

DETECTION OF RABIES VIRUS IN SELECTED TISSUES OF
NATURALLY INFECTED SKUNKS

by

DENNIS RAY HOWARD

B.S., Kansas State University, 1972

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

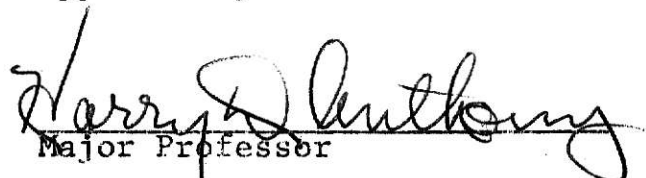
MASTER OF SCIENCE

Graduate Pathology Group

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Approved by:


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SUMMARY AND CONCLUSIONS

The FRA test was described for the detection of rabies virus antigen in the brain, salivary gland, cornea, lip, tactile follicle, and skin; a positive correlation existed in detection of rabies virus antigen in all tissues examined. Results of the study indicate that these tissues were 100% effective for the diagnosis of rabies in naturally infected skunks. Utilization of these tissues would be a practical means of determining rabies infection when sufficient brain material was unavailable because of trauma or autolysis.

INTRODUCTION

Goldwasser and Kissling¹² (1958) reported that fluorescent antibody technique was suitable for the detection of rabies virus antigen in the brains of experimentally infected mice. Since then many investigators have used the immunofluorescent method for detection of rabies virus antigen in other tissues including retina, peripheral nerve endings associated with hairs in the cheek and lip, intestine, adrenal medulla, taste buds, olfactory neuroepithelium, pancreas, stomach, lung, esophagus and cornea.^{1,3,5,6,8,10,11,14,15,16,17} Evidence of widespread rabies infection in salivary glands was demonstrated by other investigators.^{4,9,20,21}

This investigation reports the use of the fluorescent rabies antibody (FRA) test in demonstrating rabies virus antigen in tissues of naturally infected skunks, Mephitis mephitis. Tissues examined were the brain, cornea, submaxillary salivary gland, lip, tactile hair follicles, and skin from the back of the neck; the study was designed to evaluate the use of the FRA test on tissues other than brain in naturally infected animals.

MATERIALS AND METHODS

Specimens--Skunk heads for rabies diagnosis were presented to the Veterinary Diagnostic Laboratory, Kansas State University. Heads were shipped via bus, express service, mail, bankers dispatch and personal carrier. Transit time varied from four hours to 48 hours. Specimens arrived in various stages of post mortem decomposition. Examinations for rabies were conducted on day of arrival and the specimens were stored at -70° C. until further examination.

Anti Rabies Globulin--Anti-Rabies Globulin^a fluorescein labeled was used in this investigation. The reagent was an immune globulin of equine origin labeled with fluorescein isothiocyanate.

^aBBL, Division of Becton, Dickinson and Company, Cockeysville, Maryland 21030. Lot #L7GAHF.

Microscopic Equipment--A microscope^a equipped with a dark-field condenser was employed. An HBO 200 ultra high pressure mercury vapor lamp^b served as a light source. Filters included the heat suppression, (BG-38), the exciter (KP490), and the suppression filter (K510). Objectives included 10X, 25X, 43X, and 54X oil immersion and eye piece magnification of 10. A microscope^a equipped with a ploom illumination system was also used. Objectives included the 10X, 16X, 25X and 63X high dry. Eye piece magnification was 10.

Photographs were taken with a fully automatic microscope camera^a and High Speed film,^c 23 Din, 160 ASA pushed to 27 Din, 400 ASA.

Adsorbing CVS II and NMB Suspensions--Normal Mouse Brain (NMB) and Challenge Virus Standard (CVS II) Rabies Fixed Virus^d suspensions were prepared by the following method:

Rabies Fixed Virus (CVS II) 20% Mouse Brain Suspension

1. Make a 1:100 dilution of CVS virus in .01 M phosphate buffered saline, pH 7.6-7.8.

2. Inoculate 0.02 cc. of diluted virus intracerebrally into each of 30 mice.

^aE. Leitz, Inc., Rockleigh, N. J. 07647.

^bOsram, Macbeth Sales Corporation, Jeanne Drive S & G Industrial Park, Newburgh, N. Y. 12550.

^cEastman Kodak Company, Rochester, N. Y. 14650.

^dCVS II Strain of Fixed Rabies Virus, Center for Disease Control, Lawrenceville Facility, Lawrenceville, Ga. 30245.

3. As the mice become paralyzed (6-9 days), harvest brains aseptically. Weigh the mouse brain tissue.

4. Prepare Rabies Fixed Virus 20% Mouse Brain Suspension by mixing the following materials:

4.0 gm. CVS II infected mouse brains

3.2 ml. sterile horse serum^a

12.0 ml. phosphate buffered saline, (PBS) containing 0.75%
bovine albumin fraction V^b pH 7.6-7.8

2.0 mg. merthiolate^c (dissolved in the 12.0 ml. P.B.S.)

5. Grind brains, PBS and sterile horse serum in a Ten-Brook grinder.

6. Centrifuge at 1,000 r.p.m. for 5 minutes and draw off the supernatant.

7. Reconstitute Anti-Rabies Globulin, fluorescein labeled.

8. Evaluate by FA (in this investigation 1:40).

9. Incubate the final rabid mouse brain suspension for 10-15 minutes in a 37° C. water bath to insure complete reaction of the rabies antigen with labeled antibody. This adsorbed conjugate serves as a specificity control.

10. Dispense in small aliquots. Label and store at -70° C. As needed, a bottle is removed and thawed for use.

In preparing Normal Mouse Brain Suspension (NMB), follow steps 4-10, using normal mice.

^aK. C. Biological, Inc., Lenexa, Kansas 66215.

^bSigma Chemical Company, Box 14508, St. Louis, Mo, 63178.

^cEastman Kodak Company, Rochester, New York 14650.

Processing of Specimens--Methods for removal of the brain and salivary gland were previously described.¹⁹ With a pair of scissors, small sections of the hippocampus (Ammon's horn) were cut and placed on wooden tongue-depressors with the cut surface facing upward. Impressions were made by pressing the cut surface of the brain tissue against clean fluoroslides.^a Other tissue removed for study included the salivary gland, eye, lip, and neck skin. The tissues were placed on tissue holders which were covered with a mounting medium^b and placed on the quick freeze block of a cryostat.^c Specimens were sectioned at 8-10 μ m at a cutting temperature of -15° C.

Lip tissue was mounted in a position so that cross and oblique sections were produced.¹⁷ Cross sections of the back skin and salivary gland were made. The eye was mounted in such a manner that coronal (tranverse) sections of the cornea were produced. Slides were fixed in cold acetone for four hours and stored at -70° C. until staining.

Duplicate frozen sections of all tissues were cut and stained with a rapid hematoxytin and eosin method.² The Bodian's Protargol Method was also used for the demonstration of nerve fibers and neurofibrils.¹⁸

^aCurtin Matheson Scientific, Inc., Kansas City, Missouri 64111.

^bTissue-Tek OCT Compound, Ames Co., Elkhart, Indiana.

^cInternational Cryostat Model CTI International Equipment Co., Needham Heights, Mass.

FRA Staining--The direct fluorescent antibody staining method was used for detection of rabies virus antigen.^{7,12,13} Modification made was the addition of one drop of 1% evans blue per 2 ml. of adsorbed NMB and CVS II suspensions. This was used as a counter stain to give sufficient background staining.

Examination of Tissue--A positive rabies control slide was used with each group of tissues to check specificity and sensitivity of the FRA test. The positive control was examined first. The specificity of fluorescence was determined by an inhibition control slide. Autofluorescence was not a problem due to counter-staining and high specificity of the anti-rabies globulin.

The brain, salivary gland, cornea, lip, tactile hair follicles and skin from the back of the neck of thirty-two skunks were examined for the presence of rabies virus antigen. Brain tissue was examined for rabies virus by the routine techniques in which impression smears of the hippocampus were made; frozen section procedure was used for the other tissues.

RESULTS

Twenty-one of the thirty-two skunks examined were positive for rabies by the FRA test (Table I).

Submaxillary Salivary Gland--The submaxillary salivary gland of the positive skunks revealed specific immunofluorescence. Viral antigen was found in ductal lumina, but most of the antigenic material occupied acinar spaces of the salivary gland. Antigen was found in individual and in groups of acini (Fig. 2).

Cornea--Specific immunofluorescence was demonstrated in the cornea of all positive skunks. Fluorescence occurred in a dust-like pattern and many times focal areas were apparent (Fig. 3). Occasionally "strings" of fluorescence were noted in the substantia propria (Fig. 4). These areas of fluorescence were identified as nerve fibers or neurofibrils by special staining.

Lip, Tactile Follicles and Neck Skin--Sections through the lip revealed granular and diffuse fluorescence in cells associated with the large and small tactile hair follicles. The sensory innervation of many of the large tactile follicles was outlined by the viral antigen (Figs. 5, 6 and 7). Sensory nerve endings of the tactile hair fluoresced in a radial and linear array parallel to the hair shaft (Fig. 5). Nerve bundles in the lip and neck sections strongly fluoresced (Fig. 10). The nerve network surrounding the follicles of the back skin showed specific rabies immunofluorescence (Figs. 8 and 9). Fluorescence was associated with nerves in the outer root sheath. On many occasions rabies virus antigen was demonstrated in the sebaceous glands of the hair follicles (Fig. 11). Fluorescence was also noted in the epidermis of many sections.

In all rabies positive skunks, rabies virus antigen was demonstrated in brain (Fig. 1), salivary gland, cornea, lip, tactile follicles and skin from the back of the neck.

DISCUSSION

Impression smears of brain and frozen sections of salivary gland, cornea, lip, tactile follicles and skin from the back of the neck of twenty-one naturally infected skunks demonstrated the presence of rabies virus antigen in FRA stained preparations.

Many investigators have demonstrated evidence of widespread rabies infection in salivary glands.^{1,8,9,10,15,21} Rabies virus antigen was demonstrated in the cornea.^{1,5,10,14,15,16} Rabies virus antigen has also been observed in peripheral nerve endings associated with hairs in the cheek and lip.^{3,6,10,11,15,17} A variety of other organs were added to the list by other investigators.^{1,8}

Tissues for this investigation were selected from a group of skunk heads submitted for rabies diagnosis. Histories submitted with some heads were suggestive of rabies while in other cases clinical signs were either not known or not reported and the incubation periods were not known (Table 2). Rabies virus antigen distribution observed by immunofluorescence when compared with duplicate sections stained with hematoxylin-eosin and Bodian method indicated much of the viral spread in various tissues was by the neural pathway.

In all positive skunks rabies virus antigen was demonstrated in brain, cornea, salivary gland, lip, tactile follicles and skin. In this study the FRA test on these tissues was 100% reliable for the diagnosis of rabies in naturally infected skunks.

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ACKNOWLEDGEMENTS

The author wishes to express sincere gratitude to his major professor, Dr. H. D. Anthony, for help, guidance, and encouragement during his project and thesis preparation. The author also wishes to express sincere thanks to his committee members, Dr. J. L. West and Dr. H. W. Leipold, for assistance given during his project.

Special thanks is given to Mrs. Dixie Dickens for the many hours spent typing the thesis.

TABLE 1. Fluorescent Rabies Antibody Test Results of Selected Tissues From Thirty-Two Skunks

Specimen	Specimens Examined	Positive	Negative
Brain	32	21	11
Cornea	32	21	11
Salivary Gland	32	21	11
Lip	32*	21	11
Neck Skin	32*	21	11

*Duplicate samples examined.

TABLE 2. Clinical Signs and Results of Specimens

Rabies No.	Aggressiveness	Daytime Activity	Clinically Ill	FA Results
1	UNK*	UNK	UNK	POS**
2	UNK	UNK	UNK	POS
3	YES	YES	YES	POS
4	UNK	YES	YES	POS
5	YES	YES	YES	POS
6	YES	UNK	YES	POS
7	YES	YES	YES	POS
8	YES	UNK	UNK	POS
9	YES	YES	YES	POS
10	UNK	UNK	YES	POS
11	YES	YES	UNK	POS
12	YES	YES	YES	POS
13	UNK	UNK	YES	POS
14	UNK	UNK	UNK	NEG***
15	UNK	UNK	UNK	NEG
16	YES	YES	NO	POS
17	UNK	UNK	UNK	POS
18	UNK	UNK	UNK	NEG
19	UNK	UNK	UNK	NEG
20	UNK	UNK	UNK	NEG
21	NO	YES	NO	POS

*Unknown
 **Positive
 ***Negative

TABLE 2 (continued)

Rabies No.	Aggressiveness	Daytime Activity	Clinically Ill	FA Results
22	UNK	UNK	YES	POS
23	UNK	UNK	UNK	NEG
24	NO	NO	NO	NEG
25	YES	YES	UNK	NEG
26	UNK	UNK	UNK	NEG
27	UNK	UNK	UNK	POS
28	YES	UNK	UNK	POS
29	YES	UNK	UNK	POS
30	UNK	UNK	YES	NEG
31	UNK	UNK	UNK	POS
32	UNK	UNK	UNK	NEG

*Unknown
 **Positive
 ***Negative

Fig. 1. Photograph of brain impression smears of a rabid skunk, 250X.

Fig. 2. Photograph of salivary gland showing specific rabies immunofluorescence. Note the antigenic material found in individual (a) and in groups of acini (b), 100X.

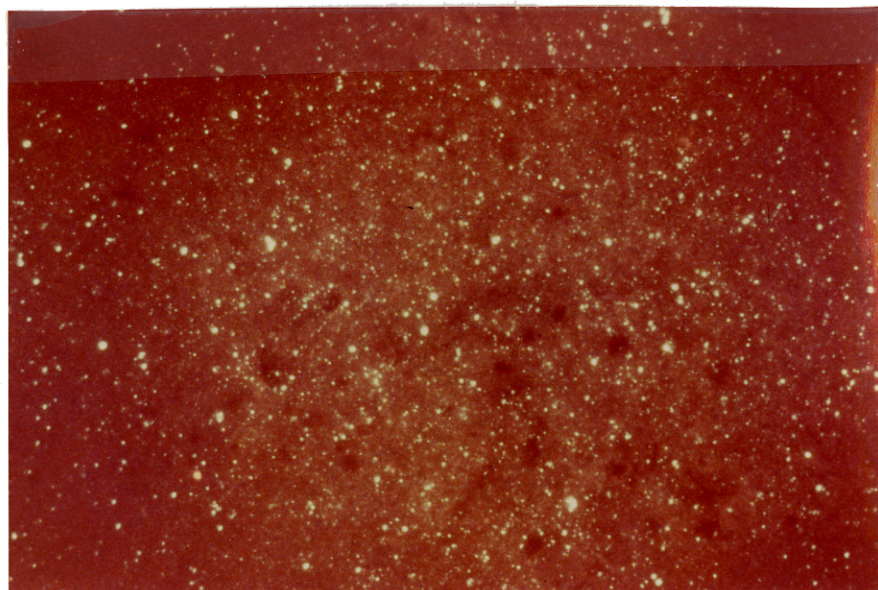


Fig. 1

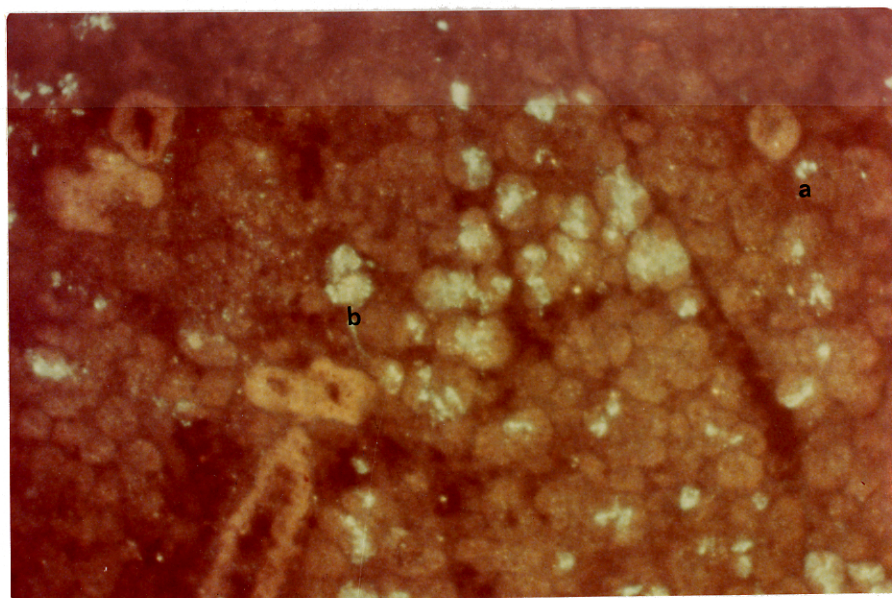


Fig. 2

Fig. 3. Photograph of cornea showing the dust-like pattern of rabies immunofluorescence. Note the apparent layer effect of fluorescence (a), 160X.

Fig. 4. Photograph of cornea. Note the fluorescence in the substantia propria (a) and the focal areas of fluorescence in the cornea (b), 250X.

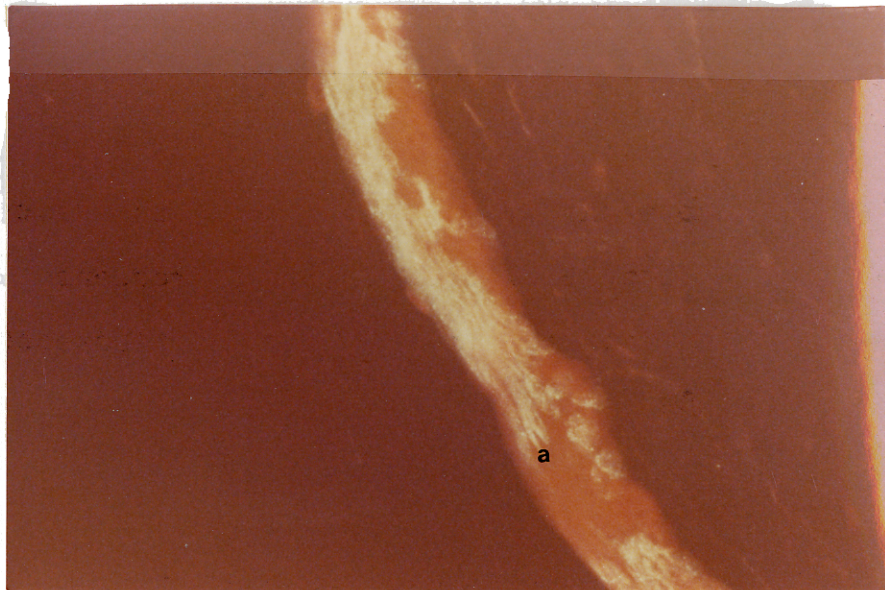


Fig. 3

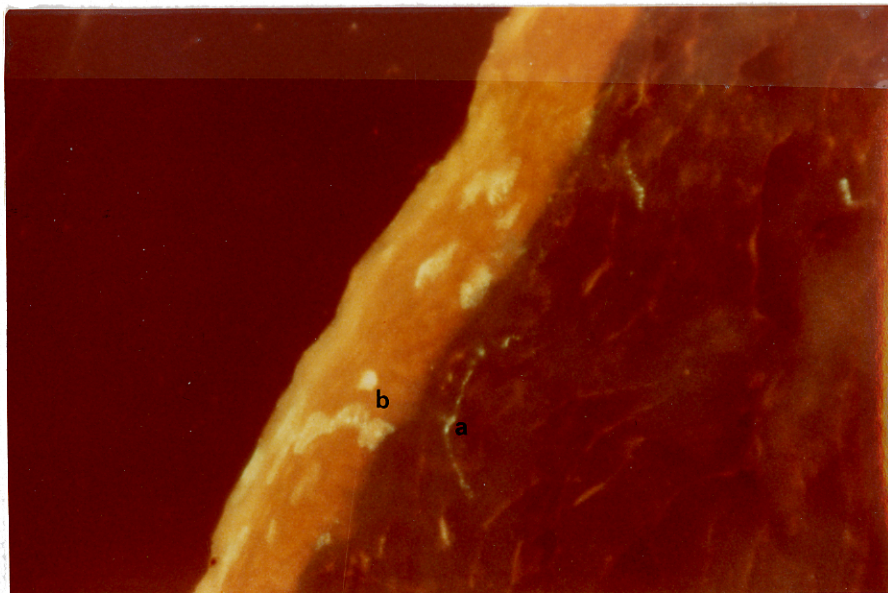


Fig. 4

Fig. 5. Photograph of tactile hair follicle showing the radial and linear array of fluorescence parallel to the hair shaft, 160X.

Fig. 6. Photograph of tactile hair follicle. Note the fluorescence associated with apparent sensory innervation, 160X.

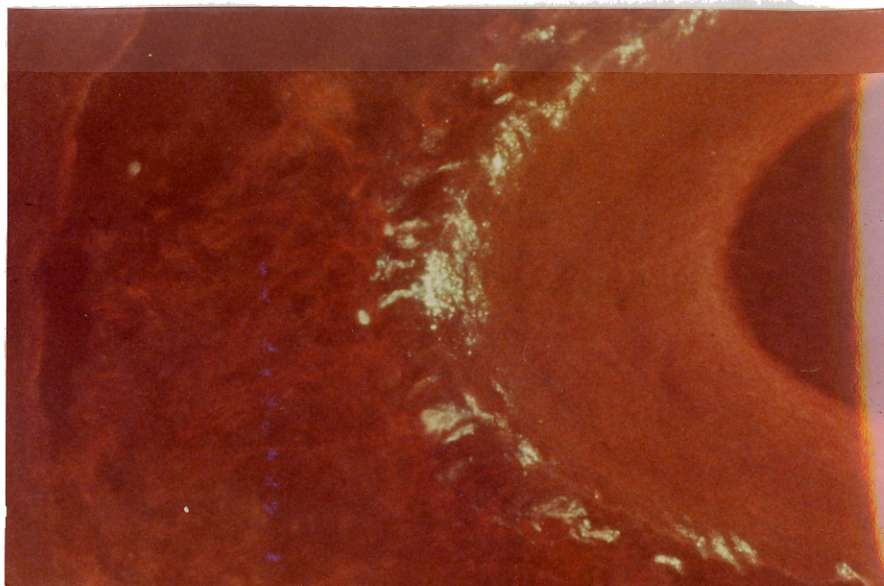


Fig. 5

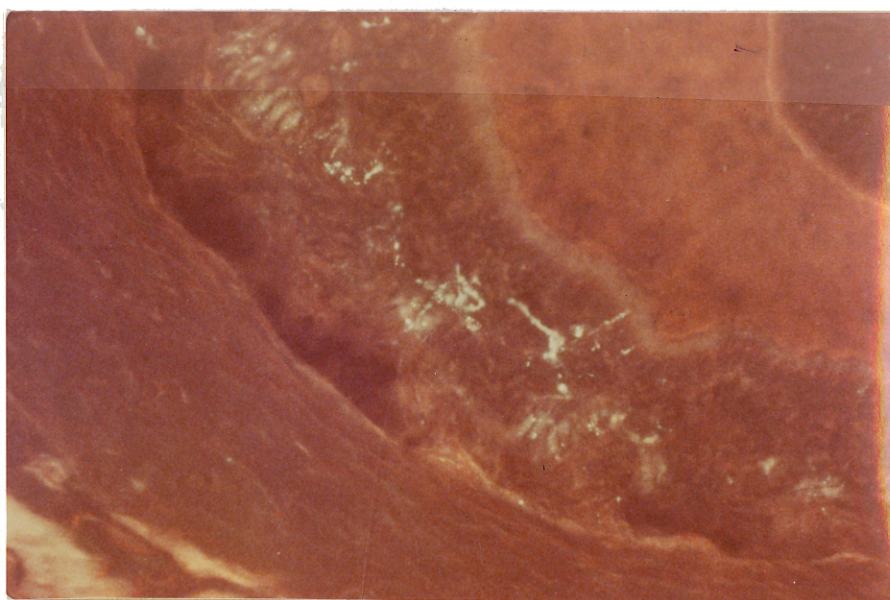


Fig. 6

Fig. 7. Photograph of tactile hair follicle. Note the abundant fluorescence surrounding follicle, 100X.

Fig. 8. Photograph of back skin showing specific rabies fluorescence in the apparent nerve network surrounding the follicles, 160X.

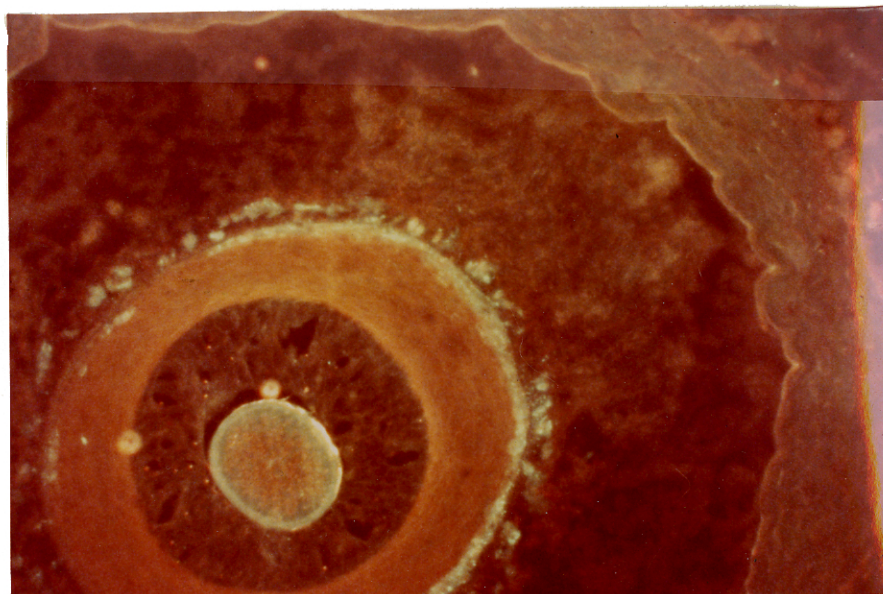


Fig. 7

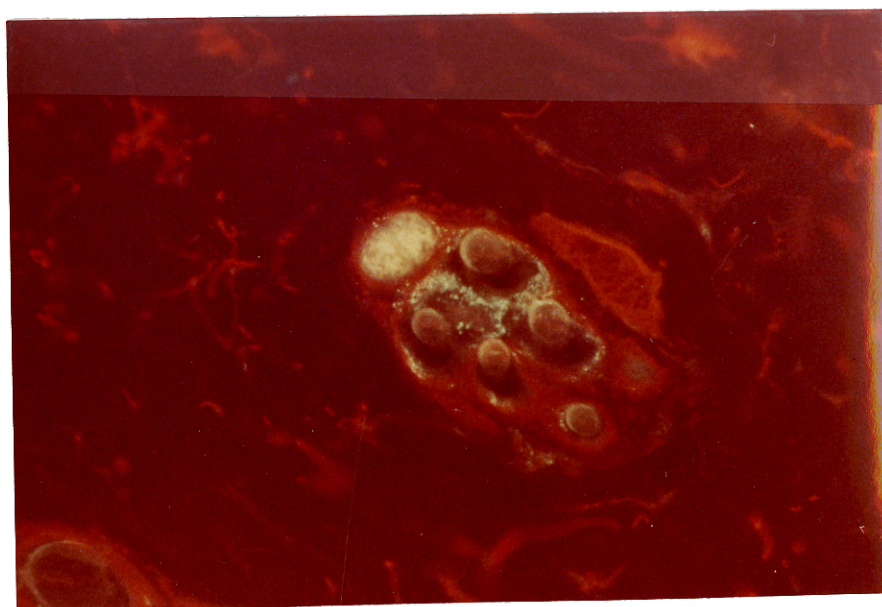


Fig. 8

Fig. 9. Photograph of follicle. Note the fluorescence of the apparent nerve network surrounding the follicle (a), 250X.

Fig. 10. Photograph of nerve bundle in lip showing specific rabies immunofluorescence, 250X.

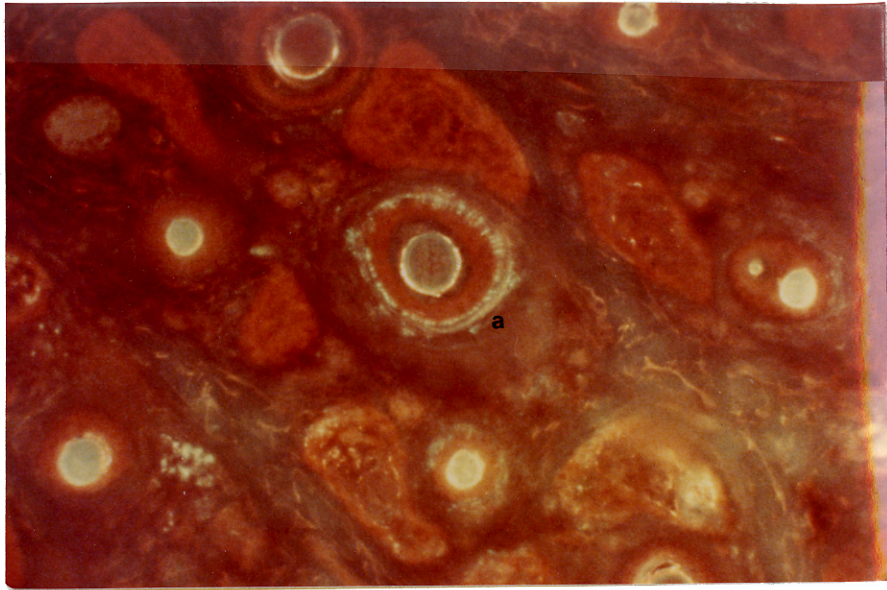


Fig. 9

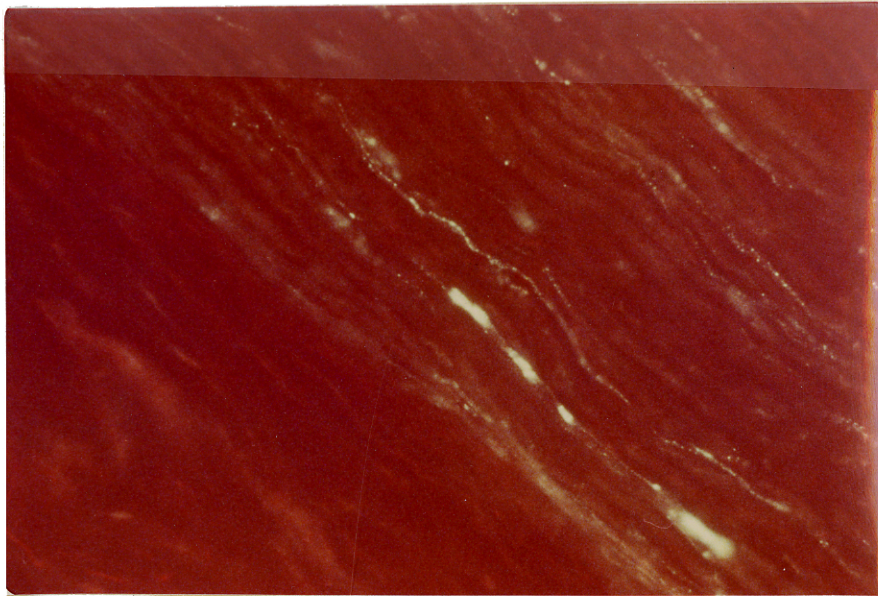


Fig. 10

Fig. 11. Photograph of lip showing rabies immunofluorescence associated with sebaceous glands (a) and hair follicle (b), 160X.

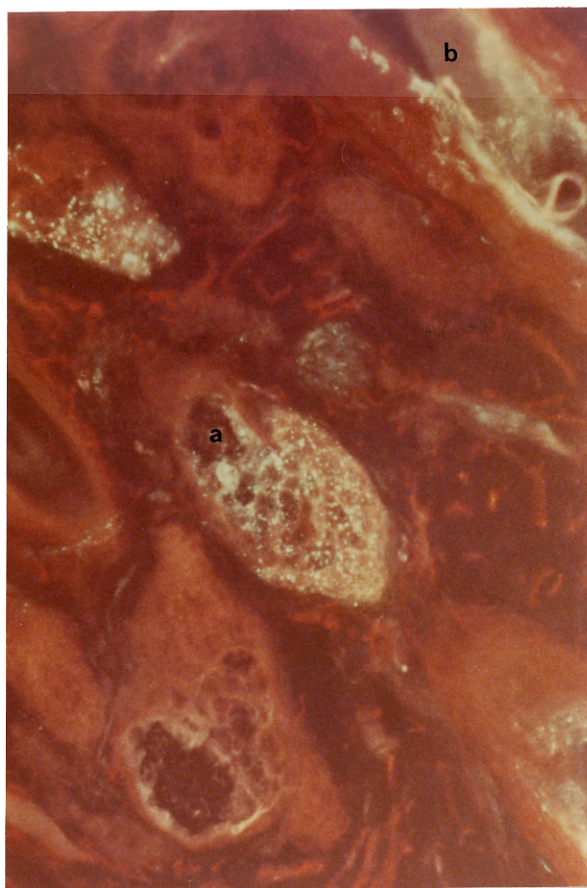


Fig. 11

APPENDIX

HISTORY OF RABIES

Rabies has a long and interesting history that is lost in antiquity. According to Athenodorus rabies was first observed in mankind in the days of the Asclepiadae, the descendants of the god of Medicine, Ascielapius.

Democritus is thought to have recorded the first description of canine rabies some 500 years B.C. Cardanus, a Roman writer, described the infectivity of the saliva of rabid dogs. The Roman writers described the infectious material as a poison for which the Latin was "virus."

Writers of the early Christian era had much to say about rabies and described it both in dogs and man. This was true of all the Roman empire but especially Greece and Crete where the disease was widespread.

From this period on the literature concerning rabies gradually expanded and with the progress of medicine became more valuable and comprehensive, however, little progress appeared to have been made in relation to the successful prevention or curing rabies

Epizootics were rare until the middle ages. Most cases were singular bites of rabid dogs and occasionally wolves, badgers, foxes, and even bears. Flemings, about the year 900, tells of the invasion of Lyon by a rabid bear which bit 20 people who attempted to kill it. In the next 27 days six people developed madness and were smothered to death.

In the nineteenth century rabies appeared to have become increasingly widespread in Europe, France, Germany and England. An extensive outbreak in foxes occurred in the Jura Alps in eastern France. This was the largest recorded outbreak.

Professor Rey from the Veterinary School of Lyons was able to serially transmit rabies by the inoculation of saliva.

Following the Civil War, rabies was widespread in most of the United States. Sylvatic rabies had been recognized in the eastern states in the eighteenth century. Rabies appears to have accompanied the pioneers during the movement to the west. Skunk rabies was reported by the mountainmen on the Great Plains in the 1830's. The United States Army reported skunk rabies was so common that the early settlers on the Great Plains referred to skunks as hydrophobia cats or phoby cats.

Pasteur published his first report on rabies during 1881, a period when many scientists attempted to transmit rabies from man to animals, and animals to animals. Joseph Meister, a nine year old boy from Alsace, bitten 14 times by a rabid dog, came to Pasteur's Laboratory. Pasteur employed a method of immunization which had been successful in dogs. The little boy did not develop rabies. Pasteur concluded the prophylaxis of rabies was established and a center for vaccination against rabies was created.

A significant advance in the diagnosis of rabies was the mouse inoculation test; mice were quite susceptible to neurotropic viruses. The mouse test demonstrated that only 85-95% of rabies positive cases were found by Negri body examination alone. The mouse

inoculation test became a standard procedure and is used to this day except where the fluorescent antibody test has replaced it. (Steele, 1975)

REVIEW OF LITERATURE

Evidence for centrifugal virus spread in the nerves has accumulated to such an extent that it is now widely accepted, though its precise mechanism is still a matter of discussion.

Systemic distribution of the virus by viremia is less well documented due to the failure of consistently demonstrating rabies virus in the blood. Since the virus is capable of replicating in a variety of nonnervous tissues, many investigators consider virus generalization to be more reasonable than neural spread. Recent experiments in which mice were injected intravenously with virus do not support the viremic hypothesis; there was no evidence of infection of nonnervous organs prior to central nervous system (CNS) involvement and peripheral organs were not terminally infected at a higher rate than after other routes of infection.

Virus isolation from peripheral nerves and nonnervous organs and demonstration of virus specific inclusions were the methods exclusively used in the past to show the presence of rabies virus in certain tissues. More recently the fluorescent antibody (FA) technique and electron-microscopy were shown to be effective tools for the study of this aspect of rabies pathogenesis. The time of appearance and the progression of rabies virus in peripheral nerves, as well as in nonnervous organs, should answer the question as to how the infection of these structures is initiated (Schneider, 1975)

Virus antigen in peripheral nerves was first reported by Dean et. al., (1963). The theory of centrifugal virus movement in nerve

was further supported by the nerve-dissection experiment. Results indicated conclusively that rabies virus is transmitted from the site of exposure to the CNS via peripheral nerves.

Yamamoto et. al., (1965) showed rabies and herpes simplex viral antigens were both present in the peripheral nerve fibers. In studies by Baer et. al., (1965 and 1968) the spread of fixed rabies virus and a street-virus isolate were investigated. Rabies virus in the CNS was first detected in the lumbar segment of the spinal cord on the sixth day after inoculation. Before this time virus could not be detected in any organ except in the foot pad immediately after inoculation. Removal of the sciatic nerve or its fasciculus prior to foot pad inoculation was a complete saving procedure in all animals, thus giving evidence for the neural spread of the infection.

In a study on experimental rabies, Johnson (1965) stated following the subcutaneous inoculation of virus in the hind-footpad, mice were sacrificed daily and blood, liver and spleen were inoculated intracerebrally into other mice to determine virus content. At no time during the incubation period could virus consistently be isolated from blood or viscera. Furthermore ligation or sectioning of the ipsilateral sciatic nerve prevented disease when 100 times the lethal dose of virus was inoculated. The failure to produce viremia and the prevention of disease by the interruption of nerves supported the hypothesis that rabies virus ascends to the CNS via nerves.

The presence of virus antigen in peripheral nerves has also been reported by Dierks et. al., (1969) and Murphy et. al., (1973). Electron microscopy and fluorescent antibody studies were used to support their findings.

Recent electron microscopic studies gave clear evidence of the maturation of rabies virus particles from virus specific matrices within axons. Virus particles and nucleocapsid-containing structures were demonstrated at the Ranvier rings, at the periphery of axon but not within Schwann cells. Rabies virus is capable of replicating in a variety of nerve structures, but it is obvious that no distinction can be made as to whether viral replication in the axoplasm of peripheral nerves is the result of centrifugal or centripetal virus movement. There is hardly any organ which at one time or another cannot be shown to yield rabies virus or antigen if careful enough search is made (Schneider, 1975).

Among nonnervous organs, the salivary glands are most frequently infected. Vaughn et. al., (1963) recovered or identified rabies virus from the submaxillary gland in 88% of 26 cats that died of rabies. Studies by Parker and Wilsnack (1966) indicated skunks emitted more virus/unit volume of saliva than foxes. Of 25 skunks dying of rabies, virus could be demonstrated in a high per cent of their saliva. One skunk shed large amounts of virus in its saliva 18 days before death. Debbie and Trimarchi (1970) demonstrated rabies virus in the salivary gland of 11 of 12 naturally infected red fox (Vulpes fulva). Other investigators have found

from experimental infections 74% dogs, 47% cattle, 20-73% foxes, 83% skunks and 63% raccoons may harbor virus in their saliva glands (Schneider, 1975).

Fluorescent antibody technique was shown to be applicable for the diagnosis of rabies using brain and salivary gland (Goldwasser and Kissling, 1958). The best single test for the rapid diagnosis of rabies today is the fluorescent antibody method (World Health Organization Expert Committee on Rabies, 1973).

In a comparison of several methods of diagnosis of rabies in experimentally infected captive skunks and foxes the most sensitive tests were FA examination of brain smears and mouse inoculation using brain suspension (Carski et. al., 1962). In studies of salivary gland specimens both mouse inoculation and fluorescent antibody methods of diagnosis displayed a lower degree of sensitivity.

Baer et. al., (1965) used the fluorescent antibody method as a sensitive tool for discovering small amounts of virus. Rabies virus in the CNS was first detected in the lumbar segment of the spinal cord after inoculation of fixed rabies virus into the rear footpad of rats.

A study was performed by Yamamoto et. al., (1965). The FA technique was employed to follow the distribution of rabies virus in infected salivary glands. They demonstrated virus antigen within the cytoplasm of the acinar cells. In the early stages fine granules of bright fluorescence appeared in the cytoplasm of acinar cells. These granules gradually aggregated at the periphery of the acinar cells by the 6th day post inoculation

and most of the antigenic material had coalesced into globular structures in the acinar spaces of the salivary glands after 9 days infection. They concluded from their observations that the rabies virus may be released from the acini of salivary glands contained in the physiological secretions. Dierks et. al., (1969) stated the fox submaxillary saliva gland is a compound tubuloacinar organ consisting of interconnecting structures: acini, acinar lumens, intercalated ducts, granular ducts, striated ducts and excretory ducts. The acini consist primarily of pyramidal mucogenic cells with occasional serous cells.

Fox salivary glands exhibited evidence of widespread rabies infection. In frozen sections stained with fluorescent conjugate, large amounts of viral antigen was found in individual and focal groups of acini. Ventral areas of glands were affected most markedly. Often the whole content of acini normally occupied by mucogenic cells was replaced by fluorescent aggregates. Where autofluorescence allow definite identification of ducts, viral antigen was detected primarily within ductal lumens; very little specific fluorescence was associated with duct cells themselves (Dierks et. al., 1969).

Virus antigen in the salivary glands first appeared in the nerves innervating the glands and only 1-3 days later in the acinar epithelial cells. Corneal epithelium and multilocular fat cells of the interscapular brown fat lobes were other nonnervous tissues found to be heavily infected. Other workers demonstrated virus antigen in the Malpighian cells of the lingual epithelium, in

nerve elements surrounding sebaceous glands, in the wall of the intestinal tract and the hair shafts in the skin of intracerebrally inoculated mice (Schneider, 1975).

Correa-Giron et. al., (1970) isolated rabies virus from various tissues of mice sacrificed between 10 minutes and 7 days after feeding virus. The presence of rabies virus antigen in the various tissues, demonstrated by the FA technique, was found most frequently in the cytoplasm of the cells of highly innervated tissues. Tissues removed for study included brain, buccal mucosa from cheek and lips, tongue, salivary glands, esophagus, trachea, lung, heart, stomach, mesentery, intestine, liver, kidney and spleen.

Mucosae of the buccal cavity were sectioned and stained with the other tissues of the cheek and lip. Specific staining was observed in the epidermis of the skin and in the tactile hairs. Specific fluorescence was observed in some of the structures of tactile hairs. Fluorescence was observed in the nerve endings in the root of papilla and in some of the cells located at nerve terminations. Fluorescence was also noted where the main sensory nerve entered the follicle and in its nerve endings. In the papilla the cells in contact with the invaginated outer root sheath showed diffuse, specific fluorescence. Specific fluorescence was also seen in those cells of the cavernous sinus in the vicinity of the nerve endings (Correa-Giron et. al., 1970).

Studies conducted by Atanasui et. al., (1970) revealed immunofluorescence, histopathological examination and mouse inoculation into suckling mice were of little value in the diagnosis of rabies

living animals. But, after death the three methods provided concordant positive results and disclosed virus in the saliva, salivary glands, cornea, retina, suprarenal glands, Gasserian ganglion and brain. Virus was abundant in the salivary glands and the cornea.

These findings were also shown by Debbie and Tremarchi (1970). The fluorescent antibody and mouse inoculation tests were employed to study rabies virus in tissues of naturally infected fox. Antigen was demonstrated in various tissues of the body, i.e., brain, spinal cord, salivary glands, esophagus, small intestine, lymph node, lung, heart, adrenal, kidney, ureter, bladder, prostate, urethra and testicle.

Specific fluorescence was seen in the cheek mucosa in cells of the stratum granulosum and stratum Malpighi in a study on pathogenesis by Fischman and Schaeffer (1971). Fluorescence in the form of single granules or granules in chains was also seen in nerve cells in the lamina propria. Nerve bundles in the submucosal tissue of the cheek were strongly fluorescent. Sections through the skin or epidermis of the cheek showed extensive granular or diffuse fluorescence in cells associated with large and small tactile hair follicles. Cells of the external root sheath of the hair papilla contained viral antigen; 'nerve cells in the lamina propria surrounding the hair papilla consistently showed evidence of infection.' Specific fluorescence was also noted in the mucosal cells in the central and dorsal surfaces of the tongue.

Murphy et. al., (1973) employed immunofluorescent microscopy to study aspects of pathogenesis of rabies infections in young hamsters from sites of invasion of the CNS to the brain and to those tissues infected via centrifugal neural spread. Centrifugal neural spread of virus terminally reached the retina and cornea and peripheral nerve endings associated with hairs, intestine, and adrenal medulla. Of the potential sites for production of infectious oronasal secretions, the many taste buds of the mouth and olfactory neuroepithelium were much more heavily infected than the salivary gland epithelium. Extraneural infection also occurred terminally in the pancreas, brown adipose tissue and myocardium.

Investigators have worked on intra-vitam diagnostic tests for the early diagnosis of rabies during the lifetime of the individual. Schneider (1969) introduced the Corneal Test (CT). Impressions of the cornea were prepared by pressing a clean microscope slide against the protruding eyeball. The adhering epithelial cells were stained and examined for fluorescence. A diagnosis of rabies was established in more than 70% of infected mice prior to the onset of clinical symptoms.

Larghi et. al., (1973) evaluated the Corneal Test from 313 subjects of different species. When the CT was compared with immunofluorescence staining and MI mouse inoculation test on brains of the same subjects a sensitivity of 41.7% and specificity of 100% was found. Specific fluorescence was clearly demonstrated in two corneal impressions from a 6-year old boy in Mexico by Cifuentes et. al., (1971). It has been observed that rabies infection of

the cornea takes place early in the course of the peripheral spread of the virus from the CNS and the virus is present in the cornea before it is found in the salivary glands (Cifuentes et. al. 1971).

Fuh and Blenden (1971) demonstrated specific rabies fluorescence in the epithelial cells surrounding hair follicles from skin of the lip of mice on the day of onset of symptoms; these mice had been inoculated intracerebrally with three different strains of rabies virus. Skin from the face of five dogs and eight skunks clinically ill with natural rabies infection also were found positive. This suggests that biopsy of skin and/or saliva gland was a useful technique to evaluate the possibility of rabies.

Other investigators demonstrated rabies virus in sections of skin from dogs, cattle, monkeys, mongooses, and human beings (Smith et. al., 1972, Blenden 1974, and Bryceson et. al., 1975).

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DETECTION OF RABIES VIRUS IN SELECTED TISSUES OF
NATURALLY INFECTED SKUNKS

by

DENNIS RAY HOWARD

B.S., Kansas State University, 1972

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Graduate Pathology Group

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

The fluorescent rabies antibody (FRA) test was used to demonstrate rabies virus antigen in selected tissues of naturally infected skunks. Tissues examined were the brain, cornea, submaxillary salivary gland, lip, tactile hair follicles, and skin from the back of the neck.

Immunofluorescence on frozen sections of cornea, salivary gland, lip and neck skin revealed the presence of rabies virus antigen in twenty-one naturally infected skunks.

The distribution of rabies virus antigen observed by immunofluorescence indicated that spread of rabies virus in selected tissues was by neural pathways. Nerves and neurofibrils were observed in fixed tissue sections stained with Hematoxylin-eosin and Bodians stains.

Thirty-two animals for this investigation were submitted by veterinarians and health departments from the State of Kansas for rabies diagnosis. Of these thirty-two specimens, twenty-one were positive for rabies. Histories submitted with some heads were suggestive of rabies while in other cases clinical signs were either not known or not reported and incubation periods were unknown. Specimens had varying degrees of post-mortem change. In all positive cases, rabies virus antigen could be demonstrated in cornea, salivary gland, lip, tactile hair follicles and skin from the back of the neck. Results of the study indicated these tissues were 100% effective for the diagnosis of rabies in naturally infected skunks. These tissues would be a diagnostic tool for determining rabies infection when sufficient brain material was unavailable because of trauma or autolysis.