis. Fluorescent antibody test is the single test currently available for rabid diagnosis of rabies. Although the WHO Expert Committee recommended other established diagnostic tests should be performed in parallel.

Afshar at al. (1972) carried out gel diffusion tests on serum samples from apparently healthy stray dogs, humans and camels. They were able to detect positive samples by this method in 3.2 per cent of healthy stray dog samples.

Lepine (1973) concluded that although gel diffusion technique is accurate and sensitive it cannot be at present be considered entirely reliable. A positive result permits a rapid diagnosis of rabies or the presence of rabies antibody. On the other hand, a negative result does not necessarily exclude rabies or the presence of rabies antibody.
In mice inoculated with street rabies virus, complement fixing antigen first appeared six-10 (average 7.7) days after infection. Negribodies first appeared seven-10 (average 9.0) days when the virus titer was highest (Ueki, 1961).

The presence or absence of Negribodies confirmed the results of the complement fixation test in 77 per cent of 272 brains of dogs, cats and wild animals, 31 being positive and 178 negative. In four cases the complement fixation test was negative although Negribodies were present. In 26 cases (9.5 per cent) the complement fixation reaction was positive in the absence of Negribodies and in 12 per cent the reaction was ambiguous (Osolina & Englert, 1962).

Baczynski (1963a) observed that between one and 20 days after artificial infection with rabies street virus, 19 of 21 dogs were positive to the serum neutralization test and/or complement fixation test and eight of 20 yielded the virus from saliva and/or urine.

Twenty one dogs aged six to 12 months were infected by intramuscular injection of 10 per cent suspension of brain tissue from a case of rabies. During a 20 day observation period, rabies virus was isolated from saliva and/or urine of eight of 20 dogs tested (40 per cent) in most cases in the first 10 days. Four of the virus excretors died subsequently from disease, four survived six months period of observation. Serum neutralization and complement fixation tests jointly were positive for 19 days (90.47 per cent) (Baczynski, 1963b).
Rudikov (1964) observed that complement fixation and fluorescent antibody tests were suitable for examining brain tissue and for differential diagnosis.

Wolter (1964) carried out complement fixation tests and histological examination of brain or mouse inoculation tests on 74 animals suspected of rabies. In 67 cases the results of CFT agreed with those of the other tests. Six animals with Negri-negative brains gave positive CFT, mouse inoculation test which were carried out on three of these six were positive. In one animal the CFT was negative when MIT was positive. The rapidly, accuracy and safety of this CFT recommended it for routine use.

Jentzsch and Winkler (1967) found no relation between the suitability for fluorescence and neutralizing, complement fixation and precipitating antibody titers of 57 sera from rabbits immunized against rabies.

Parker and Sikes (1966) reported the studies on the time when rabies inhibiting substance (RIS) developed in relation to clinical course of rabies in 16 skunks, the relationship of RIS to serum neutralization (SN) antibody and the effect of RIS on the amount of recoverable virus. Saliva samples collected from three skunks during the clinical course of the disease contained appreciable amounts of rabies virus. At postmortem, however, virus could not be isolated from the salivary glands of two of these animals and only a
trace was isolated from the salivary gland of the third. SN antibodies were demonstrated in all the skunks in which RIS was demonstrated. A relationship apparently exists between the presence of RIS in tissues and SN antibody. Less virus was recovered when SN antibody was present and even less virus was present when both RIS and SN antibodies were present. This may result in failure to isolate rabies virus by mouse inoculation although fluorescent antibody staining reveals rabies antigen. Authors concluded that since RIS seems to develop in the clinical course of the disease, it is possible that an animal might transmit the virus during the furious stage of the disease even though virus could not be isolated from the tissues after death.

Mead (1962a,b) studied SA prepared from suckling mouse brain. He confirmed the utility of precipitation at acidic pH as a concentration device, but was unable to further purify SA by chromatographic and electrophoretic techniques. At least two, and perhaps four, antigenic entities were present in SA preparations, the largest being resistant to trypsin digestion.

Wiktor et al. (1969) employed differential ultrafiltration to free the SA present in infectious tissue culture fluid from intact virions. The resulting virus depleted filtrate was concentrated and, when compared with an equivalent concentrate of rabies virions plus SA, was found to contain only 0.005 per cent of the viral infectivity and to be devoid of HA activity. On the other hand complete recovery
of CF activity was found in the concentrated SA fraction and 21 per cent of the protective activity remained. The authors concluded that SA was an immunogen for CF antibody formation as well as a vaccine which afforded protection against challenge with rabies.

Rabies virus shares some of the characteristics of myxoviruses, especially the presence of surface projections morphologically similar to those of myxoviruses (Hummel et al., 1967) it was reasonable to assume that haemagglutinating activity might occur.

Halanen et al. (1968) reported that challenge virus strain agglutinated erythrocytes from geese, two day old chicks, adult chickens, guinea pigs, rats, sheep and man (blood group O), provided the virus was grown in the absence of HA-inhibitors present in every normal serum. Optimal conditions for demonstration of rabies haemagglutination included low temperature, pH 6.2, and use of goose erythrocytes. Haemagglutination was not observed at room temperature or at 36°C. A positive haemagglutination pattern that has fully developed at 0–4°C rapidly reverts to negative at room temperature.

Murphy et al. (1968) indicated that rabies virus haemagglutination has a potential value in haemagglutination inhibition test (HAIT) for assay of immunity to rabies, since HA antigen is separate from the CF and gel precipitating
antigens, and will measure almost identical antibodies as the neutralization test.

The disruption of purified virions with an anionic detergent, sodium deoxycholate (DOC), resulted in the rapid inactivation of viral infectivity and the dramatic loss of most of the HA activity. On the other hand, CF activity remained unaltered (Sokol et al., 1969).

Wiktor et al. (1969) were able to immunize mice against rabies with virion free concentrated soluble antigen of high CF activity but no haemagglutinating activity.

Gough and Dierks (1971) observed that since all the procedures available for the measurement of rabies virus antibodies in serum have certain disadvantages, a passive haemagglutination test was developed (PHA), and the titers obtained with human sera by this procedure correlated well with those obtained by SN tests. While both, IgG and IgM classes of antibodies were measured by the procedure, it appeared to be more sensitive for detecting IgM than the SN test.

Schneider et al. (1971b) showed that the disruption of concentrated, partially purified rabies virions by the natural detergent saponin resulted in the disruption and inactivation of viral infectivity, whereas rabies HA, CF and immunogenic capacity remained largely unaltered. This close relationship was seen when mice were immunised with gradient purified saponin treated haemagglutinin. It was almost equally as immunogenic as gradient purified virion, and significantly
more potent than the reference vaccine, inspite of the low protein concentration of the purified haemagglutinin. They further observed that there is a direct relationship between the measurable complement fixing and protective activities of rabies virus preparations as long as a certain structural confirmation, especially the immunizing surface protein, remains unaltered.

Trefzger and Dierks (1971) indicated that the relationship of Lagos Bat, Mokola, and rabies viruses was distant when tested by serum dilution technique in mice, whereas the relationship was closer and readily demonstrable when undiluted hyperimmune sera were used in a constant serum varying virus dilution test.

Tignor and Shope (1972) have carried out quantitative vaccination challenge tests in mice. Mice immunized intracerebrally with rabies HEP Flury vaccine resisted challenge with Lagos Bat virus, and to a lesser extent with Mokola virus. Vaccination i.p. With Mokola, Rabies or Lagos Bat viruses did not usually protect mice against a heterologus challenge, an exception was the resistance of rabies vaccinated mice to Lagos Bat challenge.

Radioimmunoassay (RIA) for detection of viral antibody is performed either by radioisotope method (Gerloff et al., 1962) or, more commonly by a solidphase RIA (Catt and Tregear, 1967) in which viral antigen is fixed to a solid glass or plastic support, which has been found easier to
perform than RIF, and most methods for assay of viral antigens and antibodies are based upon this principle.

Hayashi et al. (1972) showed that 125I-labelled antibody could be used to demonstrate viral antigen on the surface of infected cells.

Rosenthal et al. (1972) first described the use of lysates of virus infected cells of semipurified virus, adsorbed to microtiter plates as a source of antigen for detection of viral antibodies.

Ling and Overby (1972) made use of RIA in virology for assay of hepatitis B surface antigen and antibody.

The major advantage of RIA as compared with conventional methods for detection of viral antigen or antibody is its greater sensitivity. This permits the use of highly diluted immune reagents, which in turn reduces nonspecific reactivity, and also results in economy of reagents. RIA results based upon counting isotope emissions are more quantitative and less subjective than are the results based upon visual reading. Disadvantages are the fact that radiolabelled antibodies can be used only for about three to four months, the potential hazard of radio isotopes, the need for expensive equipment for counting and the fact that localization of viral antigen in infected cells is not possible (Forghani, 1979).

Viktor et al. (1972) described radioimmunoassay (RIA) procedure which provides a new immunological tool for detecting and evaluating rabies specific antibodies. Purified rabies
virus was labelled with $^{125}\text{I}$, and the labelled virus was reacted with dilutions of rabies antibodies and the resulting virus antibody complexes were precipitated with anti IgG serum. The specific binding activity for each serum dilution could be calibrated after subtraction of the background activity determined from samples of normal serum treated in the same manner. When serum from immunized human and rabbits and a sample from a patient with a clinical rabies infection were tested, the virus binding ability of antirabies serum was shown to be similar to or higher than the values obtained by the neutralization test based on the plaque reduction method. The procedure was also used to determine the proportion of rabies specific gamma globulins in a mixture of $^{131}\text{I}$ labelled gamma globulins from immunized and normal rabbits. Ten to 12 per cent of all gamma globulins represented rabies specific antibody in this serum sample.

This method appears to be more sensitive for detection of rabies antibody than any other procedure at present in use, and it should find useful application in the detection of early antibody appearing after vaccination with the newly developed, concentrated, and purified rabies vaccines of tissue culture origin; antibody that cannot be detected by the conventional methods of virus neutralization, complement fixation, or fluorescent staining techniques (Wiktor, 1975).

Schneider et al. (1973) characterised the antibodies elicited by ribonucleoprotein (RNA - Synonymous to NC) and glycoprotein antigens isolated from rabies virus and the
rabies serogroup Mokola and Lagos Bat virus. RNP antigen was found to exhibit group specificity, being common to all of the rabies group viruses. The envelope glycoprotein antigens were found to elicit neutralizing antibodies of virus type specificity. Although the M proteins of rabies virus have not yet been obtained in pure form, comparative characterization of the M, G, and N proteins of the rhabdovirus VSV has recently been described (Dietzschold et al., 1974). The M protein of VSV elicited CF and precipitating antibodies, but no neutralizing antibody.

Kuwart (1973) stated that the CFT unlike the haem-agglutination reaction, measures all virus specific antigens and not only virions. It can be employed, therefore, for the study of the so called S-(soluble) antigens which have RV antigenicity but are not virion associated. The sensitivity threshold of the CFT is about 10 to 20 times that of immunodiffusion.

Rai Chowdhuri et al. (1973b) examined 770 brains from different species of animals suspected of rabies by complement fixation test, mouse inoculation test and presence of Negri-bodies. Out of 486 brains found positive by mouse inoculation, 455 (93.6 per cent) were positive by complement fixation test and 367 (75.5 per cent) were found positive for presence of Negribodies.

Any strain of rabies virus, regardless of cell type in which it was propagated, will agglutinate goose erythrocytes
under conditions described by Halonen et al. (1968). Rabies virus grown in CNS of laboratory animals was not suitable for HA even if it is partially purified. Cells once treated with rabies virus haemagglutinin retain their capacity to be agglutinated by the same or other strains of rabies virus. Haemagglutination Inhibition test can be performed by inactivating serum at 56°C for 30 minutes and removing nonspecific HA inhibitors by Kaolin treatment. For epidemiological surveys, one serum sample per individual animal is sufficient. For immunity studies after vaccination and for diagnostic purposes two or more samples from each individual should be available (Kuwert, 1973).

The complement fixation test was carried out on serum from 25 dogs inoculated with HEP Flury vaccine and from one control dog. Titer ranged from zero to over 256, at 15 or 90 days after vaccination. They did not correspond quantitatively with neutralization titers but 18 of the 26 serum samples produced a similar qualitative reaction in the two tests (Nilson et al., 1975).

Shope (1975) stated that by the use of hyperimmune mouse ascitic fluids, cross reaction in CF among rabies related viruses is shown readily with rabies, Mokola and Lagos Bat viruses, Obodhiang and Kotankan viruses are related to rabies virus only through Mokola and Lagos Bat. Dogs and monkeys inoculated intramuscularly with Lagos Bat and Mokola viruses usually did not form CF antibody. When antibody was found, it...
cross reacted with rabies, Mokola and Lagos Bat virus antigens. It was not possible to demonstrate any cross reaction by NT, and CFT between rabies and other 13 rhabdoviruses, which were found unrelated to rabies like viruses.

Gough

Dierks and (1973) observed that passive haem-agglutination test is a rapid, easily performed test and gives titers that correlate well with those obtained by serum neutralization tests on late post vaccination human sera. The test was performed on 347 sera with SN titers of 1:5 to 1:3000 obtained from subjects who had received pre-exposure vaccination or post-exposure prophylaxis. Only eight gave HA titers that varied from SN titers by more than one two fold dilution.

Ardoin et al. (1977a), by slight modification of the arbovirus technique of Clarke and Casals (1958) and the application of trypsin treatment obtained rabies virus haemagglutinin from the brain tissue of infected animals (cattle, foxes, rabbits and mice). The fixed and street virus strains from brain tissue of naturally or experimentally infected animals showed haemagglutination properties similar to strains cultivated in the absence of serum. Haemagglutinin of the Pasteur VP strain prepared from mouse brain appeared to be the most suitable. The HA titer was proportional to the infectivity titer by the intracerebral inoculation into mice. Vaccines are now titrated by haemagglutination (Ardoin et al., (1977b)).

Rabies antibody appears to be most closely associated with the IgM and IgG classes of immunoglobulins
During a primary response the IgM antibody can apparently be detected as early as three or four days, whereas the IgG apparently cannot be detected until approximately the 10 day. The IgM usually starts declining within 41 days, whereas the IgG appears to start declining before 225 days, but may remain detectable up to 20 years. The persistence of these antibodies has made possible the use of various serologic tests for determining the immune status of the host.

Madyarova et al. (1979) applied indirect haemagglutination reaction to serum samples from immunized guinea pigs and mice. Goose erythrocytes were sensitized with concentrated, purified rabies virus, propagated in primary culture of kidney cells from Syrian hamsters. Antibody titers were related to the titers of the neutralization tests.

Diaz and Verela-Diaz (1977) employed counter immunoelectrophoresis (CE) test to detect rabies antibody activity and the results were compared with those obtained by the SN test. The CE was more sensitive than the SN test and a good correlation was observed between the result of both techniques.

Diaz and Myers (1980) developed a modified counter immunoelectrophoresis (CIE/SN) technique as a diagnostic procedure for the detection of serum neutralizing antibodies to rabies virus in humans undergoing post exposure immunization and compared favorably with mouse neutralization test. Its advantage is speed, simplicity and low cost.
(v) **FLUORESCENT ANTIBODY TECHNIQUE:**

Coons *et al.* (1941) used fluorescent dye for detection of antigen. They used B-antracene, introduced into anti-pneumococcus type III serum by reacting the latter with B-anthryl isocyanate. The highly fluorescent conjugate agglutinated type III pneumococci, imparting a grossly visible blue fluorescence to washed clumps of bacteria. Individual cells of Type III organisms were readily visible in the fluorescence microscope after exposure to labelled antiserum for 30 minutes. On the other hand, Type II pneumococci remained non-fluorescent after exposure to Type III antiserum under the same conditions. Later on a detailed description of the preparation of fluorescein-protein conjugates and their use in detecting pneumococcal antigen in tissues of infected mice was reported (Coons *et al.*, 1942).

Coons and Kaplan (1950) described improvements in a method for the specific microscopic localization of antigen in tissue cells. The method employed antibody labelled with fluorescein isothiocyanate as a histochemical stain, the specific antigen antibody precipitate being made visible under the fluorescent microscope.

Marshall (1951) demonstrated that fluorescent antisera were specific enough to distinguish differences among native antigens comprising normal tissues, and that contaminant antibodies resulting from impure immunizing antigens could be absorbed away from labelled antiserum without affecting the
specific staining capacity of the serum. He described the localisation of native protein-adrenocorticotropic hormone in normal tissue. In addition several technical points were made: freeze drying of tissues followed by paraffin embedding for preservation of antigenicity; fixation of sections with methanol; use of dark field substage condenser in order to improve fluorescent image and that fluorescent isocyanate in acetone solution could be stored for about a year if protected from light, heat and moisture.

Weller and Coons (1954) reported on "indirect" technique. In this method, a preparation of tissue culture cells containing virus were rendered fluorescent by first exposing the cells to unlabelled human antiserum against the virus, and then to fluorescent rabbit antiserum directed against human globulin. By this technique all antigens capable of reacting with human antibodies could be stained with a single antihuman conjugate.

Another modification of basic fluorescent antibody staining procedure was a method of staining native antibody in situ. Tissue sections suspected of containing antibody-synthesizing cells were flooded with unlabelled, diluted homologous antigen. After allowing a suitable period for the antigen to react with cellular antibodies, the sections were rinsed and flooded with fluorescent antiserum directed against the homologous antigen (Coons et al., 1955).
Liu (1956) reported on rapid diagnosis of influenza as a result of staining nasal washings from suspected cases. Though it was not possible to detect all positive cases found by haemagglutination inhibition test, it was possible to give the result on the same day, whereas HA required a delay of several days before serum from convalescents could be collected.

Riggs et al. (1958) described the use of isothiocyanate derivatives of both fluorescein and tetraethylrhodamine B. Another orange fluorescent derivative of Rhodamine B the disulfonic acid was successfully conjugated to proteins by Chadwick et al. (1958).

Redetzki (1958) described labelling of antibodies with DANS, but was not used for histochemical stains.

Goldwasser and Kissing (1958) adopted fluorescein isothiocyanate for use in rabies diagnosis. The procedure consists in labelling antibody with a fluorochrome, allowing the labelled antibody to react with specific antigen if present, and observing the product of the reaction under fluorescent microscope. Staining with fluorescent antibody of street rabies antigens in smears made from the salivary glands of rabid animals was also described by Goldwasser et al. (1959).

McQueen et al. (1960) demonstrated Negribodies in 66 of 825 samples of brain material from dogs, cats, and other domestic and free living mammals. However, by mouse inoculation rabies was diagnosed in 70 samples.
Fluorescent antibody (FA) method was used for the examination of 989 specimens of rabies and results were compared with those of histological examination and mouse inoculation. The FA test and mouse inoculation were both more accurate than histological examination and the two tests were in agreement on all except six specimens (Stone et al., 1961).

Brain specimens from 424 animals of 22 species suspected of rabies were examined by fluorescent antibody technique. Fluorescein isothiocyanate gave 41 positive, 40 of which were confirmed by mouse inoculation or staining for Negri bodies (Goldwasser et al., 1961).

Carski et al. (1962) reported on the results of 46 experimentally infected foxes and skunks with rabies virus from a fox. By the fluorescent antibody test on brain smears and by mouse inoculation with brain suspension, both methods were sensitive, but not completely reliable especially when used with salivary gland material. Positive fluorescent antibody tests which do not agree with results of mouse inoculation tests should not be dismissed as false. In rare cases animals with long morbidity periods, especially wild life, develop high levels of antibodies to rabies that interfere with virus isolation by mouse inoculation (Sikes, 1962).

Schaap and Velthoën (1963) observed, by using fluorescent antibody technique that results obtained with purified globulin were as good as those obtained with labelled whole serum. Non-specific staining of brain tissue was prevented by absorbing conjugates with acetone dried mouse brain.
Villa and Alvarez (1963) demonstrated rabies virus in the kidneys of nine out of 40 Vampire bats (Desmodus rotundus), none of which had symptoms of illness. The brain, salivary glands and interscapular gland were also positive by Seller's technique, the fluorescent antibody technique or the neutralization test. Of the 40 Vampires, 26 (65 per cent) were positive in one tissue or another. The fluorescent antibody technique revealed a large number than Seller's technique. Mouse inoculation tests were negative.

Schneider (1964) reported his results on fluorescent antibody technique. He examined 525 brains from foxes, dogs, cats, deer, cattle and other animals. Negribodies were demonstrated in 128 of 153 infected brains. Rabies was diagnosed by fluorescent antibody technique in 214, and by mouse inoculation in 210 specimens. Regions most suitable for demonstration of rabies antigen by fluorescent antibody were the stem, hippocampus, cerebellum, and corpora quadrigemina. He concluded that this technique was considered superior to conventional routine methods for its accuracy and comparative rapidity.

Jentzsch (1965) stated that advantages of the fluorescent antibody test were reliability, speed, and the fact that it could be used for the examination of decomposing tissue. Adoption of this method as a routine is of considerable financial advantage. Fixation of brain tissue smears by passing two to three times through a flame and by exposure to 96°C for
one hour had no adverse effect on fluorescence serological
demonstration of Negribodies and rabies virus aggregates.
Such heat fixed preparations were safe to handle and fixation
by heat was superior to fixation by acetone.

When a specimen is found positive by the fluorescent
antibody test but no virus can be isolated by mouse inocu-
ation, the possibility must be considered that the animal
might have had virus in the saliva prior to death, although
no virus could be isolated from the salivary glands at death.
This has been shown experimentally in skunks (Wilsnack and
Parker, 1966), foxes (Carski et al., 1962) and bats (Baer and
Bales, 1967).

Wachendorfer (1966) indicated that with an increased
number of putrid specimens there is greater difficulty in FA
diagnosis, and the diagnosis of such specimens must be
reviewed very carefully in the diagnostic laboratory in order
not to consider a positive specimen negative and thus without
danger for an exposed individual; treatment in those cases
must depend on epidemiologic findings surrounding the exposure,
and very often has to be initiated inspite of negative FA
finding.

The cytopathic effect (CPE) of rabies virus is difficult
to detect on tissue cultures. It does not become obvious until
several days after maximum virus production has occurred.
Fluorescent antibody staining has proven useful in following
the progression of rabies virus and antigen in infected tissue
cultures in the absence of readily detectable CPE (Leonard et al., 1967).

Jentzsch (1967) compared the FA technique with mouse inoculation. He demonstrated an efficiency of 98 per cent with 30 of 1,537 total positive cases negative by FA technique, yet confirmed by animal inoculation; there were some cases in which FAT was positive and mouse inoculation negative. He concluded that the fluorescent method is on principle equal to animal experiment. The early stages of infection which cannot be diagnosed by fluorescence could under no circumstances actively endanger any contact persons.

Fischman (1969) observed that fluorescent methods have been instrumental in elucidating many events that occur in the pathogenesis of rabies.

The presence of infective rabies virus on acetone fixed impression smears used in fluorescent antibody diagnostic examinations was confirmed. Although the amounts of virus on the microscope slides that were examined were small, street viruses with higher titers than the strains used would probably be left in greater quantities. The destruction of such residual viruses dangerous to laboratory personnel could be ensured by using 95 per cent alcohol as fixative at 4°C for four hours. Results of an eight month study with four strains of rabies virus indicated that this procedure did not impair staining quality (Fischman and Ward, 1969).
Roslakov et al. (1970) concluded that the immunofluorescence procedure is highly specific and more sensitive than the mouse inoculation test and should be used in the differential diagnosis of rabies in vaccinated animals, but not of course to the exclusion of mice and the gel diffusion method.

Larghi and Jimenez (1971) modified FAT by eliminating preliminary acetone fixing and reducing the period of staining from 30 minutes at 37°C to 10 minutes at room temperature (22° - 25°C). The procedure did not show any effect on the diagnosis of rabies by FAT and reduced the test time from five hours to 45 minutes.

Veararaghavan et al. (1971) reported the results of 4,055 specimens received for diagnosis of rabies. Out of them, 2,623 (64.7 per cent) were detected by fluorescent antibody technique, while 1,844 (45.4 per cent) were detected by smear or section for presence of Negri body.

A review of 389 cases in 18 animal species led to the conclusion that the specificity and reliability of the fluorescent antibody test depended on the distribution of the specific antigen throughout the sample, the state of preservation, the quality of the conjugate and of the apparatus, and the skill and the experience of the operator. A complete correlation between the results of the FAT and biological test was achieved only after two years of parallel study (Wojciechowski and Trippenbach, 1971).
Pilot (1972) stated that the non-specific fluorescence reactions which may occur and confuse the interpretation in FAT are; due to autofluorescence to the presence of free fluorochrome or of serum fractions other than IgG in the conjugates, to the interactions of the immunoglobulins with cell constituents and IgG cytophilia or to electrostatic charges. False negative reactions may occur through masking of specific fluorescence, by autofluorescence through diffusion into the added fluids of hydrosoluble antigens or through quenching effects of heavy metals. Also the presence of unconjugated IgG molecules may block the fluorescent antibodies from the antigen sites.

Mataouch and Sodja (1973) used FAT to study the developmental dynamics of rabies antigen in central nervous system of mice infected with various strains. By intramuscular injections, they were able to detect the virus within 24 hours in lumbar spinal cord, 48 hours in medulla oblongata and in all parts of the CNS on fourth day after injection.

Mouse brain impressions of Mokola and Lagos Bat viruses tested with the conventional direct FAT reacted strongly and were diagnosed as rabies virus. Inhibition of staining reaction by prior absorption of the conjugate by rabies infected mouse brain suspension confirmed the specificity of the staining reaction. Obodhiang virus when thus tested was negative (Lennette, 1970).
Trefzger and Dierske (1971) compared rabies, Mokola and Lagos Bat viruses using mouse brain impression smears and conjugates of a goat antirabies serum, and rabbit anti Mokola and anti Lagos Bat sera. Brain impression slides of all three viruses reacted with the rabies, conjugate and were specifically inhibited by rabies virus. The heterologous systems gave less intense fluorescence than the homologous.

The currently practiced FA technique does not distinguish rabies from Mokola and Lagos Bat viruses, yet these differ by NT and in cross challenge experiments. Comparison of other strains of rabies virus including Derriengue from Vampire bats, arctic rabies and standard laboratory strains have shown minor antigenic differences among rabies strains, the differences were not as great as those of classic rabies and Lagos Bat or Mokola virus. Oulou Fato virus of West Africa and street rabies virus strain were antigenically same, but rabbit immunized with Oulou Fato virus succumbed to rabies challenge while rabies immunization protected against Oulou Fato virus challenge. The rabies like viruses isolated from rodents in Czecholovakia are serologically related to rabies, but it has not yet been reported whether they are also antigenically distinct from classic rabies virus (Shepe, 1975).

Cornea test:

Schneider (1969) described a test which involved the staining of corneal impression slides with fluorescent antibody prepared from antirabies horse serum and marked with
fluorescein isothiocyanate. It can be applied to living as well as dead animals and gives a positive diagnosis before the onset of clinical symptoms.

Kovalev and Shashen'ko (1970) also described the fluorescent antibody technique using corneal impression to detect rabies virus in corneal cells of rabbits and sheep infected experimentally with street rabies virus. The specificity of the technique was demonstrated by the failure of uninfected cells from control animals to show specific fluorescence and the failure of conjugates prepared from normal gamma globulin to stain infected cells. There was a high degree of correlation between the results of FA and the result of inoculation of mice with saliva. With this technique rabies can be diagnosed 3-5 days before the appearance of clinical signs and also during the course of disease and post-mortem.

Reis et al. (1971) conducted fluorescent antibody tests on 52 specimens from rabid animals and man. Forty-eight per cent were positive by immunofluorescence and 35 per cent by mouse inoculation. 13.5 per cent of the inoculated mice died. Comparative figures for the aqueous humour were 65 per cent, 72 per cent and 55 per cent, and for optic nerve 88 per cent, 80 per cent and 66 per cent. Corneal impressions from live subjects were all negative by immunofluorescence.

Examination of corneal impression smears by the fluorescent antibody technique was applied to six cows, a horse
and a dog, all showing clinical signs of rabies, subsequently confirmed by finding viral antigen in brain material. Three ewes and the horse were positive to the test while the other four animals were classified as negative. It was concluded that the cornea test may be of value in the early diagnosis of rabies but that negative results do not necessarily indicate freedom from infection (Zimmerman, 1971).

Schaaf and Schaaf (1971) carried out cornea test on 81 foxes, five sheep, four deer, four cats, three martens, a dog, a cow and a horse. They observed that the test which is reliable in the living patient was unreliable in deteriorated material. Immunofluorescent examination of the nasal mucosa was not as effective as corneal test but demonstrated the establishment and multiplication of virus in the nasal mucosa.

Beseda (1972) examined FAT positive 136 naturally infected animals (including 92 foxes) and found that 116 (85 per cent) gave positive and five gave doubtful corneal test.

Reis et al. (1972) reported the results of 54 brains, 52 corneas, 54 aqueous humour and 49 optic nerves examined by immunofluorescence and mouse inoculation (IF and MI). The pattern of agreement was as follows:
Larghi et al. (1973) evaluated corneal test (CT) for rabies diagnosis in samples from 313 subjects of different species. Some of the subjects were inoculated experimentally and others were naturally infected. When the CT was compared with immunofluorescence staining and mouse inoculation tests on brains of the same subjects, a sensitivity of 41.7 per cent and a specificity of 100 per cent were found. The authors concluded that positive CT result would confirm the diagnosis of rabies but a negative one would not exclude the possibility of the disease.

Kissling (1975) summarised the results of 25,569 brains examined for presence of Negribodies by using FAT by various workers. Out of these 3,049 were positive and 87 of these were negative by FAT (2.9 per cent).

Horyna and Kabelik (1973) isolated rabies virus from fox and passaged ten times in mice. It was then injected in 20 mice (Group I) intracerebrally, in 50 mice (Group II) intramuscularly and in 12 rabbits (Group III). In group I, corneal test became positive one to three days before the
appearance of symptoms in 17 mice, at the same time as symptoms in two mice, and two days after the appearance of symptoms in one mouse. In group II the corneal test was also positive one to three days before appearance of symptoms in 31 mice, at the same time as symptoms in 11 mice and one to two days after symptoms in two mice. Six mice remained negative and did not show symptoms. In group III positive tests were given by five rabbits at the same time as symptoms appeared and in four rabbits one to five days after the appearance of symptoms. Two rabbits remained negative and symptomless.

Foxes that were positive by FAT on brain were also positive by corneal test. The corneal test gave false negative results only when the specimen was in a macerated state. Out of 133 foxes in which rabies virus was demonstrated in the optic nerve by animal inoculation, only one was negative to the corneal test - this may have been a case of centripetal spread of the infection which was examined at a stage when virus was present in optic nerve but not in cornea. False positive results seem unlikely to occur, for only one fox was negative upon inoculation of animals with optic nerve and vitreous humour, while the FAT of corneal cells was positive. Animal tests reveal virus inhibitors in the vitreous humour of this animal. All the other cornea test positive foxes were also positive in tests on brain, lacrimal gland and optic nerve (Wiegand, 1975).
Tillotson et al. (1977b) observed in a human case corneal impression smears after about a month of the onset of disease, and the skin biopsy were negative by FAT, when CSF titer was 1:16,225.

Vasconcellos et al. (1978) examined 52 corneal smears by direct immunofluorescence and 52 saliva samples by intracerebral inoculation in mice. In the incubation period, 94 per cent and 97 per cent in the clinical phase were positive to the cornea test, while for the same two phases, 56 per cent and nine per cent respectively gave positive saliva test.

Cortes et al. (1978) applied indirect FAT to corneal samples from 18 cattle with experimental rabies and saliva from same animals were injected intracerebrally in mice. Indirect FAT gave 94 per cent positive and were positive till death, whereas only 25 per cent saliva were positive and no positive saliva tests were obtained from animals with a negative corneal test. The false corneal test suggest that a negative result does not exclude the possibility of the disease.

Vasconcellos (1978) infected eight dogs experimentally with rabies virus. Starting 10 to 18 days later, daily until dog's death, corneal impression smears were taken for the direct FAT and samples of saliva for MIT. Seven of eight dogs gave positive corneal test as soon as clinical symptoms appeared, some sooner, subsequently all eight remained positive to this test. With the saliva test, only one of the eight
dogs was positive at the appearance of clinical symptoms and subsequent tests were either negative or showed only one positive result.

Bradley (1979) found a correlation of almost 99 percent between the PAT and MIT.

Kalra and Singh (1980) studied fluorescent antibody technique (FAT) using horse and guinea pig conjugates and four histological stains were employed to demonstrate rabies antigens/Negribodies in the brain tissues of mice experimentally infected with street rabies virus. Out of 58 infected mouse brain smears tested, rabies antigen was detected in 52 by FAT using horse antibody conjugate. Only 26 of these 58 smears were tested with guinea pig conjugate and 22 were found positive. The remaining four smears found negative by guinea pig conjugate were negative by horse conjugate also. Of the remaining 30 FAT brain positive tissue samples stained with histological stains, Negribodies could be demonstrated in paraffin sections/smears of 26, 22, 22 and 22 cases by Schleifstein, acid fuchsin methylene blue, modified Van Gieson and Seller's stain, respectively. Negribodies were found more frequent in cerebrum than in any other part of the brain.

**FAT on skin:**

Smith et al. (1972) stated that rabies virus antigen can be detected in the skin of the mice that have been inoculated with rabies virus of bovine, feline and canine origin. The antigen seems to be in neural elements of the skin.
The direct FAT using labelled antirabies globulin on frozen sections of skin, gives this result. Antigen is reliably found in the skin of the face or neck one day, and occasionally as early as four days prior to onset of clinical signs. Homogenised skin, when injected intracerebrally into young mice, has induced confirmed rabies in all of several attempts. A biopsy technique for specifically diagnosing or predicting the onset of rabies would be useful to better evaluate exposures of human beings and to better define the natural history of rabies.

Several strains of rabies virus inoculated by various routes into laboratory mice can be identified in neural elements of the skin on or slightly before the day of onset of disease. Frozen sections of the skin of the head region particularly the muzzle or cross sections of ear are stained with FITC labelled antirabies globulin and Evan's blue counter stain (Blenden, 1974).

Blenden (1978) made further report on his work and stated that the rabies virus antigen was identified by immunofluorescence in histological sections of the skin from mouse, dog, cat, bat, fox, skunk, raccoon, mongoose, cattle, horse and rhesus monkey. The findings of antigen was well correlated with the results of tests on brain tissue.

Blenden (1981) reported his studies on skunks. Four juvenile skunks were vaccinated against rabies, descented, and then placed in four different households. An illness
subsequently developed in three of the skunks that was confirmed as rabies by biopsy examination of muzzle skin by immuno-fluorescence. In one clinically normal skunk the skin was found to be test positive, predicting that rabies would develop, the signs of rabies became evident in 48 hours. The infection was considered to be field acquired rather than vaccine induced.
(vi) IMMUNOPEROXIDASE TECHNIQUE
(vi) **IMMUNOPEROXIDASE TECHNIQUE:**

Kellin and Hartree (1951) reported that horse radish peroxidase was a hemoprotein with a molecular weight of about 40,000. In comparison, the molecular weight of hemoglobin is about 68,000 (Adair, 1925).

Mitsui (1960) first reported the use of benzidine in ultrastructural cytochemistry in a study of peroxidase in salamander leucocytes.

Graham and Karanovsky (1966) observed that benzidine and O-dianisidine can be used as substrates in ultrastructural cytochemistry of injected peroxidase in mouse kidney. The localization of enzymatic activity obtained with these substances is the same as that obtained with 3,3'-diaminobenzidine, the clear superiority is due to the greater electron opaqueness of its reaction product.

Singer (1959) developed a method whereby antibodies could be conjugated with ferritin for specific immunochemical identification of viruses and virus proteins by electron microscopy. Rabies virus was tagged for such study by Atanasiu et al. (1963).

Acid phosphatase has been used successfully for the light and electron microscopic localization of antigen (Nakane et al., 1966) but in contrast to the peroxidase method, the conjugation of acid phosphatase and antibody often resulted in inactive materials. Whereas, acid phosphatase
antibody conjugates were denatured by freezing and lost their activity in only a few days when stored in refrigerator, peroxidase antibody conjugates are stable and have been stored for several months at 4°C and indefinitely in frozen state (Nakane and Pierce, 1966).

Nakane and Pierce (1966) observed that enzyme labelled antibodies have distinct advantages over the fluorescent antibody method. The preparations stained with peroxidase antibody are permanent and may be examined under light microscopes. It was found as specific as immunofluorescent techniques.

Breese and Hsu (1971) recommended ferritin tagged antibody method as it allows high resolution in electron microscopy and can reveal accurately the fine structure of viral antigens at ultrastructural level.

The main disadvantage of immunoferritin technique is its high molecular weight (690,000). Thus the ferritin antibody complex has a molecular weight of at least 800,000, which increases considerably the difficulty of its penetration into the tissues or cells. Therefore, the use of this technique for the detection of viral antigens at the ultrastructural level is limited. This technique is excellent for demonstration of extracellular antigens. Other heavy metals proposed like mercury and uranium is not in use (Kurstak, 1971).
Dixon and Webb (1964) stated that horseradish peroxidase has relatively low molecular weight (40,000) compared to 100,000 for alkaline phosphatase from calf intestine, and 150,000 for glucose oxidase from penicillium. Alkaline phosphatase and glucose oxidase have been used for histochmical identification and the ELISA assay, although they are far better suited for the latter (Benjamin, 1979).

Levaditi et al. (1971) reported that when a purified antibody conjugated with peroxidase is allowed to react with fixed specimens, (impression smears, tissue sections, culture cells) on slides, and examined under the high power of ordinary microscope, cells containing rabies antigen were clearly visible.

Kurstak (1971) observed that because of the low molecular weight the penetration of peroxidase labelled antibodies into the cells is very satisfactory and better than those antibodies labelled with other enzymes or with ferritin. The enzyme is not altered during reaction with its substrate, each antibody molecule is marked with several enzyme molecules and its great sensitivity and specificity makes it popular method.

Mason et al. (1969) described immunoglobulin enzyme bridge method. In this method the antigen X is first labelled with rabbit anti-\(X\) IgG and this antigen antibody complex binds indirectly to rabbit anti HRP-antibody, using as a bridge, unlabelled swine antirabbit IgG in excess. The variable
regions of the anti HRP rabbit antibody bind free HRP and the whole complex is then demonstrated with the standard DAB reaction.

Sternberger et al. (1970) introduced a modification of the bridge method in order to optimise the immunological conditions. They combined the last two stages of the previous method, that is the rabbit anti HRP antibody and free HRP (Horse radish peroxidase), by reacting them together forming a soluble PAP complex (peroxidase anti peroxidase complex). This complex then acted as the final antigenic system which was linked to the initial rabbit anti X-IgG by the swine antirabbit IgG. This method is called the unlabelled antibody HRP-anti-HRP method or PAP method.

Levaditi et al. (1973) showed the importance of using animal inoculation, histology and immunofluorescence in rabies diagnosis and discussed the merits of immunoperoxidase technique in that relation.

Kurstak and Kurstak (1974) stated that the immunoperoxidase technique is potentially useful for the detection of virus infection in tissue culture.

Atanasiu (1973) described immunoperoxidase reaction for demonstration of rabies virus.

The ideal application for this rapid diagnostic procedure is for the direct detection of viral antigen in patient material. This has been successfully accomplished in herpes simplex infection (Benjamin and Ray, 1975).
Burns (1975) demonstrated hepatitis-B antigen in formalin-paraffin sections by immunoperoxidase and immunofluorescence methods.

The two factors which may interfere with the interpretation of the result of the immunoperoxidase technique, particularly in formalin paraffin sections, are endogenous peroxidase activity in red blood cells, granulocytes (especially eosinophils) and acid hematin, and background staining (uptake of the immune reagents by other tissue components besides the antigen under investigation (Burns, 1978).

Krusman et al. (1975) were able to reduce endogenous staining of the red cells by employing a weak concentration (0.001 per cent) of hydrogen peroxide in DAB solution.

The background staining can be reduced by using purified HRP (Rule et al., 1975), or by using improved conjugates (Ternynck and Avrameas, 1976) or by enzyme digested paraffin sections (Reading, 1977).

Gerna et al. (1976) detected 16 cytomegalovirus isolates from ill and healthy patients by either direct or indirect immunoperoxidase technique (DIP/IIP). In 13, confirmation was done by immunofluorescent antibody. The IIP was preferred over DIP, since the latter showed a certain amount of background staining. The technique can detect individual cell CMV infection at 24 hours. It is sensitive, specific and allows direct identification of infected cells in primary
isolates in as little as 90 minutes. It can be performed in standard isolation tissue culture tubes, whereas IFA requires transfer of infected cells on slides or leighton tubes.

Immunoperoxidase techniques or variations of them have been applied to a rapidly increasing range of tissue antigens, not only in formalin but also in acid alcohol, picric acid and Zenker fixed material processed to and embedded in paraffin wax. These antigens include hormones (Erlandsen et al., 1975), enzymes (Burns, 1977), intracellular immunoglobulins (Burns et al., 1974), tumour associated antigen (Struss and Pascal, 1975) hepatitis surface and core antigen (Huang, 1975) soil amoeba (Culburton, 1975) and carcino-embryonic antigen (Primus et al., 1975).

Atanasiu et al. (1977) used antigen from semipurified tissue culture virus and peroxidase conjugated antihuman immunoglobulins. Sera from immunized humans were evaluated by this procedure and the values obtained showed good correlation with seroneutralization titers. The detection of antibodies by this method is earlier than by seroneutralization.

ICMR Annual Report (1977) stated that the immunoperoxidase test used in the diagnosis of rabies at Central Research Institute, Kasauli have shown that the test is satisfactory in case of brains with a high concentration of rabies virus, but it is not dependable when the virus concentration is low.
Burns (1978) indicated that the use of diaminobenzidine in immunoperoxidase reaction can be substituted by oxidative coupling reactions of aromatic amines in the presence of phenols which is a suitable non-carcinogenic substitute as suggested in recent studies.

Enzyme linked immunosorbent assay - ELISA - is similar to the indirect immunoenzymatic procedure, but it is rendered more quantitative by absorbing the antigen (or antibody) to a solid phase (e.g. base of microtiter plate wells, agarose beads) and measuring the amount of enzyme - labelled antibody reacting with it. Two basic criteria are to be required to be fulfilled for the development of a satisfactory assay. The first is, the antigen or antibody must be attached, usually adsorbed, on to a solid phase support without altering immunologic activity. The second is; the antibody enzyme complex must retain both immunologic and enzymatic activity (Benjamin, 1979).

Taylor (1978) listed the disadvantages of the fluorescent antibody technique like the need of a specialised microscopic equipment, impermanancy of the stained slides, nonspecific reactions etc. These disadvantages are largely avoidable by use of immunoperoxidase technique. The advantages of the immunofluorescence method are that it is less time consuming, wide range of FITC conjugates are available commercially, simultaneous demonstration of two antigens by contrasting colours by using rhodamine conjugates.
These aspects of study of immunoperoxidase methods are only in their infancy.

Genovose and Andral (1978) compared the relative efficiency of the immunofluorescence and immunoperoxidase tests in 500 samples from both wild and domestic animals with suspected rabies. These results were also compared with those obtained by mouse inoculation which was positive in 230 (immunofluorescence 223 and immunoperoxidase 217). The accuracy of the immunofluorescence was 1.2 per cent higher than that of the immunoperoxidase test. However, the immunoperoxidase test remains a useful and sufficiently sensitive diagnostic method particularly in laboratories not able to carry out fluorescent microscopy.

Atanasiu and Perrin (1979) described a micromethod of the immunoenzymatic test for rabies antibody, suitable for use in mammalian samples. This method made use of protein A of Staphylococcus aureus or anti immunoglobulin antibodies conjugated to peroxidase to demonstrate antirabies immunoglobulins. The antigen used is either purified rabies virus or its glycoprotein. Good reactions were obtained with serum from dogs, foxes, hamsters, horses, rabbits and cats after vaccination against rabies. Preliminary tests showed good correlation with the results of serum neutralization in mice.

Benjamin (1979) listed the advantages of selecting enzyme labelled methods over fluorescent methods, which include; reaction is detected under light microscope, the
reactions are electron dense making electron microscopy possible, preparations are permanent, by prolonging the substrate incubation the sensitivity can be enhanced, endogenous enzyme activity can be easily blocked, reagents and conjugates are more easily standardised, few nonspecific reactions, and particularly with soluble PAP method there is no chemical alteration of the antibody. In routine diagnostic virology the major value of this method lies in direct detection of viral antigen in clinical samples, detection and identification of viral antigen in tissue culture, typing of viruses in tissue culture and serology.
(vii) PASSIVE CUTANEOUS ANAPHYLAXIS

(PCA) REACTION
(vii) PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) REACTION:

Anaphylaxis and allergy are two manifestations of hypersensitivity. The word anaphylaxis is derived from a Greek word and is an antithesis of prophylaxis and is coined by Richet (Portier and Richet, 1902).

The term allergy meaning an altered reaction was coined by Von Pirquet (1906) to indicate the hypersensitivity reaction of the body to an allergen.

The term Passive Cutaneous Anaphylaxis (PCA) and the prototype experimental procedure were introduced by Ovary (1952).

PCA is closely related to Prausnitz-Kustner (P.K.) reaction in man, which detects homocytotropic antibody where antisera from the same species (human) is used. However, in PCA reaction, as is usually carried out may involve antibodies of both reaginic and other types.

Systemic anaphylaxis is a manifestation of antigen antibody reaction in vivo. Benacerraf and Kabat (1949) reported that 0.03 mg, 0.06 mg, 0.24 mg, 1.02 mg and 2.0 mg of antibody nitrogen (AbN) per ml. given intravenously (i/v) to guinea pigs will produce sensitisation after 5 hours, 2 hours, 1 hour, 30 minutes and immediate, respectively against the shocking dose of 0.15 mg of antigen given also by i/v route.

Dean et al. (1936) stated that in Passive Cutaneous Anaphylaxis reaction it is necessary to permit a few hours
between the sensitization injection of antiserum and shocking
dose of antigen, because the antibodies should be fixed to
the tissues before the reaction can take place.

Chase (1943) stated that Passive Cutaneous Anaphylaxis
reaction is admirably suited to the detection of small quan-
tities of weakly precipitative antibodies of certain kinds.
This is an in vivo reaction which depends upon the fact that
antibodies deposited in the skin of a suitable animal and
given time presumably to attach to the tissues will then
react with intravenously injected antigen to produce a rapidly
occurring evanescent wheal and erythematous response.

This response can be much better seen if, along with
intravenously injected antigen, an intense dye such as Evan's
blue is included. At the site of the reaction where capillary
dialatation occurs with increase permeability of vessel walls,
leakage of the dye makes the reaction readily apparent
(Ramsdell, 1928).

This mode of carrying out a local hypersensitive
reaction is referred to by Ovary (1958) as local anaphylaxis
because it presumably depends upon the same immunogenic
considerations as determine anaphylactic shock. Since the
reaction is passively induced by transferred serum and
because it is carried out in the skin, it is referred to as
passive cutaneous anaphylaxis.

The passive cutaneous anaphylaxis (PCA) reactions is
considered to be due to the local release of the mediators,
the chief mediator substance released by the antigen-antibody reaction in vivo to be histamine or similar substances referred to as H-substances (Dragstedt, 1945).

Other pharmacologically active substances released besides histamine or histamine-like substances, (Raffel, 1953) are possibly the slow reacting substances (SRS-A) (Weiser et al., 1969), serotonin (Wasilkes, et al., 1957), or plasmakinins like bradykinin (Baraldo, 1950) or anaphylotoxin (Ovary and Tigelaar, 1971).

Fisher and Cooke (1957) stated that influence of antihistamines varies considerably in different hypersensitive manifestations of animals and man. The PCA reaction in guinea pigs is reduced in intensity but not entirely subdued by these agents.

Humphrey (1958) observed that Lysergic acid diethylamide (LSD) is an effective antagonist of serotonin and in rat skin dibromo-LSD reduces the intensity of PCA response.

Rosenberg et al. (1958) were able to reveal antibodies in the earliest phase of production by splenic cells removed from a donor three to four days after a primary vaccination and injected intracutaneously in lieu of serum. Thus PCA reaction under appropriate circumstances, is a sufficiently sensitive detector of antibodies.

Osler et al. (1959) believed that complement is required in vivo for the occurrence of PCA reaction.
However, Lauenstein (1969) conducted a complement inhibition study with the poly-B-oxide of the nicotinic acid ester of polyvinyl alcohol (PVA-NA) and reported that the PCA reaction is not dependent on the presence of complement.

Ovary and Briot (1951) reported that the immediate cutaneous anaphylaxis can be better visualised by injecting intravenously certain dyes of large molecular size, which are not normally diffusible through capillaries. Trypan blue or Evans blue, to which normal capillaries are impermeable, will diffuse through the capillaries if they get dilated by the local release of histamine or histamine like substances formed due to antigen antibody combination. These vasoactive substances increase the permeability and the dye pass out of the capillaries at the site of antigen antibody reaction. The cutaneous anaphylaxis test can detect as little as 0.003 µg or less of AbN in 0.1 ml of serum.

Miles and Miles (1952) reported that since the test is dependent upon the proper blood flow in the skin, the animals used for the test should not be frightened or excited and the injection should be done quickly. They also mentioned that after the intradermal injection of antiserum, the bleb formed should be clearly visible and should persist for about five minutes. They further stated that the reaction depends upon a good blood flow in the skin, it is most satisfactory in animals which are not anaesthetised and are not frightened or excited.
A comparison of PCA test in guinea pigs with the Arthus phenomenon was reported by Ovary and Bier (1953).

Anaphylaxis is an immediate type of hypersensitive reaction. In anaphylactic shock a series of events start immediately after the entrance of the antigen preferably intravenously into the animal which has been sensitised by active or passive immunisation. The combination of antigen with specific antibody in vivo causes the sudden release of histamine and other pharmacologically active substances which may lead to sudden death of the animal if not treated immediately. The histopathological changes noticed in anaphylaxis are the contraction of the smooth muscles and an injury to the vascular endothelium with subsequent increased vascular permeability (Cushing and Campbell, 1957).

According to Ovary (1958) guinea pigs are not sensitized by cow, chicken, horse, and sheep antibodies.

Raffel (1961) stated that antibodies of heavy globulin \(19_s\), the gamma globulin) are unsuccessful in sensitizing, whether from human beings or rabbits. The sera of some animals will not induce the reaction regardless of the kind, quality or quantity of antibody. This is true for horse and goats.

Albino guinea pigs are best suited for the PCA reaction, since these animals react to very low doses of rabbit antibody. The PCA reaction can also be elicited in rabbit, cat, dog, monkey, mouse, rat, hamster and chicken (Ovary, 1964).
Rose et al. (1964) reported that the monkeys can be used more than once for the PCA test.

Brocklehurst (1967) used monkeys for the PCA test with human IgA.

Janoff et al. (1961) reported a sensitive method, for the detection of antigen by utilizing PCA test. In this test, antibody and the unknown antigen is mixed before intradermal injection. The neutralized antibody will fail to give the reaction or will be smaller compared to the reaction with untreated antibody.

Antibodies produced by human beings, rabbits, and guinea pigs can induce the reaction with appropriate antigens, but not all antibodies from these species are suitable. Thus in human serum the peculiar reagents associated with the atopic hypersensitive state are unsatisfactory, as are some of the human antihuman erythrocyte antibodies (Raffel, 1961).

Circulating antibodies reactive with thyroid substance may be found in lower incidence in the subacute thyroiditis, accompanying viral infections, in hyperthyroidism, in goiters of diverse kinds and in carcinoma of gland. The circulating antibodies found in these disorders are gamma globulins and they react with thyroglobulin or thyroid extract in a variety of serologic procedures including the Boyden haemagglutination, the agar diffusion precipitation, the passive cutaneous anaphylaxis and complement fixation tests (Ovary et al., 1958; Roitt and Doniach, 1959).
Judah and Willoughby (1962) reported correct method for the quantitative estimation of dye leakage in the skin tests is not by measuring the diameter of blueing or intensity of blueing, but by the extraction of the dye from the tissues and determining the quantity of dye in absolute amounts.

Sevitt (1958) stated that Evans blue is used as 2 per cent solution and the dose is 20-100 mg per kg of body weight.

Sepector (1958) used trypan blue 40 mg per kg body weight as one per cent solution mainly in experiments involving chemically induced lesions.

Frimmer and Muller (1962) described the method for the extraction and evaluation of Evans blue.

Miles (1959) has summarised the PCA reaction as the endothelium that separates the circulating blood from the tissues in the normal animal is nicely adjusted to maintain a highly selective exchange of material between the two. One common feature of nearly all allergic and inflammatory reactions is a dislocation of this endothelial barrier.

Broeklehurst (1967) stated that modifications of the tests have been employed to test the antigen, like P.K. reaction in man. The PCA is said to be "reversed" when the antigen is given before the antibody. The reversed PCA has been used to demonstrate that certain antigenic substances such as Y globulins, Y globulins - heptan conjugates, and Y globulin fragments produced by partial proteolysis, are
retained in the skin. "Fixation" in the injection site is shown by a PCA response to the injection of antibody after 3 or 4 hours.

Smith and Jones (1966) stated that diagnostic use is made of the hypersensitivity existing in the diseases. They give an example of tuberculin reaction, in which an intradermal tuberculin is injected and after a usual incubation period for antigen antibody reactions, a localised inflammatory swelling develops. It is composed of usual ingredients of acute, non purulant inflammatory exudates, serum, a little fibrin, lymphocytes, mononuclears of the reticulo-endothelial group and a scattering of other inflammatory cells.

Goose and Blair (1969) reported that disodium cromoglycate (DSCG) inhibited the PCA in rats when tested with homologus reagin like antibodies.

DSCG inhibited the antigen induced release of histamine from mast cells while it did not affect the release of slow reacting substance A from leucocytes (Morse et al., 1969).

Macher (1969) applied PCA test for detecting the heptan specific circulating antibody and reported the absence of antibodies in tolerant group.

Ovary et al. (1964) reported that gamma-1 is active in the homologus species and gamma-2 is active in heterologus species (homocytotropic and heterocytotropic antibody respectively).
In man homologus IgE passively sensitizes the skin to PCA reaction (Ishizaka and Ishizaka, 1967).

Ishizaka et al. (1967) reported that monkeys are also sensitized by human IgE antibody.

Vaz and Ovary (1968) reported that mice are not sensitized by guinea pig gamma-1 or gamma-2 antibodies.

The counterpart of human IgE responsible for PCA reaction in other species (heterocytotropic antibody) has been reported in rat (Mota, 1964), rabbits (Zvaifler and Becker, 1966), mouse (Prouvost-Danon et al., 1966), and guinea pig (Catty, 1969).

Two gamma-1 heat stable globulins and one heat labile reaginic antibody present in the guinea pig serum are capable of producing PCA reaction in guinea pig (homologus or homocytotropic) tissues (Parish, 1970). PCA active antibody belongs to either an IgG subclass or can be a heat stable homocytotropic antibody (Vanés et al., 1970).

Weiser et al. (1969) stated that the PCA reaction is considered to be due to the local release of the mediators from the mast cells.

Hawrowitz (1963) reported that the Fc portion of the antibody molecule is essential for the development of PCA reaction, because it is supposed to fix to the skin of the injected animal through the Fc portion.
Ovary and Tigelaar (1971) have also reported that the Fc fragment of the IgG or IgE is the one responsible for the biological activity of sensitization.

It seems clear that mast cells become coated by a particular type of antibody whose Fc region can bind specifically to sites on the mast cell surface. The most effective homocytotropic antibodies belong to the IgE class but it is clear that IgG antibodies can also act as reagins although the extent of their contribution to the allergic state in the human is not yet resolved. IgG reagins differ from IgE in their relative insensitivity to mild heat and 2-mercaptoethanol reduction and especially in their lower binding affinity for mast cells; whereas IgE antibodies can be detected at the site of intradermal injection into a normal individual for several weeks, IgG disperses within a day or so. The technique of passive cutaneous anaphylaxis utilises this dermal reaction as a highly sensitive indicator for reaginic antibodies (Roitt, 1977).
(viii) COMPARATIVE EVALUATION
OF
VARIOUS METHODS
Kokles and Wittman (1964) reported that the demonstration of Negribodies in brain smears stained by the method of Murmotskev agreed with results of mouse inoculation test in 93.5 per cent cases, and by complement fixation test in 90.3 per cent of cases. They observed that smears fixed in methyl alcohol for 30 minutes at 50°C or for 90 minutes at room temperature did not contain infectious virus.

Schneider and Wachendorfer (1964) compared three diagnostic methods using brains of 224 domestic and wild animals. Rabies was confirmed in 110 by FAT, and 109 by mouse inoculation (MI). Negribodies were present in 91 samples. By all methods, rabies was confirmed in 111 samples.

Beauregard et al. (1965) examined 750 domestic and wild animals submitted for rabies diagnosis by William's stained impressions, mouse inoculation and FAT. Out of the 175 positive samples only 58 (33 per cent) were detected in Williams stained impressions. On the other hand two rabies cases were missed by mouse inoculation and four by FAT.

Neuman (1965) made comparative evaluation of three diagnostic methods on 62 positive specimens of rabies. FAT detected 90 per cent of cases as against 58.3 per cent revealed by Seller's stain. However, mouse inoculation was the most accurate method. FAT yielded two false positives and it failed to detect six infected animals, one of them positive by Seller's method.
Histochemical staining, mouse inoculation and FAT were compared to determine the presence of rabies antigen in brains of skunks and foxes dying of rabies by Wilsnack and Parker, (1966).

Histochemical staining and FAT were used on salivary glands also. Results indicated that FAT proved to be the most sensitive in brain as well as salivary glands. Mouse inoculation was not a definitive test in salivary glands or brain because of the presence of "Rabies Inhibiting Substance" (RIS) which rendered the virus non-lethal for mice when they were inoculated intracerebrally. However, in presence of RIS, rabies antigen was detectable by FAT.

Hurter (1966) studied 1673 brain samples from rabies suspected domesticated and wild animals, ruminants, carnivora and birds by FAT, mouse inoculation and Negribody detection. Mouse inoculation and Negribody staining was confirmed by FAT in 498 and 607 cases respectively. The FAT yielded best results.

The importance was shown of histological examination and fluorescent antibody staining of Gassarion ganglion in the diagnosis of rabies. The value of these procedures is enhanced when damaged or putrification of the brain prevents the detection of Negribodies. Among 28 dogs, an ox and a fox, all but eight dogs were positive. Only two animals were positive to one test and not the other. Histological studies of the adrenal glands showed cellular infiltration in the medulla of 11 animals, but Negribodies were not observed.
The salivary glands showed fluorescence in 12 cases (54.5 per cent). A doubtful fluorescence was observed in three cases in adrenals, but inoculation of mice with these glands gave negative results (Reis and Lamas, 1967).

Three diagnostic methods, the FAT, mouse inoculation and Seller's staining, were compared in 1,108 specimens received in varying states of preservation. All were (100 per cent) positive to mouse inoculation (346 of the total). Of the 201 positive specimens in a good state of preservation, 91 per cent were positive to FAT and 64 per cent to Seller's staining. Of the 93 in a fair state of preservation, 79 per cent were positive to the FAT and 60 per cent to Seller's staining and of the 52 in a poor state of preservation, 34 per cent were positive to the FAT and 19 per cent to Seller's staining (Salido and Romero, 1967).

Lucas et al. (1970) observed that out of the 247 confirmed cases of rabies, Seller's staining was positive for 65.6 per cent, histological examination for 73.2 per cent and FAT was the most certain technique with good specimens, it was possible to make a diagnosis within 48 hours in 89.5 per cent of cases. They concluded that several techniques should be used in routine diagnosis and the inoculated mice should be kept under observation for at least 28 days.

Levaditi et al. (1973) compared animal inoculation, histology and immunofluorescence on 2104 animals submitted for rabies diagnosis. All the three tests were negative in 1926 and all three were positive in 147. Histology was
negative in 13 cases, FAT in seven cases and in one case only animal inoculation was negative. The authors while discussing the merits of the immunoperoxidase techniques, emphasized the need to perform all three tests for rabies diagnosis.

da Silva et al. (1973) found that the FAT was of comparable efficiency to mouse inoculation and superior to demonstration of Negribodies in diagnosis of rabies.

Weigland (1973) stated that between 1953 and 1965, 15,159 specimens from domestic and wild animals were submitted for rabies diagnosis. According to different combinations of the results of the three diagnostic tests (histological, fluorescent antibody and mouse inoculation) 6.4 per cent were positive, 37.8 per cent were conditionally positive, 30.7 per cent negative, 20.8 per cent conditionally negative, 3.1 per cent doubtful and in 1.2 per cent no diagnosis was made. Statistical analysis of the data showed that positive diagnosis are more certain than negative and that negative diagnosis have an average of 2.25 per cent uncertainty and conditionally negative 7.13 per cent.

Koch et al. (1974) compared the virus neutralization test in mice (N) and the immunofluorescent antibody (IFA) test as method of estimating rabies antibody on 296 serum samples. The samples were taken at different times from 85 patients immunised with Hempt vaccine. Statistical analysis of the result showed 98 per cent agreement between
the test on preimmunization sera. In samples taken up to 10 days after vaccination began, antibody was detected in more samples by IF A than by N test. No statistically significant correlation was found between the tests, when the results of the later samples were analysed. The authors suggested that both the methods have advantage and disadvantage although neither necessarily provides an index of protection against rabies, they do indicate experience of rabies antigen.

Germano (1976) evaluated three techniques, Seller's FAT and MI on 2242, samples submitted for examination at Pasteur Institute, SaoPaulo, Brazil. He found direct FAT and MI most sensitive and agreed best with each other. Seller's was the least sensitive and results correspond poorly with those of the other two methods. It was suggested that two or more diagnostic methods should always be used together. Great sensitivity was achieved with a combination of MI and direct FAT (Germano et al., 1977).

A group of 18 half bred Zebu bullocks, 18-32 months of age, were experimentally infected with rabies. From the appearance of clinical symptoms, samples of saliva and corneal impression smears were taken daily. All the cattle were killed in agonic phase of the disease, and the hippocampus, cerebellum, bulb and medulla were examined histologically to confirm diagnosis. The corneal test was significantly more sensitive than intracerebral inoculation of mice and
immunofluorescence gave more consistent results in successive tests. However, 17 per cent of the false negative by immunofluorescence indicated that negative results by this test cannot exclude the possibility of rabies (Cortes, 1977).