CLINICOPATHOLOGICAL CHANGES IN CATTLE, SHEEP, AND RABBITS CAUSED BY A NORTH DAKOTA BOVINE VIRAL ISOLANT

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ABSTRACT

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by Hansjakob Rothenbacher

Mucosal disease, a sporadic but highly fatal condition in cattle resembling rinderpest has been recognized for the past ten years. In spite of extensive research efforts its cause and mode of transmission have remained unknown. In 1958 the isolation of a virus from fecal material of affected cattle was announced and it was considered the causative agent.

The characterization of this new bovine viral isolant--the North Dakota mucosal disease virus--was attempted in this dissertation. Studies included clinical, hematological, immunological, serological, gross and histopathological observations on experimentally inoculated cattle, sheep, and rabbits. All experimental animals proved susceptible to the bovine embryo kidney-cell-culture propagated virus. The experimental infection in cattle was characterized by an acute monophasic febrile reaction combined with a severe leukopenia and heteropenia. Other clinical signs of the experimental infection included partial anorexia, nervousness, tachycardia, increased respiration, ocular discharge, depression and

constipation. The experimental infection in cattle lasted from six to ten days and resulted in the production of virus neutralizing antibodies measurable in tissue culture systems and in immunity to challenge 14 days after the initial inoculation.

The major gross and histopathological lesions produced by the North Dakota virus in experimental animals were confined to the adrenal glands, the gastrointestinal tract, the lymphatic tissues, the kidneys, the liver, and the bone marrow. The epitheliotropism of the virus was evidenced by inflammatory and necrotizing changes of the epithelial tissues of the gastrointestinal tract and of the parenchymatous organs. Inflammatory, necrotizing and regressive changes were noted in the lymphatic system. Similar regressive changes were also seen in the bone marrow.

Contact transmission did not occur. Experimental cattle inoculated with fresh blood and organ emulsions of infected animals failed to contract the infection. Three or more inoculations of calves with organ emulsions resulted in immunity to challenge with the tissue-culture-grown virus.

Numerous virus isolation attempts failed to reveal the presence of the virus in fresh blood, nasal and ocular swabs, mucosal scrapings and fecal specimens of infected cattle. The virus was recovered from the spleen, kidneys, and lungs of two calves six and nine days, respectively, after virus inoculation.

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The experimental North Dakota virus infection in cattle did not resemble any of the classical descriptions of Iowa mucosal disease, malignant catarrhal fever, Ume disease, parainfluenza-3 respiratory infection, virus diarrhea, or infectious bovine rhinotracheitis.

The experimental North Dakota virus infection in sheep and rabbits was similar but milder in degree and of shorter duration.

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INTRODUCTION

The name "mucosal disease" was chosen by Ramsey and Chivers (1953) for an apparently new disease syndrome recognized in Iowa since 1951, in North Dakota (Schipper and Noyce, 1959C) possibly since 1945, and in other states for several years prior to the above mentioned report in 1953.

Pritchard (1955) stated that the difference between the easily transmissible virus diarrhea and mucosal disease was quantitative rather than qualitative, though he erroneously thought the described so-called "Indiana" virus diarrhea (Pritchard et al., 1956) to be different from the virus diarrhea earlier reported by Olafson et al. (1946) in New York. It was later proved by Gillespie and Baker (1959) that the existing strains of Indiana and New York virus diarrhea were identical.

With the increasing recognition of clinically and pathologically similar transmissible disease syndromes in this country by Wheat et al. (1954) in California; Hoag et al. (1956) and Rooney (1957) in Virginia; Huck (1957), Dow et al. (1956) and Jarrett (1958) in England; Hedstrom and Isaksson (1951) in Sweden; Reinders (1959) in Holland; and Johnston (1959) in Australia--to name just a few--the term "mucosal disease complex" has been used by many authors to

include the "Iowa" type mucosal disease and the easily transmissible virus diarrheas resembling the description by Olafson et al. (1946).

Due to the similarity of both mucosal disease and virus diarrhea to the potentially dangerous foreign disease, rinderpest, extensive investigational work has been carried out in several states to find exact means of differentiation. To date, four doctoral dissertations have been written describing the clinical, gross, and microscopic pathology of the Iowa-type mucosal disease (Ramsey, 1956; Whiteman, 1960; Trapp, 1960; and Bajwa, 1961). In spite of vigorous research efforts the cause of this mucosal disease is still an enigma and all attempts to experimentally reproduce the disease seen in the field, or isolate its etiological agent have failed to yield conclusive results. Serological studies have shown (Olafson and Rickard, 1947; Ramsey, 1956) that mucosal disease and virus diarrhea have no antigenic and/or immunological relationship to rinderpest.

Schipper and Noyce (1959A) and Noyce and Schipper(1959) announced the isolation of a mucosal disease agent in bovine embryo kidney cell culture. This was the first report of a cytopathogenic virus isolated repeatedly from field cases of mucosal disease, and subsequent investigations by Schipper and Noyce (1959B) and Barner et al. (1959) indicated a possible etiological relationship of this virus to the mucosal disease syndrome.

It was the purpose of this investigation to characterize this North Dakota mucosal disease virus by its clinical, gross, and microscopic pathology produced in calves, sheep, and rabbits. The virus was generously made available by the original investigators to Dr. R. D. Barner under whose guidance this work was conducted.

CHAPTER I

REVIEW OF THE LITERATURE

General Remarks

During the past few years many "new" viral agents have been isolated from the bovine species. In order to correlate research and outline standard methods of classification of animal viruses, a Committee on Virus Research was formed by the United States Livestock Sanitary Association (1960). A proposed standard method for serological identification of viruses was drawn up by this committee in order to facilitate comparison of results obtained by different groups of research workers. A coordinator for each of the following animal species was chosen: bovine, porcine, feline, canine, and poultry.

As a part of the future program, it was recommended that investigators should be willing to supply virus and antiserum to a repository for use as reference in future research. It was also recommended that the National Animal Disease Laboratory at Ames, Iowa, act as the repository and as a central agency for these viruses and antisera. In studying the causative agents of any disease the first step, after the isolation of the agent, should be to determine whether the new isolant is identical to, or different from,

agents already obtained by other groups of investigators.

Our present methods to identify a virus still vary widely and comparisons between laboratories are therefore difficult. Too frequently, when a virus is isolated, it is studied from various approaches without too much consideration to its possible relationship to agents isolated elsewhere from the same species of animal.

In the rapidly expanding field of virus infections in the bovine, an effective virus classification method is urgently needed to overcome the already existing confusion between the various etiological agents isolated to date and to avoid similar confusion in the future.

Viral Isolants from Syndromes of the Virus Diarrhea-Mucosal Disease Complex with Probable Viral Etiology

Since the original reports by Schipper and Noyce (1959A), and Noyce and Shipper (1959), research workers from Indiana (Claflin *et al.*, 1959) and Iowa (Ramsey *et al.*, 1959) announced the isolation of two nonculturable and noncytopathogenic infectious agents from mucosal disease field cases. These agents were named the "Merrell" (Indiana) and "Sanders" (Iowa) mucosal disease agents. Both agents are under investigation and their comparison in cross protection studies proved them to be similar or identical (Claflin *et al.*, 1959; Ramsey *et al.*, 1959) and possibly also identical to the noncytopathogenic virus diarrhea agent (Rothenbacher *et al.*, 1961) described by Lee and Gillespie (1957). All three agents are noncytopathogenic

and produce a characteristic diphasic temperature elevation combined with similar hematological and clinical changes in experimentally inoculated cattle. The experimental disease resembles the description of virus diarrhea by the American (Olafson et al., 1946; Pritchard et al., 1956; Carlson et al., 1957; and Runnells et al., 1960), British (Huck, 1957; Dow et al., 1956; and Jarrett,1958) and Australian (Johnston, 1959; Edwards and Sier, 1960) workers. Kniazeff (1960) reported the neutralization of Sanders post-inoculation sera by the Oregon C-24-V virus diarrhea virus (Gillespie et al., 1960) indicating a close relationship between the two agents.

More recently, Cunningham and Church (1960) announced the isolation of a bovine cytopathogenic enterovirus in Michigan from a field case of mucosal disease clinically diagnosed by Beck (1959) and reported by Rothenbacher and Barner (1960) and Barner et al. (1960). Subsequent studies by Barner et al. (1960) showed that this "Dibble" virus was nonpathogenic to calves and that it had no clinical, antigenic, or immunological relationship to the North Dakota mucosal disease virus. Its possible etiological role in a bovine dysentery syndrome reported by Moore et al. (1962) is in doubt. Antisera against the Dibble virus did not show any close relationship with the Oregon C-24-V virus (York, 1961) or with the Michigan LC-4 strain ECBO virus (Cunningham and Church, 1960).

With the discovery by Gillespie et al. (1960) that the cytopathogenic Oregon C-24-V virus was antigenically and

immunologically identical to the noncytopathogenic strains described by Lee and Gillespie (1957) a valuable tool was obtained for future serological studies. For many years this comparative research on bovine virus diarrhea had been hindered by the fact that all the isolated agents were noncytopathogenic in tissue culture systems. Kniazeff and Pritchard (1960) used this new strain (C-24-V) to investigate the antigenic relationships in the "virus diarrhea-mucosal disease complex." Dow et al. (1956) and Kniazeff and Pritchard (1958) first used this term of wider definition which has been adopted by many workers. Numerous recent reports from all over the world describe mucosal-type disease syndromes which are easily transmissible and, in their epizootiology and pathology, resemble virus diarrhea rather than the classical description of mucosal disease by Ramsey and Chivers (1953).

Virus diarrhea antisera from Florida, Indiana, New York, and Nebraska all showed high and approximately equal neutralizing capacities against Oregon C-24-V strain virus. Similar results were obtained with antisera to mucosal disease syndromes from Indiana, Iowa, North Dakota, and England. On the other hand, Kniazeff and Pritchard (1960) did not discover any degree of neutralization from antisera against the following diseases: bluetongue, hog cholera, infectious bovine rhinotracheitis, infectious ulcerative stomatitis, mycotic stomatitis, malignant catarrhal fever, sporadic bovine encephalitis, and winter dysentery. In their discussion of the

immunological evidence from these tests, the authors concluded that the virus diarrhea and mucosal disease agents considered are members of an antigenically related group. This serological relationship, in conjunction with the highly similar pathogenicities exhibited, led the authors to consider all the agents (as tested by their antisera) as the etiological agents of a single disease. They also felt that differences in epizootiological and pathological characteristics did not invalidate their conclusion. They speculated that the apparent immunological relationship between these agents did not imply that they must be antigenically related. They conceded, however, that studies by York (1960) have already shown that a certain percentage of antisera collected in field outbreaks of virus diarrhea show no neutralization titer against the C-24-V prototype virus. Kniazeff and Pritchard (1960) also conceded that their experimental design did not permit exact evaluation of antigenic interrelationships between the viral agents in question. Such evaluations could only be accomplished by quantitative crosstitration tests, tissue-culture studies and animal inoculations. Results of such studies presently in progress have not been reported at this time.

The reported antigenic relationship between hog cholera and mucosal disease by Darbyshire (1960) which seems in contradiction to the negative relationship reported by Kniazeff and Pritchard (1960) was explained by the latter authors to

be due to antibodies not participating in the virus-serum neutralization phenomenon. Beckenhauer et al. (1961) seem to confirm Darbyshire's (1960) report to the extent that they consider the hog cholera virus as another serological type of the virus diarrhea group of agents. This group of agents, in their opinion--in contrast to Kniazeff's and Pritchard's (1960) findings of a close relationship between all members of the group--shows quite a wide divergence of serological types. This is evidenced by their finding that only one of three bovine enteroviruses, the C-24-V virus diarrhea prototype, was able to protect swine against hog cholera challenge. The immunity produced in swine by the virus diarrhea agent (C-24-V) was shown to be due to antibody formation and not to some blocking mechanism. The vaccination of pigs with the Oregon C-24-V virus did not cause any clinical symptoms and no contact infection of the vaccinal virus from pig to pig could be observed. Beckenhauer et al. (1961) emphasized that if titered against the hog cholera virus, various virus diarrhea agents did not seem closely related.

Serum neutralization tests conducted by York (1961) between antisera to the Michigan (Dibble) and the North Dakota mucosal disease viruses revealed no close relationship of either virus to the Oregon C-24-V prototype virus. Also, convalescent sera from a dysentery syndrome in young cattle and dairy cows reported by Moore et al.(1962) did not

show a significant neutralization titer to the C-24-V virus. York's (1961) finding pertaining to the North Dakota virus was not in agreement with Kniazeff's and Pritchard's (1960) data but was a confirmation of clinical and pathological findings as presented in this thesis.

The controversial views of various authors point out the confusion in the field of bovine enteric virus infections. It is obvious that our present means of classifying and comparing viruses need standardization and refinement. The relative accuracy of present serological tests and their questionable comparative value is self-evident. It is hoped that the work presented in this thesis will fill a research gap in the investigation of the virus diarrhea-mucosal disease complex. It should further point out the need for clinical and pathological characterization of an individual virus and its pathogenicity in the experimental animal as a necessary supplementation of *in vitro* virological studies.

York *et al.* (1960) evaluated a modified-live, and a rabbit-adapted vaccine produced from the prototype Oregon C-24-V virus. Of 112 vaccinated feedlot cattle, nine later developed signs of virus diarrhea after field exposure while 67 of 265 control animals became ill. Their extensive serological studies of vaccinated cattle and of cattle from field outbreaks confirmed the findings made by Robson *et al.* (1960) that antibodies determined by neutralization tests are indicators of immunity to virus diarrhea. This is also in

agreement with the studies on infectious bovine rhinotracheitis by Schwarz et al. (1957), and on canine distemper by Gillespie et al. (1958), both of whom presented evidence that neutralizing antibodies when present denote resistance to the specific virus.

It was the opinion of Hagan (1958) that Olson and Hoerlein (1956) probably saw virus diarrhea in their observations on mucosal disease epizootics in Nebraska, involving mainly beef calves. Hagan (1958) also believed that Hoag et al. (1956) and Rooney (1957) probably described virus diarrhea in Virginia, though no virus comparisons were made. Also, the Swedish epizootic enteritis described by Hedstrom and Isaksson (1951) and the British reports by Dow et al. (1956) and Huck (1957), according to Hagan (1958), resembled virus diarrhea even though some of their reported features did not exactly match the description by Olafson et al. (1946), who first saw the clinical signs of epizootic diarrhea primarily in adult dairy cows. Olafson and Rickard (1947) named the disease "virus diarrhea."

Hagan (1958) enumerated the original descriptions of the separate disease entities which were later grouped together in the virus diarrhea-mucosal disease complex as follows: virus diarrhea (New York), virus diarrhea (Indiana), mucosal disease (Iowa), infectious bovine rhinotracheitis, mycotic stomatitis, ulcerative stomatitis (Indiana), epizootic enteritis (Sweden), malignant catarrhal fever, and rinderpest.

1961; Robson et al., 1961) who assumed a subclinical form of the disease. York and Rosner (1961) furnished the serological confirmation for this assumption. It is now generally concluded that a large percentage of all cattle show an antibody titer against the prototype C-24-V virus as reported by Runnells et al. (1960).

Underdahl et al. (1957) emphasized that many cases of what they considered to be mucosal disease obviously went undiagnosed. For their transmission studies they reported difficulty in finding animals without some degree of immunity. They succeeded in isolating a viral agent from tissues collected in Iowa and Nebraska outbreaks. The cytopathogenic effect of the virus on bovine embryo kidney cells was the same from both tissue pools. The virus could be passaged in tissue culture but not egg-adapted. Preliminary neutralization tests indicated a cross neutralization of the two isolants. They also found antibody titers commonly in herds with no history of mucosal disease or virus diarrhea. The authors concluded that the evidence presented in their studies did not prove this virus to be the causative agent of mucosal disease. It is hoped that further studies in progress will soon show the possible relationship between this virus and the already mentioned Dibble virus (Barner et al., 1960), the North Dakota mucosal disease virus (Schipper and Noyce, 1959A; and Noyce and Schipper, 1959) and the cytopathogenic prototype virus diarrhea strain C-24-V (Gillespie et al., 1960).

Van Bekkum (1959) in Holland reported a cytopathogenic agent isolated from a cow suffering from a mucosal disease syndrome. This agent was isolated on bovine fetal skin epithelium. Virus passaged on skin epithelium proved pathogenic also for embryo kidney cells, while primary culturing attempts with the original material on kidney cells were negative. This fact led Van Bekkum (1959) to speculate that skin cells might be more suitable for primary isolation attempts. Experimentally inoculated calves showed fever combined with a leukopenia between the second and fourth days after inoculation. The described lesions in the oral cavity and the occasional diarrhea compared well to the existing descriptions of experimental virus diarrhea by Baker et al. (1954) and Carlson et al. (1957). Contact transmission was also seen in experimental animals and the discrepancy between the condition in the field and the experimental disease was pointed out. Van Bekkum (1959) also acknowledged the similarity of his experimental disease with the virus diarrhea reported in North America. From this description one cannot deny the possible relationship to the C-24-V virus described by Gillespie et al. (1960), which future serological studies may well confirm.

Bögel and Mussgay (1960) reported the isolation of a cytopathogenic virus from calf feces which differed serologically from the "ECBO" LC-R4 virus, and caused blood-flaked feces in colostrum-deprived calves after experimental infection.

In his characterization of an ECBO virus isolation from healthy cattle, Soliman (1958) observed no signs of illness after inoculation of a three-month-old calf. However, there was a significant rise in antibody titer after inoculation and the virus could be cultured from the calf's feces for 13 days. Intracerebral inoculation of suckling mice and hamsters produced paralytic symptoms similar to those induced by Coxsackie A or Lansing-type polio viruses. Soliman (1958) did not inoculate colostrum-deprived calves. This latter procedure, if used, might shed more light on the obscure nature of the numerous isolations of enteric cytopathogenic bovine orphan (ECBO) viruses.

Another instance of an incompletely characterized virus is the isolation of a cytopathogenic agent from bovine feces by Moll and Finlayson (1958). Their isolant was grown in bovine kidney cell cultures and produced high fever and respiratory symptoms in calves. Other clinicopathological data are not given, and the etiological role of the virus in calves or cattle has not been established.

Referring back to Hedstrom and Isaksson's (1951) description of a virus-diarrhea-like epizootic in Sweden, Bakos and Dinter (1960A) reported the isolation of a cytopathogenic agent from respiratory tract exudate of animals with a mucosal type disease. This disease outbreak at Umeå, Sweden, was investigated by Bakos, Isaksson and Nystedt (1958), and showed a mortality of up to 70% in individual herds. Outstanding

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clinical signs were conjunctivitis, rhinitis, fever, salivation, diarrhea, coughing, and buccal erosions. In comparing this disease outbreak with the one described by Hedstrom and Isaksson, they pointed out a primarily respiratory involvement in the outbreak at Umeå 1958, while Hedstrom and Isaksson in 1951 saw predominantly gastrointestinal signs with respiratory involvement in only some cases. The virus isolated by Bakos and Dinter (1960A) from this so-called "Ume" disease was cultivated in bovine embryo kidney cells and, when injected into experimental calves, produced a mild form of the disease seen in the field. Serological studies also revealed an antibody titer in convalescent sera. The results of cross-neutralization tests indicated a close relationship between the Umeå virus and the parainfluenza-3 virus strain HA-1.

Further work on Ume disease was reported by Nystedt (1960). A cytopathogenic strain of the virus diarrhea virus (C-24-V) isolated in the U.S.A. was used to test cattle sera from Umeå. Sera taken at the later stage of Ume disease, as well as convalescent cattle sera, contained antibodies against the virus diarrhea agent in addition to antibodies against the parainfluenza-3 virus. Sera from the early stages of the disease were generally negative.

The discovery of neutralizing antibodies against the C-24-V prototype virus in Ume disease convalescent sera tends to confirm Hagan's (1958) earlier supposition that epizootic diarrhea in Sweden (Hedstrom and Isaksson, 1951) is identical

with the American virus diarrhea described by Olafson et al. (1946). However, it does not seem clear at this time whether two viruses, parainfluenza and virus diarrhea, are involved in Ume disease, or whether the C-24-V virus is closely related to the parainfluenza virus; there might be only an immunological relationship without antigenic similarity or identity. Such immunological relationships have been found to exist between rinderpest and canine distemper (Goret et al., 1960), hog cholera and mucosal disease (Darbyshire, 1960; Beckenhauer et al., 1961), and between human measles and canine distemper (Carlström, 1960).

Further studies by Bakos and Dinter (1960B) revealed antibodies against the parainfluenza-3 virus in 39 out of 41 suspect herds with primarily respiratory symptoms. A positive titer was found in 70% of random cow sera from different parts of Sweden and in 50% of all calf sera. The calves had lower titers than did adult cattle. Experimentally inoculated calves developed fever and rhinotracheitis very similar to the disease seen in the field. The antibody response in the calves was prompt and reached high titers.

This finding of respiratory signs in calves was similar to the report by Moll and Finlayson (1958) before the parainfluenza relationship was discovered. Runnells et al. (1960) stated that "there is considerable evidence to indicate that infectious rhinotracheitis in many instances, is probably nothing more than virus diarrhea." While details to this

mentioned evidence are not given, the authors proceed to state that there is also reason to believe that outbreaks of calf diphtheria may be preceded by a mild infection of virus diarrhea. Again, the reasons for this belief are not given. Runnells et al. (1960) go so far as to say that not only infectious bovine rhinotracheitis, but mucosal disease (Ramsey and Chivers, 1953), mycotic stomatitis (Pritchard and Wassenaar, 1959), and malignant catarrhal fever are "similar if not identical" to virus diarrhea. While the similarity of mucosal disease (Ramsey and Chivers, 1953) and mycotic stomatitis (Pritchard and Wassenaar, 1959) with virus diarrhea may be well established (Olson and Hoerlein, 1956), the author of this thesis takes exception to the inclusion of malignant catarrhal fever as a disease similar to, if not identical with, virus diarrhea.

A clear differential diagnosis between the virus diarrhea-mucosal disease complex and malignant catarrhal fever has been given by Berkman (1958) and Berkman et al. (1960). According to Smith and Jones (1957), malignant catarrhal fever can be differentiated from virus diarrhea by its low morbidity and the characteristic ocular, nasal and brain lesions.

That our present definition of some of the viral diseases formerly counted to the mucosal disease complex is still somewhat arbitrary was shown by Abinanti and Plumer (1961), who recovered the infectious bovine rhinotracheitis (IBR) virus from field cases of keratoconjunctivitis. Two-

thirds of 125 yearling cattle showed signs of keratoconjunctivitis but without respiratory involvement or corneal opacity. Koch's postulates were fulfilled with the isolated virus and neutralizing antibodies discovered after inoculation. Since the IBR virus may cause infectious pustular vulvovaginitis (IPV), conjuctivitis and rhinitis, the authors concluded, together with Baker et al. (1960), that the original descriptive name, rhinotracheitis, may be too restrictive. Abinanti and Plumer (1961) stated that the diverse pathogenicity of the IBR-IPV virus resembled the human adenovirus III.

Dmochowski (1961) recently also isolated a cytopathogenic agent from the eyes of cattle with a mucosal type syndrome, and comparative studies to classify this virus are in progress.

Abinanti et al. (1960) attempted to clarify the role of the myxovirus parainfluenza-3 in bovine infections. In seven outbreaks of respiratory infections of calves and cattle they recovered parainfluenza virus on 15 trials. Serological evidence of infection was demonstrated in some cattle. The authors concluded that at this time it could not be said whether the calves became infected from exposure to man or to other cattle. Serological comparisons to the IBR-IPV virus or to the C-24-V virus were not made. Rosen and Abinanti (1960) showed that calves could be infected with each of four types of human reoviruses. They also proved serologically that infected animals could transmit their

infection to uninoculated calves by contact. Reoviruses antigenically identical with the human parainfluenza-3 were isolated from a group of calves which became infected under natural conditions. Again, serological comparisons to the virus diarrhea virus or to the IBR-IPV virus were not made.

The great importance of a serological differential diagnosis in the virus diarrhea-mucosal disease complex becomes evident from these reports. It seems quite possible in the near future to exclude the purely respiratory infections; on the other hand, as soon as large scale serological surveys are feasible they might also show that what was called virus diarrhea yesterday may turn out to be an infection caused by more than one virus.

The Swedish reports (Bakos and Dinter, 1960A and 1960B; Bakos, et al., 1958) might be the first in a series where serological studies have shown that more than one possible etiological agent existed.

In the description of virus diarrhea or virus diarrhea-like transmissible diseases, respiratory signs were mentioned by Hedstrom and Isaksson (1951), Pritchard et al. (1955), Hoag et al. (1956), Rooney (1957), Carlson et al. (1957), Bakos et al. (1958), and Bakos and Dinter (1960B).

For further clarification, clinical, gross and histopathological studies of the individual pure virus infection in experimental animals--as was attempted with the North Dakota mucosal disease virus in this thesis--would be highly

desirable. While Carlson et al. (1957) gave a classical pathological description of what they called "Indiana" virus diarrhea, it would be interesting to test serologically whether they worked with a pure virus since they used defibrinated whole blood for their experimental infections.

Syndrome of the Virus Diarrhea-Mucosal Disease Complex with Questionable or Undetermined Viral Etiology

The last section discussed primarily viral agents--cytopathogenic, noncytopathogenic, or nonculturable--from virus diarrhea syndromes which are able to reproduce a predictable sequence of clinical and pathological changes and an immunological response demonstrable by cross-immunization or serum-neutralization tests. The syndrome discussed in this section has not yet been reproduced experimentally, according to Ramsey (1956), and its cause and transmissibility are to a large extent as yet unknown.

In spite of vigorous research efforts, including four Ph. D. theses (Ramsey, 1956; Whiteman, 1960; Trapp, 1960; Bajwa, 1961) describing the gross and microscopic pathology of mucosal disease as reported in Iowa (Ramsey and Chivers, 1953), its cause and pathogenesis are to date not known. The search for a viral agent immunologically distinct from virus diarrhea, as suggested by Smith and Jones (1957), has not led to any conclusive evidence. None of the viral agents discussed in the previous chapter has reproduced the classical syndrome as described by Ramsey and Chivers (1953).

Nielsen et al. (1955) described a similar or identical disease in Canada. Transmission attempts were reportedly unsuccessful. Swope and Luedke (1956) had similarly negative results in their transmission experiments of a mucosal disease in Pennsylvania.

Voss (1959) gave the first description of the Iowa or classical type mucosal disease from Germany. Ten animals died out of a herd of 13 six- to ten-months-old calves. A clear differential diagnosis from malignant catarrhal fever was given by the author. Stöber (1959) made further clinical observations of mucosal disease in Germany and reported transmission attempts with 100 ml. of citrated blood from acute cases as inconclusive or negative. He also reported negative results in differential diagnostic examinations for paratuberculosis and salmonellosis. His finding of hand-sized skin encrustations with epidermal sloughing and alopecia in two animals that recovered corresponded with Rothenbacher's and Barner's (1960) report of a clinical mucosal disease case from Michigan. Stöber (1959) confirmed a mortality rate of 90% from several disease outbreaks. An interesting statement was his remark that at the date of the report (1959) the other members of the mucosal disease complex had not been diagnosed in Germany.

Schulz (1959) gave a pathological-anatomical description of mucosal disease in Germany which closely correlated to Ramsey's (1956) findings. Both Schultz (1959) and Stöber

(1959) pointed out the greatest similarity to (or identity with) rinderpest lesions as presented by Albrecht (1929), and Hutyra et al. (1954). Stöber (1959) also mentioned the possibility that some of the cases formerly diagnosed as atypical malignant catarrhal fever (MCF) may actually have been mucosal disease.

Goret and Pilet (1958) reporting on "mucosal diseases" from France called attention to the fact that what they termed the "true mucosal disease" could also in certain aspects resemble foot and mouth disease.

Seibold (1956) in Alabama described what Rooney (1957) called the "Ramsey-type" mucosal disease. The lesions reported matched those reported from Iowa.

Barner et al. (1959, 1960) reported 23 Iowa-type mucosal disease cases from Michigan in a two-year period. Attempts to transmit the disease were unsuccessful.

While these classical reports described the Iowa-type mucosal disease with its clinical and post mortem changes they also agreed with Ramsey's (1956) differential diagnostic exclusion of virus diarrhea on the basis of high morbidity, low mortality, and easy transmissibility. It is interesting, however, that in the progress reports of the regional NC-34 research project on "mucosal diseases in cattle" virus diarrhea was not reported from those collaborating states which reported mucosal disease (Ramsey et al., 1959, 1960; Barner et al., 1958, 1959, 1960; Schipper and Noyce, 1959C; Jones,

1959). The only state of the North Central region which for the last six years has consistently reported virus diarrhea and mucosal disease is Indiana (Claflin et al., 1957; Pritchard, 1955).

Pritchard (1955) and Pritchard et al. (1955) were the first to state that the differences between the two diseases are quantitative rather than qualitative. This view was later supported by the research workers in Great Britain (Huck, 1957; Dow et al., 1956; Jarrett, 1958), who described mild syndromes resembling the descriptions of virus diarrhea (Olafson et al., 1946; Baker et al., 1954) and more severe syndromes resembling mucosal disease.

According to Pritchard (1955) it was Ramsey and Chivers (1953) who first presented evidence that mucosal disease might be transmissible. They obtained a temperature rise in seven calves two to eight days after inoculation.

Pritchard (1955) reproduced mucosal disease by the intravenous inoculation of defibrinated whole blood from mucosal disease field cases taken during the leukopenic early phase of the illness. A typical temperature and leukocyte curve presented in Pritchard's (1955) paper for the experimental mucosal disease is identical with the clinical course reported for experimental virus diarrhea (Carlson et al., 1957; Baker et al., 1954) as well as for the Merrell or Sanders experimental mucosal disease (Claflin et al., 1959; Ramsey et al., 1959). A diphasic temperature response was

described by Pritchard (1955) with a first peak and leukopenia around the third day and a second higher fever peak for a short period (24 hours) around the seventh day. The symptoms included depression, lacrimation, nasal discharge and mouth lesions. Cross protection tests with two strains of virus-diarrhea virus were reported as inconclusive by Pritchard (1955). Later tests by Rothenbacher and Whiteman (1961) indicated a cross immunization between the Sanders, Merrell, and virus diarrhea (Indiana) agents. Reports from North Dakota (Schipper et al., 1955; Schipper, 1957), and South Dakota (Harshfield, 1957) confirmed Pritchard's (1955) earlier transmission studies.

Schipper et al. (1955) described mucosal disease in cattle and found a morbidity of 1 to 89% and a mortality of 100% in sick animals. They reported subacute and chronic cases with hyperkeratosis correlating with later reports from Michigan (Rothenbacher and Barner, 1960) and Germany (Stöber, 1959). The fact that hyperkeratosis was noted in herds two to three weeks after mucosal disease outbreaks, was interpreted by Schipper et al. (1955) to suggest that numerous mild mucosal disease cases go unnoticed with perhaps only a transient temperature elevation.

In their study on the incidence and mortality of mucosal disease in Iowa, Ramsey et al. (1958) gave a mortality rate of 1 to 50%. They admitted that insufficient herd observations were primarily responsible for insufficient

information on morbidity. Ramsey et al. (1958) also conceded that a necropsy is usually necessary to make a specific diagnosis of mucosal disease. In some instances they failed to diagnose the condition in the antemortem state.

Discussing the virus diarrheas of cattle and similar diseases in Australia, Johnston (1959) stated that mucosal disease was first seen in 1956 with high mortality, and that there was some evidence of a high incidence of inapparent infections. Transmission attempts with defibrinated blood succeeded in some animals and not in others. Epizootic diarrhea (Edwards and Sier, 1960), an apparently new disease in Australia, spread throughout that country, affecting cattle of all ages. Whittem (1959) pointed out that the lesions of mucosal disease--as first observed in Sidney 1956--looked like rinderpest. On the other hand, the epizootiology of epizootic diarrhea closely resembled rinderpest. Whittem (1959) wondered whether the three viruses (rinderpest, epizootic diarrhea, mucosal disease) had arisen from a common ancestor and developed different degrees of pathogenicity.

In their studies on bovine mucosal disease, Schipper and Noyce (1959C), reported the reisolation of their earlier reported mucosal-disease virus (Schipper and Noyce, 1959A) from six animals showing typical signs of mucosal disease during the first nine months of 1959. They emphasized that in all instances the tissues prepared for virus culturing were obtained from diseased animals immediately prior to,

or upon the first appearance of, the acute symptoms. In no case were they able to isolate the virus when the animal had displayed the signs of the disease for several days or after death of the animal.

Summary

In summarizing the review of the literature pertaining to this thesis it can be said that virus diarrhea as first described in New York seems to be a better explored syndrome than the Iowa mucosal disease. Its viral etiology has been confirmed and Koch's postulates fulfilled. There remains, however, a certain discrepancy between the severity of the syndrome seen in the field and the reportedly mild experimental disease. The possibility of more than one etiological factor in the pathogenesis of virus diarrhea has to be considered. There is indication that in severalcases a combination of two viruses was involved in the syndrome.

The pathogenesis of Iowa-type mucosal disease has remained a challenge to many investigators. While several viruses have been isolated from field cases, these viruses are either nonpathogenic in pure infection, or resemble the experimental virus diarrhea, causing only mild and transient symptoms.

The supposition by several research workers that Iowa mucosal disease represents nothing but a severe form of virus diarrhea is being carefully investigated. The possibility of additional and as yet unknown factors in its pathogenesis must be considered.

CHAPTER II

MATERIALS AND METHODS

Materials Used for Animal Inoculation

The North Dakota mucosal disease virus isolated and reported by Schipper and Noyce (1959A)and Noyce and Schipper (1959) was grown and maintained in tissue culture by Cunningham and Church (1960). Bovine embryo kidney cells in primary culture according to the procedure given by the original investigators (Noyce and Schipper, 1959) were required for viral multiplication. Virus was harvested 24 hours after inoculation of a six-day-old tissue culture when approximately 75% of the cells showed cytopathogenic effects (CPE). The virus was then stored at -60 C until used. The titer of the virus was generally 10^5 tissue culture infectious doses (TCID).

Tissue culture fluid from a preliminary experiment aimed at adapting the virus to rabbit kidney cell culture (Rothenbacher, 1960) was harvested, stored, and used in like manner.

Sterile lactalbumin hydrolysate plus 2% horse serum, the growth medium for the virus, was used to inoculate control animals to test for possible antigenicity or pathogenicity.

Organ emulsions of spleen, liver, lungs, kidneys, adrenals, and lymph nodes of infected calves euthanatized during the acute, subacute, or recovery phase of the experimental disease were similarly used to inoculate calves. A mixture of approximately equal amounts of the ground tissues was suspended 1:10 in sterile physiological saline solution; antibiotics were added to the emulsion to a concentration of 1000 units of penicillin and 1000 micrograms of streptomycin per ml. Organ emulsion was used fresh, or stored like the virus until further use.

Fresh blood, without addition of preservative, from animals showing acute signs of the experimental disease was used for transmission studies. The blood was taken from donor calves two to nine days after their inoculation with the virus.

Experimental Animals

Thirty-seven healthy dairy cattle with no history of virus diarrhea or mucosal disease were used for experimental inoculations. The majority of these were calves from three to six months old. The youngest animal inoculated was a one-day-old colostrum-deprived calf and the oldest animal was a two-year-old Herford cow that had recovered from clinical signs of mucosal disease. Thirty-one of the 37 animals originated from the Michigan State University dairy farm; the rest were purchased from outside sources.

Ten sheep (five six-year-old ewes, four 18-month-old lambs, and one 4-year-old ram) served as experimental animals for studies with the North Dakota virus. The six-year-old ewes originated from the Michigan State University sheep herd. Four lambs were purchased from outside sources, and one five-year-old ram was donated by the United States Department of Agriculture.

Ten rabbits ranging in age between one and three years were used for similar studies with the North Dakota virus. All rabbits were raised in the Department of Veterinary Pathology animal colony.

Case histories and materials of 11 calves from negative malignant catarrhal fever transmission studies (Barner et al., 1958) served as controls for comparative studies.

Hematological Studies

It was found that in the present literature only sparse data on the hematological values of the young calf exist (Dukes, 1955; Coffin, 1953; Krölling and Grau, 1960; Schalm, 1961). Since data were available in this study, the preinoculation values of 41 healthy experimental cattle were divided into four age groups, averaged and considered as normal values for the respective age groups.

The average hematological data of five healthy sheep were used as normal values.

Eight rabbits were similarly used for normal preinoculation blood data.

Blood examinations including total erythrocyte (RBC) and leucocyte (WBC) counts, hemoglobin and hematocrit determinations and a WBC-differential count were made once to twice daily. The hemoglobin determination was done by the acid-hematin (Dukes, 1955) and the cyanmethemoglobin (Schalm, 1961) spectrophotometric methods. Hematocrit values were determined by means of a microhematocrit centrifuge. Wright's stain was used on the blood smears for the differential counts.

Blood sugar and nonprotein nitrogen were determined in some animals on Folin Wu protein-free filtrates according to the methods given by Bray (1957).

Virus Inoculations and Transmission Studies

On the first five calves, various routes of inoculation including intravenous, subcutaneous, intracutaneous, intramuscular, intranasal, intramucosal, and intranodal (prescapular lymphnode) were used. After the infectivity of the virus and the susceptibility of the experimental animals were well established, the main routes of inoculation used were intravenous and subcutaneous.

Five ml. of the freshly thawed virus constituted the basic dose of inoculum for calves and sheep. A few drops of the virus which remained in the inoculation needle and syringe were given intraocularly and/or intranasally in some animals.

Inoculation with virus. Twenty-six calves from two to eight months old, four yearling cattle, one two-year-old cow, and one day-old calf were inoculated with the North Dakota mucosal disease virus. Four calves received repeated injections of the virus: one of the four (#25) received three virus injections at weekly intervals. Another calf (#04) received two virus injections 69 days apart; the third calf (#35) received two virus injections 36 days apart; and the fourth calf (#02) received four virus inoculations with one each on days 0, 23, 51, and 121 of the experiment. Epinephrin was usually given at repeated injections to counteract anaphylactic reactions.

Inoculation with rabbit kidney cell culture harvest. Rabbit kidney cell culture harvest (Rothenbacher, 1960) was similarly inoculated into three calves.

Inoculations with fresh whole blood. Six calves were inoculated with fresh, whole blood (without addition of preservative) from animals showing acute signs of the experimental North Dakota mucosal disease (Chp.II, p.28). Twenty ml. of the fresh blood were given intravenously as a standard dose. The blood was drawn and reinjected within ten seconds. One of the six calves received blood from a sheep showing acute signs of the experimental infection. One calf received a second blood inoculation five days later. To test for immunity all calves were challenged with the pure virus ten to 60 days later.

Inoculations with organ emulsions. Three calves were given organ emulsions from seven animals euthanatized from two to eight days after inoculation, during the acute or subacute phase of the experimental North Dakota mucosal disease. Each inoculated animal received an organ emulsion mixture (Chp. II,p.28)from one or several donor animals. The inoculum for sheep and calves consisted of 20 ml. of the emulsion given subcutaneously or intramuscularly. One of the four animals (#32) received three consecutive injections of the organ emulsion mixture at weekly intervals. Another (#27) received consecutive injections on days 0, 14, 32, and 39. One ml. epinephrin was usually administered subcutaneously at the time of repeated injections. To check for the onset of immunity, two out of these three calves were challenged with the pure virus from seven to 60 days after inoculation.

Inoculations with sterile tissue culture medium. Three calves and one sheep received the sterile tissue culture medium (Chp.II, p.28) in the identical dosage and by the same routes of inoculation as for the virus.

Contact transmission studies. Two calves were kept in intimate contact with an experimentally inoculated animal during the acute phase and recovery period of the experimental disease. Each calf was placed into the same stall with an experimental calf that had just been given the virus. The calves drank and ate from the same troughs and were kept

in contact for ten days. Both calves were challenged with the pure virus from five to eight weeks after this contact period to check for a possible subclinical infection resulting in immunity.

Inoculation of sheep. Seven sheep ranging in age from one and one-half to six years were inoculated with the same virus dose subcutaneously. One 18-month-old lamb was inoculated with sterile tissue culture medium.

Inoculation of rabbits. Eight rabbits from one to three years old each received 0.2 ml. of the virus intravenously, subcutaneously, intramuscularly, and intraocularly.

Clinical Observations

All experimental animals were allowed to adjust to the isolation quarters for one to several weeks before inoculation. No change in diet, environment, or management occurred during the experimental period. Preinoculation observations included those on body temperature, pulse, respiration, food and water consumption, and the consistency of the feces. Blood samples for hematological observations were taken daily for several days prior to inoculation.

During an experiment, strict isolation of the experimental animals was maintained. The temperature of inoculated animals was taken from one to three times daily.

Other clinical observations before and after inoculation included complete daily hemograms (Chp.II, p.30) and periodic but less frequent urinalysis and fecal examination.

Gross Pathological Observations and Necropsy Procedures

At various time intervals ranging from one to 134 days after inoculation, animals were euthanatized by exsanguination, usually following electrical stunning. Gross lesions were observed as soon as possible after death. The necropsy procedure was modified routinely as to the sequence of body tracts and regions examined; alternatively, gastrointestinal tract, respiratory tract, or urogenital tract were examined first, in order to allow an immediate and fresh post-morten view of each tract. In the case of the gastrointestinal tract, different parts of it were examined first on various necropsies, in order to obtain a clear picture of the pathological changes occurring.

Eighteen calves were necropsied during the acute clinical phase of the experimental North Dakota mucosal disease infection from one to six days after inoculation. One calf was necropsied eight days and another 14 days after inoculation.

Three calves that were allowed to recover from the acute experimental disease were necropsied 54, 64, and 107 days after inoculation. All three of these calves had been hyperimmunized with additional inoculations of virus, rabbit cell culture harvest, or organ emulsion.

One calf was necropsied six days after inoculation with sterile lactalbumin hydrolysate, the tissue culture growth medium.

Five calves and one two-year-old cow were commercially slaughtered at the M. S. U. meats laboratory 22, 33, 40, 45, 118, and 218 days, respectively, after virus inoculation. All animals had completely recovered from the experimental disease and none showed signs of illness. Gross observations were made and tissue specimens obtained at the time of slaughter.

Six calves, after complete recovery from the North Dakota experimental disease (21, 22, 28, 32, 33, and 67 days after inoculation) were released to Dr. C. K. Whitehair* for nitrate-nitrite toxicity studies and were necropsied in the course of this work by Dr. R. Naghshineh.*

Of seven sheep inoculated with the North Dakota virus, two six-year-old ewes were necropsied six days, another two six-year-old ewes five days, and two 18-month-old lambs four days after inoculation. Three sheep (one six-year-old ewe, and two 18-month-old lambs) were necropsied as healthy control animals. One of the control lambs was necropsied four days after inoculation with sterile tissue culture growth medium.

Two one-year-old rabbits were necropsied two days, and another three one-year-old rabbits three days after the North Dakota virus inoculation. Three three-year-old rabbits were necropsied three days after inoculation. One two-year-old

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and one one-year-old rabbit were necropsied as normal control animals.

Histopathological Observations

Representative tissue sections of the adrenal, thyroid, parathyroid and pituitary glands, brain, spinal cord, liver, kidneys, spleen, oral and nasal mucosae, lung, heart, stomach(s), duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, urethra, vagina, uterus, ovaries, testes, penis, musculature, skin, bone marrow, pancreas, salivary glands, thymus, aorta and pulmonary arteries, and lymph nodes from various body regions were fixed in buffered 10% formalin solution (A.F.I.P., 1960).

In view of the distribution of the gross lesions, special emphasis was given to the sections of the posterior small intestine, adrenal glands, spleen, bone marrow, kidneys, liver, tonsils, and mesenteric, mandibular, parotid, supra-pharyngeal, bronchial, mediastinal, prescapular and pre-femoral lymph nodes.

Sections of the sternum were decalcified after fixation in buffered 10% formalin. Decalcification was accomplished by the formic acid-sodium citrate method (A.F.I.P., 1960).

The histological technique used for all tissue sections followed the outline given by the Manual of Histologic and Special Staining Technics (A.F.I.P., 1960) unless otherwise noted. After fixation the tissues were dehydrated in a

series of graded alcohol solutions, cleared in xylene and embedded in paraffin. The paraffin sections were cut at six to seven microns thickness and stained with hematoxylin and eosin (A.F.I.P., 1960).

Special staining procedures used on selected tissue sections included the periodic acid-Schiff reaction (PAS), Mallory's iron reaction, Lendrum's, Heidenhain's anilin blue, crystal violet, Schorr's, Weil's, Verhoef's, Von Kossa's, Scharlach red (Sudan IV), and oil-red-O stains. The latter two stains were used on frozen sections of adrenal glands.

Serological Studies

Pre- and post-inoculation sera were saved from each animal and stored at -60 C for serum neutralization studies and virus comparison work.

Bacteriological and Virological Examinations

Fresh tissue specimens were submitted for examination to the microbiological diagnostic laboratory at Michigan State University.

For virus isolation attempts fresh tissue specimens and fecal scrapings of the intestines were submitted to the virology section, Department of Microbiology and Public Health, within ten minutes after death of the experimental animal.

Other Post-Mortem Observations

Fecal samples were routinely checked for parasite eggs, Strongyloides, nematode larval stages, coccidia and other protozoa.

Urine specimens were also taken in some cases during necropsy of the experimental animal and submitted for examination.

CHAPTER III

RESULTS

General Clinical Observations in Virus-Inoculated Experimental Animals

Cattle. Thirty calves (five weeks to eight months old), two yearling bulls, two yearling heifers, one two-year-old cow and one day-old calf were inoculated with the North Dakota virus by various routes of inoculation (see Chapter II, p. 31). A predictable and rather constant sequence of symptoms was produced in all cattle including the day-old calf.

Pyrexia started after 16 to 48 hours and reached a peak after 24 to 72 hours. The average preinoculation temperature of the experimental cattle was 102.28 F. The average peak temperature reached after 24 to 72 hours was 105.24 F. The peak temperature was 107.0 F. in one calf and 11 out of 30 calves had peak temperatures of 106 F or higher. Concomitant with the rising temperature were nervousness and restlessness evidenced 16 to 48 hours after inoculation, slight to profuse serous ocular discharge, tachycardia and an increased rate of respiration. These clinical signs appeared to be more pronounced in the younger calves. Usually there was an erythema of the oral mucous membranes during

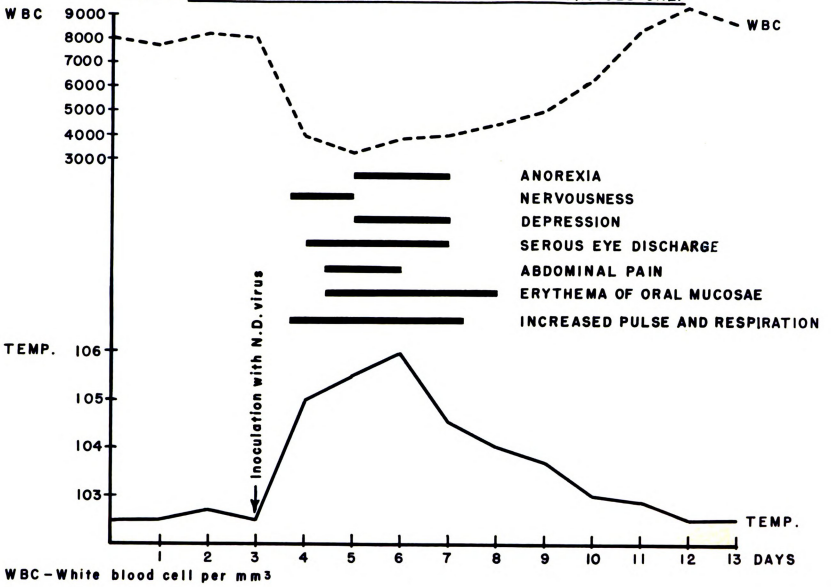
the period of high fever. Increased salivation and champing were also observed in some calves during this period. Starting generally at the time of the highest temperature (24 to 48 hours after inoculation), partial to complete anorexia, lasting from one to three days, was observed in the experimental cattle.

Colic was noted in some calves, evidenced by restlessness and by kicking of the abdomen with the hind feet. In several calves a moderate degree of bloating of the rumen was also seen beginning during this period of high fever, and lasting from one to four days.

Dehydration and constipation during the time of high temperature was evidenced by a decrease in the number of defecations to one-half or less and by the passing of small amounts of dry feces in formed spheres two to three cm. in diameter. The water consumption decreased during the time of the highest temperature elevation and increased one to three days after the peak in temperature was reached. Pulse, respiration, water and feed consumption, and amount and consistency of the feces, as well as frequency of defecation, returned to normal approximately two to five days after the highest temperature elevation.

The whole course of the experimental infection lasted from five to nine days (see Tables 1, 4, 7, 9, 10, and 11). The initial period of nervousness and restlessness, which occurred between one and two days after inoculation, while

Table 1. ***NORTH DAKOTA VIRUS INFECTION OF A 4-MONTHS-OLD CALF***



WBC – White blood cell per mm3

the fever was rising, generally gave way to a period of depression lasting from one to two days, usually until the high fever began to subside.

Diarrhea was never seen in observations extending up to 60 days after virus inoculation. Respiratory symptoms were not observed except for a transitory increase in respiration during the period of restlessness and high temperature. The ocular signs described were very mild in the older calves and rarely lasted longer than 48 hours.

Sheep. Four six-year-old ewes, one four-year-old ram, and three one-and-one-half-year-old lambs were inoculated with the North Dakota virus by various routes of inoculation (see Chp.II, p.34). The clinical symptoms produced in sheep were milder in degree than in the cattle and less obvious to the observer. Temperature rises of one to two F. were observed from one to four days after inoculation. The average preinoculation temperature of the experimental sheep was 102.80 F. The average peak temperature reached from one to four days after inoculation was 104.15 F. The highest temperature in one sheep was 104.8 and two other sheep had peak temperatures of 104 F. No temperature elevation was detected in one sheep.

Depression and partial anorexia were observed from two to four days after inoculation. The visible mucous membranes appeared slightly congested during the febrile period. Simultaneous to the period of depression and anorexia, dehydration

and constipation were evidenced by a decrease in the defecation rate and by dehydrated and smaller amounts of feces with a smaller than normal pellet size.

In general, the onset of the temperature rise occurred later in sheep and the course of the experimental infection was shorter than in cattle, lasting only from three to five days.

Rabbits. Eight rabbits (three two-and-one-half year-olds, two one-and-one-half-year-olds, and three one-year-olds) received the North Dakota virus by various routes of inoculation including intravenous, intramuscular, subcutaneous, and intraocular.

Similarly to the sheep, the rabbits displayed a very mild degree of clinical disturbance. Pyrexia occurred from one to three days after inoculation and temperature rises from 0.8 to 2.5 F. were noted. The average preinjection temperature in rabbits was 102.5 F. and the average peak temperature measured three days after inoculation was 104.9 F. No temperature elevation was noted in one rabbit. Partial anorexia was seen during the febrile period from one to four days after inoculation. The feces passed during this period were less in amount, smaller in pellet size and showed a marked dehydration as compared to those of control animals.

Hematological Findings

Due to the paucity of specific hematological data for the young bovine (see Chp. II), the preinoculation data of

34 healthy experimental cattle were divided into four age groups and the hematological values averaged for each group. These values are compared with the hematological levels observed during the period of lowest depression of the total leukocyte count and the highest temperature elevation, both of which occurred from two to four days after inoculation.

TABLE 2

AVERAGE HEMATOLOGICAL DATA FROM HEALTHY 1- TO 2-MONTHS-OLD CALVES BEFORE AND AFTER NORTH DAKOTA VIRUS INOCULATION

Average	6 Calves Before Inoculation		4 Calves 78 Hrs. After Inoculation	
	Per cu. mm.	%	Per cu. mm.	%
Hemoglobin (Gm./100 ml.)		9.99		7.52
Hematocrit (volumes %)		29.0		27.5
Total leukocytes	8200		3687	
Heterophils	2186.66	26.66	617.65	16.75
Stab cells	273.33	3.33	138.28	3.75
Lymphocytes	5589.66	68.16	2996.09	81.25
Monocytes	369.00	4.50	55.31	1.50
Eosinophils	54.66	0.66	18.43	0.50
Basophils	0.00	0.00	0.00	0.00

TABLE 3

AVERAGE HEMATOLOGICAL DATA FROM HEALTHY 3-MONTHS-OLD CALVES BEFORE AND AFTER NORTH DAKOTA VIRUS INOCULATION

Average	15 Calves Before Inoculation		9 Calves 51 Hours After Inoculation	
	Per cu. mm.	%	Per cu. mm.	%
Hemoglobin (Gm./100 ml.)		11.6		10.86
Hematocrit (volumes %)		31.06		32.0
Total leukocytes	7323.00		3111.1	
Heterophils	1854.91	25.33	601.44	19.33
Stab cells	375.66	5.13	152.06	4.88
Lymphocytes	5008.93	68.40	2309.04	74.22
Monocytes	263.62	3.60	159.00	5.11
Eosinophils	185.49	2.53	37.95	1.22
Basophils	9.76	0.13	3.45	0.11

TABLE 4

HEMATOLOGICAL AND CLINICAL CHANGES IN A TYPICAL NORTH DAKOTA VIRUS EXPERIMENTAL INFECTION OF A 3-MONTHS-OLD CALF

			Cells/cu. mm. of Blood and (Percentages)							
Days	Temp.	Hb.	RBC	WBC	H	S	L	M	E	B
-2	102.8	9.0	7.7	9,400	3478	188	5358	376	---	---
					(37)	(2)	(57)	(4)	(0)	(0)
-1	103.0	9.0	7.0	10,800	3240	648	6372	324	216	---
					(30)	(6)	(59)	(3)	(2)	(0)
0	102.2	10.1	7.1	11,100	4773	---	6216	---	111	---
					(43)	(0)	(56)	(0)	(1)	(0)
+1	104.8	8.8	6.7	3,150	756	63	2268	63	---	---
					(24)	(2)	(72)	(2)	(0)	(0)
+2	105.6	7.9	9.3	5,450	1635	381.5	3324.5	109	---	---
					(30)	(7)	(61)	(2)	(0)	(0)
+3	105.2	9.6	9.0	6,500	2600	130	3770	---	---	---
					(40)	(2)	(58)	(0)	(0)	(0)
+4	104.0	9.1	7.8	6,450	1999.5	64.5	4192.5	193.5	---	---
					(31)	(1)	(65)	(3)	(0)	(0)
+5	103.2	9.5	7.3	7,300	1606	146	5256	292	---	---
					(22)	(2)	(72)	(4)	(0)	(0)
+6	103.0	8.9	7.3	7,500	1725	75	5400	300	---	---
					(23)	(1)	(72)	(4)	(0)	(0)
+7	103.0	8.8	6.4	6,600	1716	264	4290	264	66	---
					(26)	(4)	(65)	(4)	(1)	(0)
+9	102.6	10.8	8.5	8,700	1827	---	6177	609	---	87
					(21)	(0)	(71)	(7)	(0)	(1)

Abbreviations: Day 0 = day of inoculation; Hb. = Gm. hemoglobin/100 ml.; RBC = millions of red blood cells/cu. mm.; WBC = white blood cells; H = heterophils; S = stab cells; L = lymphocytes; M = monocytes; E = eosinophils; B = basophils.

An additional hematological finding in animals of this age group was made through the experimental virus inoculation into two calves suffering from pneumonia and coccidiosis. The changes in their preinoculation blood picture of leukocytosis, heterophilia, and eosinophilia are shown in Table 5.

TABLE 5

HEMATOLOGICAL CHANGES 72-HOURS AFTER NORTH DAKOTA VIRUS INOCULATION OF 3-MONTH-OLD CALVES WITH CLINICAL SIGNS OF PNEUMONIA AND COCCIDIOSIS

	Calf 1 (Pneumonia)		Calf 2 (Pneumonia and Coccidiosis)	
	Pre-Inoculation	Post-Inoculation	Pre-Inoculation	Post-Inoculation
Hemoglobin (Gm./100 ml.)	8.8	7.2	12.1	10.8
Hematocrit (volumes %)	28	27	37	33
Total Leukocytes/cu.mm.	12,700	9,650	17,000	5,800
Heterophils/cu. mm.	6,096	3,360	7,990	1,508
%	48	35	47	26
Stab cells/cu. mm.	1,524	1,344	680	522
%	12	14	4	9
Lymphocytes/cu.mm.	5,842	6,144	5,440	3,712
%	46	64	32	64
Monocytes/cu.mm.	635	0	1,020	116
%	5	0	6	2
Eosinophils/cu.mm.	127	0	2,550	406
%	1	0	15	7
Basophils/cu.mm.	0	96	0	0
%	0	1	0	0

TABLE 6

AVERAGE HEMATOLOGICAL DATA FROM HEALTHY 4- TO 8-MONTHS-OLD CALVES BEFORE AND AFTER NORTH DAKOTA VIRUS INOCULATION

Average	17 Calves Before Inoculation		11 Calves 60 Hours After Inoculation	
	Per cu. mm.	%	Per cu. mm.	%
Hemoglobin (Gm./100 ml.)		10.62		10.42
Hematocrit (volumes %)		31.70		29.72
Total leukocytes	8732.00		4940.99	
Heterophils	2136.72	24.47	789.90	15.99
Stab cells	61.56	0.70	31.42	0.63
Lymphocytes	6034.68	69.11	3754.40	76.00
Monocytes	210.52	2.41	251.44	5.09
Eosinophils	344.12	3.94	129.32	2.63
Basophils	5.13	0.05	17.93	0.36

TABLE 7

HEMATOLOGICAL AND CLINICAL CHANGES IN A TYPICAL NORTH DAKOTA VIRUS EXPERIMENTAL INFECTION OF A 4-MONTHS-OLD CALF

Days After Inoculation	Temp.	Hb.	Cells/cu. mm. of Blood and (Percentages)							
			RBC	WBC	H	S	L	M	E	B
0	102.6	10.7	8.4	8700	3306	87	5046	87	174	0
					(38)	(1)	(58)	(1)	(2)	---
+1	105.4	9.8	9.5	4700	1551	188	2914	47	0	0
					(33)	(4)	(62)	(1)	---	---
+2	105.0	10.1	7.5	3550	568	71	2627	248	35	0
					(16)	(2)	(74)	(7)	(1)	---
+3	105.8	10.2	8.0	3700	1036	74	2442	148	0	0
					(28)	(2)	(66)	(4)	---	---
+4	104.2	10.0	7.8	4800	1056	96	3360	288	0	0
					(22)	(2)	(70)	(6)	---	---
+5	104.0	9.6	6.8	6800	1292	68	5236	204	0	0
					(19)	(1)	(77)	(3)	---	---
+6	102.8	9.8	8.1	8900	623	178	7921	178	0	0
					(7)	(2)	(89)	(2)	---	---

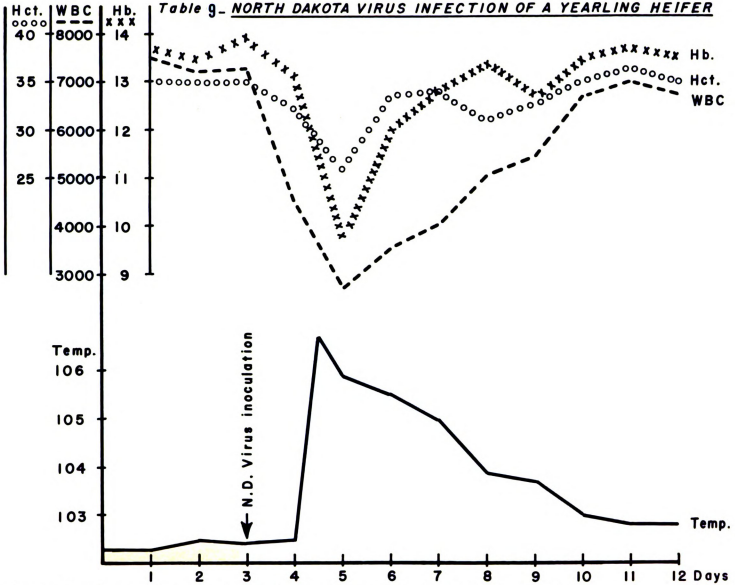
Abbreviations: Hb. = Gm. hemoglobin/100 ml.; RBC = millions of red blood cells/cu. mm.; WBC = white blood cells; H = heterophils; S = stab cells; L = lymphocytes; M = monocytes; E = eosinophils; B = basophils.

TABLE 8

HEMATOLOGICAL DATA FROM FOUR YEARLING CATTLE BEFORE AND AFTER NORTH DAKOTA VIRUS INOCULATION

Average	Before Inoculation		60 Hours After Inoculation	
	Per cu. mm.	%	Per cu. mm.	%
Hemoglobin (Gm./100 ml.)		12.35		11.45
Hematocrit (volumes %)		34		29.75
Total leukocytes	7287.00		3661.00	
Heterophils	1512.05	20.75	616.25	17.0
Stab cells	236.82	3.25	135.93	3.75
Lymphocytes	5301.29	72.75	2854.68	78.75
Monocytes	400.78	5.50	145.0	4.0
Eosinophils	72.87	1.0	9.06	0.25
Basophils	0.0	0.0	0.0	0.0

Table 9 – **NORTH DAKOTA VIRUS INFECTION OF A YEARLING HEIFER**



Hct. = microhematocrit vol. %; Hb = hemaglobin Gm. %; WBC = White blood cells per mm3.

TABLE 10

HEMATOLOGICAL AND CLINICAL CHANGES IN A TYPICAL NORTH DAKOTA VIRUS EXPERIMENTAL INFECTION OF A 1-YEAR-OLD BULL

Days After Inoculation	Temp.	Hb.	Hct.	Cells/cu.mm. of Blood and (Percentages)						
				WBC	H	S	L	M	E	B
0	102.4	13.7	35	7000	1050	0	5670	210	70	0
					(15)	---	(81)	(3)	(1)	---
+1	102.4	13.1	35	6750	1552	337	4725	135	0	0
					(23)	(5)	(70)	(2)	---	---
+1-1/2	106.4	11.3	29	3550	390	71	3053	35	0	0
					(11)	(2)	(86)	(1)	---	---
+2	106.0	9.8	26	1700	153	85	1360	85	17	0
					(9)	(5)	(80)	(5)	(1)	---
+2-1/2	107.0	12.0	33	3700	703	0	2960	37	0	0
					(19)	---	(80)	(1)	---	---
+3	106.4	13.3	35	3950	592	40	3239	0	79	0
					(15)	(1)	(82)	---	(2)	---
+3-1/2	106.4	10.4	35	4650	1442	651	2278	279	0	0
					(31)	(14)	(49)	(6)	---	---
+4	105.6	13.3	34	4800	1344	288	2784	384	0	0
					(28)	(6)	(58)	(8)	---	---
+5	104.0	11.7	31	5950	1428	297	4046	179	0	0
					(24)	(5)	(68)	(3)	---	---
+6	102.4	11.7	31	8200	820	246	6970	82	82	0
					(10)	(3)	(85)	(1)	(1)	---

Abbreviations: Hb. = Gm. hemoglobin/100 ml.; Hct. = hematocrit volume %; WBC = white blood cells; H = heterophils; S = stab cells; L = lymphocytes; M = monocytes; E = eosinophils; B = basophils.

TABLE 11

HEMATOLOGICAL AND CLINICAL CHANGES IN A TYPICAL NORTH DAKOTA V[illegible] EXPERIMENTAL

TABLE 11

HEMATOLOGICAL AND CLINICAL CHANGES IN A TYPICAL NORTH DAKOTA VIRUS EXPERIMENTAL INFECTION OF A 2-YEAR-OLD COW

Days After Inoculation	Temp.	Hb.	Hct.	Cells/cu. mm. of Blood and (Percentages)						
				WBC	H	S	L	M	E	B
-1	101.2	15.0	38	7700	2772	77	3234	77	1540	0
					(36)	(1)	(42)	(1)	(20)	---
0	101.8	15.0	37	10,200	1938	612	5508	204	1938	0
					(19)	(6)	(54)	(2)	(19)	---
+1	102.4	14.1	34	6000	1800	180	3000	180	840	0
					(30)	(3)	(50)	(3)	(14)	---
+2	106.4	11.1	34	3200	160	32	2016	32	960	0
					(5)	(1)	(63)	(1)	(30)	---
+3	104.8	15.0	37	3400	752	102	1972	34	340	0
					(28)	(3)	(58)	(1)	(10)	---
+4	104.0	13.3	33	5600	1736	392	2688	280	504	0
					(31)	(7)	(48)	(5)	(9)	---
+5	102.8	14.0	34	5900	1475	295	3186	236	708	0
					(25)	(5)	(54)	(4)	(12)	---
+6	101.8	12.3	33	6600	851	396	4686	264	396	0
					(13)	(6)	(71)	(4)	(6)	---
+7	101.4	13.5	35	7000	1820	210	4270	140	560	0
					(26)	(3)	(61)	(2)	(8)	---

Abbreviations: Hb. = Gm. hemoglobin/100 ml.; Hct. = hematocrit volume %; WBC = white blood cells; H = heterophils; S = stab cells; L = lymphocytes; M = monocytes; E = eosinophils; B = basophils.

TABLE 12

HEMATOLOGICAL AND CLINICAL CHANGES IN A 1-DAY-OLD CALF AFTER NORTH DAKOTA VIRUS INOCULATION

Days After Inoculation	Temp.	Hb.	Hct.	Cells/cu. mm. of Blood and (Percentages)						
				WBC	H	S	L	M	E	B
0	102.2	11.6	37	8200	2460	1230	4408	82	0	0
					(30)	(15)	(54)	(1)	---	---
+1	102.0	11.9	35	11700	3998	1872	4914	936	0	0
					(34)	(16)	(42)	(8)	---	---
+2	103.2	10.8	34	8750	2537.5	2450	3412.5	350	0	0
					(29)	(28)	(39)	(4)	---	---
+3	105.0	11.1	32	6900	1518	483	4623	276	0	0
					(22)	(7)	(67)	(4)	---	---
+4	104.4	11.1	31	10600	1908	1060	3714	318	0	0
					(18)	(10)	(69)	(3)	---	---
+5	105.0	9.8	30	7350	955.5	1249.5	5145	0	0	0
					(13)	(17)	(70)	---	---	---
+6	104.6	9.1	31	11700	3042	1404	7020	234	---	---
					(26)	(12)	(60)	(2)	0	0
+7	104.8	9.4	29	6850	2055	822	3562	411	0	0
					(30)	(12)	(52)	(6)	---	---
+8	103.8	10.3	31	13300	2926	2128	7714	532	0	0
					(22)	(16)	(58)	(4)	---	---

Abbreviations: Hb. = Gm. hemoglobin/100 ml.; Hct. = hematocrit volume %; WBC = white blood cells; H = heterophils; S = stab cells; L = lymphocytes; M = monocytes; E = eosinophils; B = basophils.

TABLE 13

AVERAGE HEMATOLOGICAL DATA FROM HEALTY ADULT SHEEP BEFORE AND AFTER NORTH DAKOTA VIRUS INOCULATION

Average	6 Sheep Before Inoculation		4 Sheep 96 Hours After Inoculation	
	Per cu. mm.	%	Per cu. mm.	%
Hemoglobin (Gm./100 ml.)		12.55		14.42
Erythrocytes (millions)	11.52		10.45	
Total Leukocytes	5841.66		3375.00	
Heterophils	1915.8	32.8	995.62	29.50
Stab cells	70.1	1.2	92.81	2.75
Lymphocytes	3551.3	60.8	2160.0	64.0
Monocytes	93.4	1.6	67.5	2.0
Eosinophils	198.6	3.4	59.06	1.75
Basophils	11.6	0.2	0.0	0.0

TABLE 14

AVERAGE HEMATOLOGICAL DATA FROM HEALTHY ADULT RABBITS BEFORE AND AFTER NORTH DAKOTA VIRUS INOCULATION

Average	12 Rabbits Before Inoculation		7 Rabbits 56 Hrs. After Inoculation	
	Per cu. mm.	%	Per cu. mm.	%
Hemoglobin (Gm./100 ml.)		13.05		11.64
Hematocrit (volume %)		38.08		36.14
Total Leukocytes	9087.50		5593.00	
Heterophils	3331.47	36.66	1741.66	31.14
Stab cells	7.27	0.08	7.83	0.14
Lymphocytes	4982.67	54.83	2931.85	52.42
Monocytes	454.37	5.0	758.97	13.57
Eosinophils	90.87	1.0	55.93	1.0
Basophils	219.0	2.41	95.46	1.71

Group of one-to two-months-old calves. The outstanding hematological change seen in these calves was the pronounced leukopenia between the second and fourth day after inoculation. The average depression from the preinoculation total leukocyte count was 3837.5 in all four calves. The lowest individual leukocyte count was 1200 in one calf reached three days after inoculation (Table 2).

Depression of the absolute but not the relative count of lymphocytes was evident by an increase from 68.1 to 81.2 in the relative differential percentage for lymphocytes.

The absolute and relative counts of eosinophils dropped markedly during the period of high fever and leukopenia. An absolute and relative drop of the average count of monocytes was also encountered.

Anemia was evidenced during the febrile and leukopenic period.

Group of three-months-old calves. Pronounced leukopenia was the outstanding hematological change also in this age group of calves. The average depression of the preinoculation total leukocyte count (average 7323) was 4322. The lowest individual leukocyte count was 1950 in one calf reached after [illegible] hours.

There was a severe absolute and relative depression of the total count of heterophils (Tables 3 and 4).

The average counts of stab cells revealed also an absolute and relative depression; an increase of immature

leukocytes was not seen at this time (50 hours after virus inoculation).

An absolute depression of the total lymphocyte counts was evident. This depression of lymphocytes, like in the one- to two-month-old calves was absolute; the differential percentage showed a relative increase in lymphocytes from 68.4 to 74.2.

While the absolute count of monocytes showed a drop after inoculation, this cell category similarly increased slightly in its relative differential percentage.

A constant and severe reduction of both the absolute and relative counts of eosinophils was noted in all inoculated calves of this age group. This eosinopenia was more pronounced than in the calves of the one- to two-months age group. The post-inoculation drop in the absolute and relative numbers of basophils appeared to follow the rate of reduction reported for the heterophils and eosinophils.

Fifty hours after inoculation the reduction of the average preinoculation total leukocyte count was 57.4%; however, the average reduction for the absolute heterophil count was 76.6%, for the stab cells 59%, and for the absolute eosinophil count 79.9%. The corresponding reduction of the average absolute lymphocyte count was 53.8% and for the absolute monocyte count 38.9%.

Comparison of pre- and post-inoculation values of hemoglobin revealed a slight anemia. On the other hand, the

hematocrit value increased slightly from an average of 31.0% to 32.1% volumes.

Tables 1, 7, 9, 10, and 11 illustrate that the inception and degree of hematological changes closely follow the temperature curve of the clinical course of the experimental infection.

The depression of the total leukocyte counts in the two calves with pneumonia (Table 5) as well as the depression of their absolute heterophil, eosinophil, and monocyte counts followed the pattern seen in the inoculated healthy calves. The relative lymphocytosis was also noted. Calf No. 2 was euthanatized three days after virus inoculation and calf No. 1 made a slower but uneventful recovery, the clinical course of both the experimental virus infection and the pneumonia taking about ten days.

Group of four- to eight-months-old calves. Like in the previous age groups, a pronounced leukopenia was the outstanding change in the four- to eight-months-old calves. The average depression of the preinoculation total leukocyte count (average 8732) was by 3790. The lowest individual WBC count in one calf was 3550 (Tables 6 and 7).

A severe absolute and relative depression of the total count of heterophils was evident. The pre- and post-inoculation comparison of the stab cells also showed an absolute and relative depression. An increase of immature leukocytes was not seen at this time (60 hours after inoculation).

There was an absolute depression of the total lymphocyte count as in the two previous age groups of calves, but the differential percentage showed a relative increase. Similarly to the lymphocytes, the relative percentage of monocytes also showed an increase. As in the previous age groups of calves, the depression of the absolute and relative counts of eosinophils was a constant finding also in this age group.

The average counts of basophils showed an absolute and relative increase in the calves of this age group.

Sixty hours after inoculation the reduction of the average pre-inoculation total leukocyte count was 43.4%; however, the reduction of the average total heterophils was 64.1%, that of the average total stab cells 49.1%, and that of the average total eosinophils 63.2%. The corresponding reduction of the average total lymphocyte count was 37.8%.

Group of yearling cattle. Comparing the pre- and post-inoculation hematological averages of this age group, similar changes were encountered as in the previous groups of calves. An average depression of 3626 from the pre-inoculation total leukocyte count was noted.

A slight anemia was also evident in this age group of cattle.

Also in this group the most outstanding hematological change was the absolute and relative depression of the heterophil counts (Tables 8 to 10).

While there was a depression of the absolute count of lymphocytes, the relative percentage value of this cell category showed a substantial increase as in the other groups of calves.

Monocytes decreased in absolute and relative numbers.

A marked drop was noted in absolute and relative numbers of eosinophils.

Two-year-old cow. As can be seen from Table 11, the experimental infection of a two-year-old cow with North Dakota virus produced similar clinical and hematological changes as seen in the experimental cattle reported previously. The depression of the absolute and relative counts of heterophils was most outstanding during the febrile period. A corresponding increase of the relative lymphocyte count was noted. The erratic behavior of the relative and absolute eosinophil count in this case may be explained by a chronic pediculosis of the animal.

One-day-old calf. Even in a one-day-old colostrum-deprived calf (Table 12) the clinical and hematological changes were similar to those in older cattle.

Sheep. A pronounced leukopenia was evidenced in the experimental sheep. The lowest individual WBC count was 2700 and the average depression from the preinoculation total leukocyte count was by 2466 or 42.2%. As in the cattle,

but somewhat less pronounced, there was an absolute and relative depression of the total heterophil count. The depression of heterophils was 48.1%. Corresponding to similar findings in the experimental cattle, the relative lymphocyte and monocyte counts showed increases after inoculation. The depression of the absolute and relative counts of eosinophils was a constant finding also in the sheep. Post-inoculation hemoglobin values showed a slight increase and post-inoculation RBC counts a slight decrease as compared to the pre-inoculation normals. A slight increase in the number of stab cells was also evident (Table 13).

Rabbits. As can be seen from Table 14, the rabbit post-inoculation hemogram showed essentially similar changes after North Dakota virus inoculation as did the hemograms of cattle and sheep.

The absolute and relative depression of the heterophils and basophils was the most outstanding change in the post-inoculation hemogram of rabbits. The depression of the eosinophils and lymphocytes was absolute and not relative. A pronounced absolute and relative monocytosis was an additional finding in the rabbits. The depression of the average total leukocyte count from the pre-inoculation average was by 3494 cells or 38.48%. The corresponding depression for the average heterophil count was by 1590 cells or 47.75%, and the depression of the average lymphocyte count from the

pre-inoculation average by 2051 cells or 41.0%. A mild anemia was also seen.

Transmission Attempts

Routes of transmission and infectious agent. In order to establish the susceptibility of the available native calves to the North Dakota virus, the following routes of inoculation were used simultaneously: intravenous, subcutaneous, intracutaneous, intramuscular, intranasal, intramucosal, rectal, intratesticular, and intranodal (prescapular lymph node). After the susceptibility of the experimental calves had been proved in two calves, the routes of inoculation were restricted to either the intravenous or subcutaneous in the following calves. The infectivity of the bovine tissue-culture virus was confirmed by the inoculation of 26 calves from two to eight months old, four yearlings and one two-year-old cow. All of these experimental cattle showed the typical sequence of clinicopathological changes outlined earlier.

Attempts to transmit the experimental disease by using materials other than the bovine tissue-culture-grown virus failed. Fresh blood (Chapter II) without preservative, taken from experimental calves during the febrile and leukopenic period of the experimental infection, and reinjected within ten seconds, did not reproduce the experimental infection in six calves. The blood was taken from the

experimental calves from one to nine days after inoculation. One of the six calves received the blood of a sheep donor. The challenge of the blood-inoculated calves two to four weeks later with the pure virus produced the typical clinicopathological signs of the experimental North Dakota virus infection in each case.

Other attempts were aimed at transmitting the experimental disease by the inoculation of organ emulsions prepared from spleen, kidneys, lungs, liver, lymph nodes, and adrenals of experimental cattle sacrificed during the acute, subacute, or recovery period of the experimental infection. Three calves from two to four months of age were inoculated with organ emulsion and showed no clinical signs of infection. One of these calves (No. 17) was challenged seven days after organ-emulsion inoculation with the pure virus and developed the typical sequence of clinicopathological changes as described. The second calf (No. 27) received repeated injections of organ emulsions on days 0, 14, 32, and 39 and showed no signs of infection. Sixty days after the last injection the calf was challenged with the pure virus and proved to be resistant. The third calf (No. 32) was given three injections of organ emulsion at weekly intervals and showed no signs of infection. Challenge with the pure virus two weeks after the last inoculation with organ emulsion proved this calf to be immune also.

Rabbit kidney-cell culture harvest from an experiment to adapt the North Dakota virus to rabbit

kidney-cell culture (Rothenbacher, 1960) was inoculated into three three-months-old calves. A three-months-old calf (No. 16) was given 10.0 ml. of this inoculum intravenously. While no clinical signs of infection developed, this calf proved resistant to challenge with the original (bovine-embryo-kidney-cell culture) virus three weeks later. The second three-months-old calf (No. 25) received the same rabbit-cell-culture inoculum and developed mild clinical signs consisting of slight leukopenia and a temperature elevation of 1.5 F. Challenge of this calf with the bovine-kidney-cell virus seven days later did not produce any clinical changes. A second challenge with the virus after one more week was also negative.

The third three-months-old calf (No. 26) was given 6.0 ml. of the rabbit-cell culture intravenously. No clinico-pathological changes were observed within four days after inoculation. Challenge of this calf after two weeks with the bovine-cell-culture virus did not produce any of the characteristic changes of the North Dakota experimental infection. The inoculation of one calf and one sheep with sterile tissue-culture medium did not produce any clinical, gross and histopathological changes in the experimental animals.

Contact transmission studies. A three-months-old and a five-months-old calf were placed in a stall together with an experimental calf which had just been inoculated with the

North Dakota virus. The calves ate and drank from the same trough and were kept in contact for ten days during the whole course of the experimental infection. While the inoculated calf in each case developed the typical signs of the experimental infection, none of the contact calves showed any clinicopathological changes. Both contact calves proved susceptible to the North Dakota virus challenge when inoculated five and eight weeks, respectively, after their contact period. No accidental contact transmissions occurred during the two-year period of experimental work with the North Dakota virus. During this period an accidental transmission of virus diarrhea was reported by Rothenbacher et al. (1961) working under similar experimental conditions with the nonculturable virus diarrhea agents from Iowa and Indiana.

Koch's postulates. From the results of experimental studies reported, it is obvious that the typical acute experimental North Dakota virus infection could not be reproduced by any material other than the virus grown in bovine-embryo kidney-cell culture. The inoculation of sterile lactalbumin hydrolysate plus 2% horse serum, the growth medium for the virus, did not produce any clinicopathological changes in two calves and one sheep. Reisolation of the virus (Barner et al., 1959) proved very difficult. Numerous specimens of internal organs, heparinized blood, nasal and ocular swabs, mucosal scrapings, and fecal specimens taken from experimental calves from two to 15 days after North Dakota virus inoculation

failed to reveal the presence of the virus. The virus was recovered in only two instances from the lung, spleen, and kidney of experimental calves six and nine days after inoculation (Barner et al., 1959). The recovered virus was neutralized by North Dakota virus antiserum, and, upon reinoculation into susceptible calves, produced the typical changes of the described experimental infection.

Gross Pathological Findings

Experimental animals were euthanatized at various time intervals ranging from one to 134 days after virus inoculation (Chapter II). The most pronounced gross lesions were encountered in animals necropsied during the acute phase of the experimental infection from two to six days after inoculation.

Cattle. Sixteen calves from one to six months of age, one yearling steer and one yearling heifer were necropsied from one to 14 days after North Dakota virus inoculation and showed the following gross lesions:

External body surface:--The conjunctivae showed variable degrees of congestion. Occasionally, mucopurulent exudate and/or encrustations were present in the medial canthi of the eyes. Serous discharge was evidenced by matting of hair below the eyes of some animals necropsied from one to two days after virus inoculation. A moderate degree of edematous swelling was usually seen in the submaxillary, prefemoral, and prescapular lymph nodes.

Digestive tract.--The oral mucosae showed moderate to severe and patchy congestion. The parotid and the supra-pharyngeal lymph nodes were enlarged and congested. The tonsils similarly appeared swollen and congested. No lesions could be discovered in the pharynx, rumen, reticulum, and omasum. A moderate to severe catarrhal gastritis was present in the abomasum. The duodenum and the anterior half of the jejunum showed catarrhal enteritis with patchy areas of marked congestion. A severe catarrhal to catarrhohemorrhagic enteritis was noted in the posterior half of the jejunum and extending occasionally to the ileum. Areas of marked congestion were more numerous in this region and, in some animals, gave a "tiger stripe" appearance. An abundance of catarrhal exudate ranging from clear to blood-tinged was usually encountered in the jejunum. The Peyer's patches of the posterior jejunum and ileum appeared hyperplastic. Lesions in the Peyer's patches varied from scattered petechiae and ecchymoses to patchy congestion and hemorrhagic marbling in the lymphatic tissue layer. Hemorrhagic seams of 4 to 8 mm. width were observed in the submucosa and subserosa around Peyer's patches of the posterior jejunum. The mucosa covering the Peyer's patches showed mild degrees of fibrinous exudation and/or fibrinonecrotic changes.

Similar changes in the form of a blackish-brown hemorrhagic to fibrinonecrotic seam of 5 to 10 mm. width were observed on the ileocecal ostium protruding into the cecum. Scattered ecchymoses were often found in the submucosal lymphatic tissue of the ostium (Fig. 10 and 11).

The large and continuous Peyer's patches of the ileum were occasionally covered with cream-colored fibrinonecrotic epithelial layers that wiped off easily from the underlying lymphatic tissue. The latter showed varying degrees of hemorrhage and/or congestion (Fig. 7 and 11).

Only a mild catarrhal inflammation was noted in the cecum. From 30 to 45 cm. posterior to the ileocecal ostium, an area of small cystic degeneration interspersed with ecchymotic hemorrhages was regularly found in the mucosa of the colon over an area from 10 to 25 cm. in length. Only mild catarrhal changes were encountered in the colon. Increased amounts of grayish viscous mucus were present in the colon and rectum. Longitudinal areas of marked congestion were seen on the rugae of the rectum. A band-shaped area from 2 to 5 cm. in length adjacent and anterior to the anorectal junction showed severe congestion.

The mesenteric lymph nodes draining the small intestine and the cecum showed various degrees of edematous swelling and congestion of medulla and cortex. Scattered petechial and ecchymotic hemorrhages were seen in subcapsular and cortical areas of the lymph nodes of the posterior jejunum and ileum. Lymph nodes of the duodenum, colon, and rectum were less severely affected (Fig. 1, 2, 4, 5, and 6).

Respiratory tract.--There was a slight hyperemia of the nasal mucosae with a corresponding mild edema and congestion of the suprapharyngeal lymph nodes. The lung lymph

nodes similarly showed a moderate degree of edema and congestion. No lesions could be found in the larynx, trachea, bronchi, or lungs.

Heart.--Subendocardial suffusions were seen in two calves. No other visible lesions could be noted.

Liver.--The liver showed a generalized congestion and a moderate swelling as evidenced by rounded ventral edges. The gall bladder was distended up to twice its normal size. The bile appeared dark and thickened.

Spleen.--The spleen was enlarged. A few petechial hemorrhages were occasionally seen subcapsularly. The cut surface bulged and showed congestion.

Thymus.--Numerous petechial and ecchymotic hemorrhages were scattered subcapsularly and on the cut surface of the thymus gland. To a lesser degree these lesions were also present in glands from control animals.

Urogenital tract.--A few petechial hemorrhages were occasionally seen subcapsularly and in the cortex of the kidneys (Fig. 13). Cortex and medulla of the kidneys appeared anemic. No gross pathological changes could be seen in the ureters, bladder, urethra, testes, or ovaries.

Endocrine glands.--The adrenal glands appeared enlarged and showed variable subcapsular hemorrhage in the form of suffusions or scattered ecchymoses (Fig. 14). On cut section, areas of hemorrhage and/or dark brown necrotic foci of 1 to 5 mm. in diameter were discovered in the zona fasciculata. No

gross lesions were seen in the thyroid gland and in the hypophysis.

Central nervous system.--No gross lesions could be found.

Sheep. Six adult sheep from one and one-half to six years of age were necropsied from four to six days after North Dakota virus inoculation.

The gross pathological findings seen in sheep compared closely to those described in cattle. The degree of the lesions was somewhat milder. No gross lesions could be found in the eyes, in the upper digestive tract, and in the respiratory tract.

A catarrhal abomasits was present in all sheep and, unlike the syndrome in cattle, there was severe catarrhal duodenitis. The jejunum showed a moderate catarrhal inflammation with areas of pronounced congestion. The Peyer's patches of the posterior jejunum appeared hyperplastic and some were covered with a fibrinonecrotic pseudomembrane. In contrast to the mild involvement of the large intestine in cattle, the spiral colon of the sheep was empty of feed and showed a severe catarrhal colitis with abundance of clear and foamy viscid mucus. The mesenteric lymph nodes appeared slightly swollen and congested. Ecchymotic cortical hemorrhages were noted occasionally.

The liver was congested and the gall bladder distended. A moderate swelling and congestion were seen in the spleen. The kidneys appeared anemic. No other gross lesions were present in the urogenital tract. The central nervous system

did not reveal any gross lesions. On cut section, the adrenals revealed similar lesions as were described in cattle except that no subcapsular or cortical hemorrhages were present.

Rabbits. Seven adult rabbits ranging in age from one to three years were necropsied two to three days after North Dakota virus inoculation. The stomachs of the rabbits were empty and various amounts of hair were balled up in the fundus. No characteristic gross lesions could be definitely associated with the experimental North Dakota virus infection.

Histopathological Observations

Histopathological findings in North Dakota virus inoculated cattle.

Digestive tract.--Mild patchy hyperemia of the lamina propria could be occasionally found in the stratified squamous mucous membranes of the digestive tract. The mucous adnexal glands of these membranes were found in a state of hypersecretion or secretory exhaustion in animals euthanatized during the acute or subacute phase of the experimental infection. Occasional hydropic changes in the stratum lucidum and stratum corneum of the esophagus, rumen, reticulum, and omasum were found. Rarely encountered focal hyperkeratotic changes of the rumen and esophagus were also present (Fig. 15).

Abomasum.- The abomasum showed an acute catarrhal gastritis with a variable degree of inflammatory edema and

congestion in the lamina propria and in the submucosa. Copious amounts of seromucous exudate were found in the fundic and pyloric regions.

Small intestine.- A similar acute catarrhal enteritis was seen in the duodenum. Numerous cystic submucosal glands (Brunner's glands) were observed filled with seromucous exudate and desquamated epithelial cells. The degree of catarrhal enteritis increased posteriorly in the jejunum and was found to be catarrho-hemorrhagic to fibrinous in areas of the posterior jejunum. Inflammatory edema and hyperemia were marked, especially in the lamina propria of the villi and in the submucosa. Necrosis and desquamation were noted in the epithelial covering of the mucosal villi. Cellular infiltrations were observed in the propria, including the villi, and consisted primarily of lymphocytes, macrophages, plasma cells, histiocytes, eosinophils, and a few neutrophils. The goblet cells appeared to be increased in size and number, especially in the posterior part of the jejunum and in the ileum.

Catarrhal to hemorrhagic exudate of the posterior jejunum also contained monouclear cells, heterophils, and a few eosinophils. The hemorrhagic exudation was found to be primarily diffuse in distribution and probably derived from diapedesis through the extremely congested capillaries of the denuded intestinal villi. Occasionally, petechial and ecchymotic hemorrhages could be seen in the lamina

propria and submucosa of the jejunum and ileum(Fig. 16 & 17).

The posterior jejunum and ileum, in many instances, also presented the histopathological picture of a fibrinous enteritis. This was particularly frequent in the mucosal areas covering the larger Peyer's patches. Fibrinous exudate on the mucosal surface was mixed with mucus, necrotic inflammatory cells as described, and desquamated epithelial cells. Villi showed inflammatory edema, hyperemia, and cellular infiltration similar to that described earlier. The Peyer's patches showed inflammatory edema and hyperemia and were found in a state of lymphocytic hyperplasia during the acute phase of the experimental infection. Lymphocytic depletion and/or exhaustion was seen in animals euthanatized during the later stages of the acute phase. The severe and patchy congestion corresponded well to the described gross findings.

Scattered petechial and ecchymotic hemorrhages were often present in the lymphatic tissue of the ileal Peyer's patches. Occasionally necrotic foci were observed in reaction centers forming micro-abscesses with lymphorrhexis and heterophilic infiltration.

Extensive submucosal edema and congestion, combined with a fibrinonecrotic to hemorrhagic inflammation of the mucosa, were consistently observed in sections of the ileo-cecal ostium.

Large intestine.- The cecal mucosa showed similar patchy congestion with a relatively mild catarrhal typhlitis. This

mild catarrhal enteritis continued into the colon. The area over an approximately 25-cm.-long stretch of colon mucosa near the ceco-colic junction (as described in gross findings) contained numerous cystic mucosal crypts filled with mucus and cells, primarily heterophils and lymphocytes. Fibrinonecrotic changes of the mucosa of this area were similar to the ones described in the ileum and posterior jejunum. The underlying Peyer's patches also showed occasional foci of lymphorrhexis and heterophilic infiltration of the reaction centers. The mucosa of the remaining posterior portion of the colon displayed only mild catarrhal changes with increased mucous secretion. Areas of congestion were seen especially on the crests of the rugae of the rectum. Severe congestion was also noted in the rectal mucosa immediately anterior to the anorectal junction.

Lymph nodes of the digestive tract- Similarly to the lymphatic tissue of the Peyer's patches, the lymph nodes of the digestive tract showed the histopathological picture of a reactive stimulation with variable degrees of inflammatory hyperemia, edema, and petechial and ecchymotic hemorrhages. In the cortical and medullary sinuses of the nodes, reticulohistiocytic proliferation and lymphocytic hyperplasia frequently created the classic picture of the "sinus-catarrh", (Pallaske, 1960) with all sinusoidal spaces crammed by proliferated reticulohistiocytic (mononuclear) cells,

mature lymphocytes, heterophils, plasma cells, and eosinophils. The nuclei of these cells, congesting the marginal sinuses, were often found flattened in the tangential plane of the capsule. The capsule frequently showed various degrees of thinning and infiltration with mononuclear cell elements. Petechial hemorrhages were most commonly encountered in the reaction centers of secondary lymph nodules (Krölling and Grau, 1960) and in the vicinity of small cortical arterioles showing mild hyalinization of their wall structure (Fig 18).

The medulla of the lymph nodes commonly showed an inflammatory edema with an increase of reticulohistiocytic, plasmocytic and heterophilic elements. Increased hemosiderosis was occasionally seen in macrophages in the medullary sinuses, cortical reaction centers of secondary lymph nodules, and in cortical sinuses. Circular to fusiform bodies with onion-skin structure of 20 to 50 microns in diameter were also occasionally found in reaction centers. These bodies stained blue on hematoxylin-eosin stains and gave a positive reaction for calcium with the Von Kossa stain. They were also found in control animals and were considered a fortuitous finding (Trapp, 1960). A state of lymphocytic depletion was often seen in the primary and secondary lymph nodules of the cortex as evidenced by a severe narrowing or complete disappearance of the polar "caps" of maturing lymphocytes. In some lymph nodes the reactive reticulohistiocytic proliferation led to a uniformalization of the cortical tissue with a

resulting disappearance of the primary and secondary follicular structures. Balled-up proliferating histiocytic cells and an increased amount of nuclear debris was often seen in such lymph nodes. This was a common finding in animals euthanatized during the later stages of the acute phase of the experimental infection (Fig. 21 and 22).

Reaction centers in many lymph nodes showed an increase in nuclear fragments and a decrease of reticular cells and mitotic figures. Occasionally necrotic microfoci of pyknosis and karyorrhexis were encountered in primary lymph nodules and reaction centers of the cortex. Heterophilic aggregations were usually associated with these necrotic foci. The most pronounced of these described lesions were seen in the lymph nodes of the ileocecal region, the ileum, the posterior jejunum, and the head region (Fig. 19).

Respiratory tract.--The upper respiratory tract showed mild hyperemic and hypersecretory changes similar to those described for the upper digestive tract.

Lungs.- Even as the gross pathological examination of the lungs did not yield any pertinent findings, the histopathological findings were primarily confined to the pulmonary lymph nodes which showed lesions similar to those described for the lymph nodes of the head and the digestive tract. Slight evidence of peribronchial and interstitial mononuclear infiltration and congestion was occasionally noted.

Cardiovascular system.--Besides a few subendocardial ecchymoses and suffusions no other histopathological changes were seen. Such endocardial hemorrhages are frequently present in electrically stunned animals.

Liver.--A mild edematous swelling of the liver parenchyma was evidenced by intra- and extracellular edema and edematous distention of the Disse-spaces (Krölling and Grau, 1960). The sinusoids and intralobular capillaries appeared distended and were sometimes filled with a serous fluid. Microfoci of reticulohistiocytic and mononuclear cells could be seen intralobularly in the parenchyma or perilobularly, adjacent to the portal triads. The Kupffer cells appeared in a state of active proliferation. Occasionally the liver parenchyma showed microfoci of necrosis with pyknosis, karyorrhexis, and karyolysis. Infiltrations of mononuclear cells tended to assemble around the necrotic foci and sinusoidal and capillary congestion was common in the vicinity of the foci (Fig. 23 and 24).

Lymphatic system.--Similarly to the lymphatic tissue of the digestive tract, the spleen showed lymphocytic hyperplasia with extensive reticulohistiocytic proliferation. In the later stage of the acute phase of the experimental infection, lymphocytic depletion and exhaustion, edema of the reaction centers and depression of the reticulocytic mitotic activity could be noted. Microfoci of lymphorrhexis

with neutrophilic infiltration were a fairly common occurrence in the spleen. Petechial and ecchymotic hemorrhages were also seen in splenic follicles as described in the reaction centers of mesenteric lymph nodes. An increased hemosiderosis of the free macrophages and sessile reticulocytes could also be seen during the acute phase of the infection (Fig. 20).

As in the spleen, numerous petechial and ecchymotic hemorrhages were noted in the thymal medullary light substance or at its periphery. A depletion of the medulla was evident and focal neutrophilic infiltration was frequently seen. The Hassall bodies of infected animals appeared to be fewer in number and at an immature stage.

The other body lymph nodes showed similar or identical histopathological changes as already described for the gastrointestinal and respiratory lymphatic tissue.

Bone marrow.--Limited histopathological studies of the bone marrow of experimental cattle appeared to correlate with the hematological findings described earlier in this chapter. A depression of the myelogenous activity was evident and there was a paucity of mature and immature heterophils and eosinophils as compared to the control animals.

Kidneys.--As in the case of the liver, the kidney responded with mild focal or diffuse mononuclear proliferation in the cortex. Large numbers of the glomerular tufts

were shrunken and appeared nonfunctional. Bowman's capsules were often found to be thickened. There was capillary hyperemia in the vicinity of the affected glomeruli. Petechial hemorrhages in the cortex were rarely observed (Fig. 25 and 26).

Ballooned Bowman's capsules were occasionally seen. A focal interstitial nephritis was evidenced by proliferated interstitial cells obstructing tubules and capillaries, expecially in the area of the corticomedullary junction, and resulting in severe focal congestion in this region and distention of renal tubules anterior to the obstruction sites. Obstructed tubules frequently contained hyaline or granular casts. A small number of hyaline casts resulting from focal interstitial nephritis was observed to a lesser degree in tissues from control animals.

Endocrine glands.--

Adrenal gland.- The most pronounced histopathological changes in the experimental North Dakota virus infection were encountered in this gland. In accord with the gross lesions, suffusions could be seen in the loose pericapsular connective tissue. What appeared on gross inspection as petechial or ecchymotic capsular hemorrhages turned out to be numerous distinct foci of necrosis with severe peripheral capillary congestion and extravasations in the cortex close to the capsule.

These distinct cortical foci of necrosis were present in 12 out of 14 calves euthanatized during the acute phase of

the experimental infection. They consisted of spherical foci in the zona fasciculata showing coagulation necrosis, karyolysis, and occasional karyorrhexis in the center and primarily vacuolar, cytolytic, and karyolytic changes at the periphery. Pyknotic nuclear changes in dark red- to purple-staining cells were also occasionally seen at the periphery of a necrotic focus. The necrotic foci were surrounded by severe congestion, infiltration of mononuclear and heterophilic cells and serous exudation into interstitial spaces in the vicinity of capillaries. In some cases the picture of coagulation necrosis predominated while in others karyorrhexis and more extensive heterophilic infiltration could be seen. These necrotic foci varied from 100 to more than 1,000 microns in diameter. They were always located in the zona fasciculata, sometimes infringing upon the peripheral zona glomerulosa and the more central zona reticularis (Fig. 29).

The parenchymal cells of the zona fasciculata and its neighboring zones also showed marked alterative parenchymal changes consisting of vacuolation and reticulation of cytoplasm and numerous eccentric nuclei. A foamy reticular cytoplasmic structure and partial cytolysis were frequently seen. This change was most pronounced in the peripheral zona fasciculata. The zona glomerulosa rarely showed the vacuolar changes (Fig. 28).

Small colloid-like droplets were occasionally seen in the zona fasciculata. These were probably similar to or

identical with the hyaline-like droplets described by Whiteman (1960) in Iowa mucosal disease field cases. Whiteman (1960) ascribed these droplets to dehydration and hemoconcentration, clinical signs also present to a moderate degree in the North Dakota virus experimental infection.

Between the cell cords of the zona fasciculata showing vacuolar and/or cytolytic-reticular changes, strands or microfoci of elongated, histiocyte-like dark red- or purple-staining cells with pyknotic nuclei could frequently be observed, primarily in radial arrangement. This cell type was also occasionally observed at the periphery of the necrotic foci as described previously. Similarly staining cells could sometimes be seen in adrenals of control animals. Identical cells were documented by Whiteman (1960) in the adrenal cortices of Iowa mucosal disease field cases and described as scattered cell necrosis consisting of necrotic cells with increased cytoplasmic staining, cell shrinkage, and pyknosis (Fig. 30).

Increased mitotic activity, as described by Whiteman (1960) in the peripheral zona fasciculata-intermedia of Iowa mucosal disease field cases, could not be observed in the North Dakota virus experimental infection.

A constant finding in the adrenal cortices of North Dakota virus-inoculated cattle was the marked inner progressive transformation (Matthias, 1954; Krölling and Grau, 1960) consisting of a hypertrophic widening and extension

of the zona fasciculata at the expense of the zona glomerulosa and zona reticularis. In all the adrenals studied, a narrowing and compression of the zona glomerulosa was a rather constant finding. As a result, the adrenal capsule appeared stretched and thin as compared to control animals. As a consequence of this progressive transformation, the zona intermedia could no longer be distinguished. Though Whiteman (1960) did not use the term, he accurately described a progressive transformation in 50% of the examined Iowa mucosal disease field cases. In other cases he seemed to describe regressive changes.

Griem (1955) saw similar progressive changes in hog cholera-infected pigs with acute gastrointestinal involvement.

Special stains applied to the described adrenal changes revealed rather variable findings. The lipid stains, oil-red-O and Sudan IV, yielded only minimal amounts of scattered positive-staining globules in the cells of the glomerulosa, fasciculata and in the medulla. The uniformly brown oil-red-O-stained adrenal sections appeared somewhat darker in the zona fasciculata and medulla. In no instance could a comparable lipidosis be obtained as documented by Whiteman (1960) for Iowa mucosal disease. Whiteman (1960) reported that the lipids of his oil-red-O-stained adrenal sections corresponded in location and amount with the fatty alterative changes described from the hematoxylin-eosin-stained paraffin

sections. The fatty changes seen by Whiteman (1960) are, in location and description, similar to or identical with the vacuolar and reticular degenerative changes described here. However, no lipidosis was seen in the experimental North Dakota virus infection.

An increased accumulation of lipid droplets in the bovine adrenal as a result of chronic stress situations was reported by Bell and Weber (1959). This might not result from an acute stress of short duration as caused by the experimental North Dakota virus infection.

On oil-red-O-stained sections occasionally some increased reddish-brown staining amorphous matter was noted at the periphery of the described necrotic foci. Some necrotic foci showed a slightly positive reaction for amyloid when stained with crystal violet. PAS-positive particles were often seen in the centers of the necrotic foci. Distinct foci of coagulation necrosis in the zona fasciculata, as frequently seen in the experimental North Dakota virus infection, took on similar tingibility as did the connective tissue of the adrenal capsule when stained with Heidenhain's aniline blue stain.

Globular and rod-shaped, bright red-staining bodies of 1 to 2 microns in diameter were described by Whiteman (1960) in the zona glomerulosa cells of Iowa mucosal disease field cases when stained with Crossman's modification of Mallory's connective tissue stain. Similar or identical bodies were

seen in the same cells in North Dakota virus-infected cattle as well as in control animals when stained with Heidenhain's aniline blue stain.

Thyroid gland.- Upon histopathological examination, no definite changes could be found in the thyroids of experimental animals. In several instances extensive vacuolation was seen at the periphery of the colloid in the smaller acini. The vacuoles varied in size from 10 to 50 microns. This change was present to a mild degree in the thyroids of control animals.

Hypophysis.- The histopathological changes of the hypophysis were indistinct. Severe congestion was noted in the adenohypophysis and in the pars intermedia. Occasionally microfoci of pyknosis and coagulation necrosis were present in the pars intermedia. Patchy areas of eosinophilic and basophilic degranulation were encountered in the anterior part of the adenohypophysis. Quantitative and morphological studies, such as those attempted by Whiteman (1960), were found to be unreliable and were not pursued.

Other body systems and tracts.--No significant histopathological changes could be found in the central nervous system, the spinal cord, the musculo-skeletal system, the male or female reproductive tracts, or the skin.

Histopathological findings in North Dakota virus-inoculated sheep. The histopathological changes seen in the tissues of experimental sheep resembled those described in cattle. They tended to be somewhat milder in the respiratory tract, the liver, and the kidneys. Five out of five inoculated sheep showed pronounced changes in their adrenal glands similar to those in experimental cattle. Excessive hemosiderosis of the spleen was present in all experimental sheep, but not in the controls. The changes in the gastrointestinal tract, the spleen, and the adrenals tended to be more severe than those reported in calves.

Histopathological findings in North Dakota virus-inoculated rabbits. Corresponding to the mild hematological changes in the experimental rabbits, the histopathological lesions were also slight and difficult to evaluate. The lesions in the lymphatic system and in the adrenal glands appeared to be similar to those described for the cattle and sheep. Changes found in the liver, spleen, and kidneys were variable. In general, the lesions were considered to be too mild in degree and too variable to make the rabbit a useful experimental animal.

Other Post-Mortem Findings

Urine specimens of cattle and sheep taken at the time of necropsy and submitted for urinalysis did not show any changes from the normal. Periodical fecal examinations were

similarly negative. No significant changes were found in limited blood-sugar and nonprotein-nitrogen determinations. Aseptically removed organ specimens from euthanatized experimental animals were submitted for bacteriological examination (Chapter II). No pathogenic organisms were isolated.

Serological and Immunological Findings

The antibody response to North Dakota virus inoculation was studied in several calves. Pre- and post-inoculation sera were submitted to the virology section, Department of Microbiology and Public Health.

No neutralizing antibodies were present in the pre-inoculation sera of experimental calves as indicated by tissue-culture studies. Eight days after inoculation, less than 10^1 neutralizing antibodies were present in the serum.

On days 15 and 23 after inoculation the neutralization index was 10^2. Following reinoculation on day 23, sera taken on day 33 through day 65 had indices equal to or greater than 10^4 in one animal.

Four calves proved resistant to reinoculation from 14 to 130 days after the experimental North Dakota virus infection.

One calf, inoculated with the rabbit tissue culture harvest (Rothenbacher, 1960), proved resistant to challenge with the bovine-embryo kidney-cell virus given 7 and 14 days after the original inoculation. Another calf similarly

inoculated resisted challenge with the North Dakota virus (bovine tissue-culture origin) 14 days later.

Two calves having received three or more injections of organ emulsions (three injections at weekly intervals in one instance and four injections on days 0, 14, 32, and 39 in the other) proved resistant to challenge with the North Dakota virus.

A third calf was fully susceptible to virus challenge seven days after inoculation with organ emulsions.

CHAPTER IV

DISCUSSION

Transmission Studies

Limited transmission attempts by contact and by using inoculation materials other than the tissue culture grown virus were unsuccessful. This indicated that sufficient quantities of the infectious agent were not present in the body discharges and in the blood and organ emulsions taken from experimental cattle during the febrile and leukopenic phase of the infection. The fact that two calves receiving three or more inoculations with organ emulsions proved resistant to challenge with the tissue culture virus indicated that some form of a viral antigen may have been present in these organ emulsions from experimentally infected cattle.

The difficulties in reisolating the virus from blood, organs, and body discharges of experimentally infected animals have been reported (Barner et al., 1959). In two instances, however, the virus was recovered from kidneys, spleen, and lungs of calves euthanatized six and nine days, respectively, after virus inoculation. The identity of the virus was proved by neutralization tests with North Dakota antiserum, and by reinoculation into several calves.

Comparison of the degree of gross and microscopic pathological findings in cattle, sheep, and rabbits may indicate that, of the three animal species the bovine might act as the most probable host of the virus in a possible but as yet unknown natural transmission of epizootic importance. The pathogenicity exhibited and the lesions produced by the virus in cattle strongly suggest that it does not fit into the bovine enterovirus (ECBO) classification like the one described by Soliman (1958).

Gross and Histopathological Findings

Acute catarrhal inflammation was evident in the gastrointestinal tract starting from the abomasum. Rarely seen hyperkeratotic foci of the ruminal, reticular, and esophageal mucosae were interpreted as being due to causes other than the North Dakota virus inoculation. The possibility of a hypovitaminosis A was considered as a cause (Pallaske, 1960).

Hydropic changes encountered in the stratum lucidum and corneum of the esophagus, rumen, reticulum, and omasum may be interpreted as a normal finding according to Krölling and Grau (1960). Catarrhal inflammatory changes often increased to fibrinous and hemorrhagic in the posterior half of the small intestine. The hemorrhagic to fibrinous exudation was thought to be due to diapedesis through extremely congested capillaries of denuded intestinal villi, since petechial and ecchymotic hemorrhages were rarely observed in the lamina propria and submucosa of the jejunum and ileum.

Lymphatic tissues. An initial state of lymphocytic hyperplasia during the acute phase giving way to lymphocytic depletion and/or exhaustion was the common histopathological picture seen in the Peyer's patches as well as in all lymphatic tissues of the body. Necrotic foci that were often present in secondary reaction centers of Peyer's patches and lymph nodes were considered a sequence to lymphoid exhaustion. Lymphorrhexis and an abundance of nuclear fragments with pronounced heterophilic infilitration were found in the necrotic foci. Pallaske's (1960) description of the so-called "sinus-catarrh" consisting of densely crammed peripheral sinuses with various stages of lymphoid cells mixed with neutrophils, plasma cells, eosinophils, and reticulohistiocytic cell elements was often observed in lymph nodes during the subacute and/or recovery phase of the experimental infection.

Petechial hemorrhages frequently seen in secondary reaction centers of, especially, the mesenteric lymph nodes and corresponding to those reported in Peyer's patches were thought to be due to a possible attack of the virus on the peripheral capillaries.

In lymph nodes from experimental cattle euthanatized during the subacute and recovery phases of the North Dakota virus infection, the disappearance of primary and secondary lymph nodules which took place and the uniformalization of the cortical architecture resembled the tertiary stage of

Krölling and Grau's (1960) description of lymph nodules. The cortical tissue consisted mainly of the reticulohistiocytic, large lymphoid and plasmocytic elements and a paucity of mature lymphocytes was noted in such nodes. The similarity to Raffel's (1961) description of the antibody-producing lymph node cortex was evident. The significance of conglomerated reticulohistiocytic cells into globular dark-staining structures of 20 to 100 microns in diameter was considered uncertain.

Bone marrow. In limited histopathological bone marrow studies it appeared that the hematological findings of heteropenia and eosinopenia were due, not merely to a depression of circulating cells, but to a generalized depression of the myelogenous activity resulting in a paucity of mature and immature heterophils and eosinophils. This fact correlated to the absence of immature cell forms noted in the hemogram of virus inoculated cattle and pointed to the bone marrow as a major target of the experimental North Dakota virus.

Respiratory tract. In the respiratory tract very mild catarrhal and hyperemic changes were considered secondary to the febrile reaction of the experimental infection. Focal areas of congestion and the already described lesions in the lymphatic tissues were the only findings in the lungs. Based on the questionable significance of these lesions, it is believed that the North Dakota virus is not primarily a

respiratory virus such as the parainfluenze-3 (Abinanti et al., 1960) and the infectious bovine rhinotracheitis viruses (Hagan and Bruner, 1961).

Adrenal glands. As in the liver and kidney, the epitheliotropism of the North Dakota virus was most evident in the parenchyma of the adrenal cortex. The most pronounced lesions were encountered in the zona fasciculata. The similarity of the vacuolar parenchymal change with Whiteman's (1960) description of adrenal lesions in Iowa mucosal disease was pointed out. However, a lipidosis could not be found in these affected cells. The increased accumulation of lipid droplets in the bovine adrenal as a result of chronic stress reported by Bell and Weber (1959) could be confirmed by Whiteman (1960) for Iowa mucosal disease. The reason why this change was not found in the adrenals of North Dakota virus-inoculated cattle may be due to the fact that this experimental infection constitutes a more acute stress. Distinct and extensive necrotic foci of the zona fasciculata were not common in the adrenals of cattle affected with Iowa mucosal disease as reported by Whiteman (1960).

A cell form of elongated histiocyte-like shape and with a uniformly dark red or purple staining cytoplasm and uniformly dark blue homogenous and pyknotic-appearing nucleus was seen in the zona fasciculata. The darker cells occurred individually, in small aggregations or in radial strands between the

lighter cells of the fasiculata. They were identical with the cell form documented and described by Whiteman (1960) as "scattered necrotic cells with increased cytoplasmic staining, cell shrinkage and nuclear pyknosis." This author tends to believe that these cell forms may not constitute necrosis but resting, (Matthias, 1954) regenerative, or reversible physiological exhaustion stages of parenchymal fasciculata cells. Further degeneration stages of these darkly-staining cells were not commonly seen in the adrenals of North Dakota virus inoculated cattle nor were they reported in adrenals of Iowa mucosal disease field cases (Whiteman, 1960). This cell form was not commonly involved in the necrotic foci of the vesicular lightly-staining fasciculata cells reported here.

The progressive inner transformation of the adrenal cortex was found to compare well to the descriptions by Matthias (1954) and Griem (1955) of the adrenal changes in pigs caused by the acute infectious stress of hog cholera.

Summary of Discussion

In summarizing this discussion of the clinical, gross, and histopathological changes it may be stated that the experimental North Dakota virus infection resembled that of a pantropic virus, affecting most notably the mucous membranes of the intestinal tract, the parenchymatous organs, the lymphatic tissues, and the bone morrow. The depression of circulating eosinophils, heterophils, and lymphocytes with

depletion and exhaustion of lymphatic tissues is compatible with the theory of adrenocortical hyperactivity during acute infections. The possibility of a direct effect of the virus upon the adrenocortical cells may be assumed since the described focal adrenocortical lesions have not been reported in other acute infections such as virus diarrhea, malignant catarrhal fever, infectious bovine rhinotracheitis, calf pneumonia, bovine salmonellosis, pasteurellosis, and enterotoxemia. A primary attack by the virus or a secondary hormonal effect upon the hypophysis, thyroid, the lymphatic system and the bone marrow may also be assumed.

From the difficulties in transmitting the experimental disease and in reisolating the virus, and from the absence of neutralizing antibodies in a large number of native cattle, the true origin of the virus as a bovine enterovirus may be doubted or an obscure mode of transmission assumed.

CHAPTER V

SUMMARY AND CONCLUSIONS

The characterization of a disease caused by a new bovine viral isolant--the North Dakota mucosal disease virus--was attempted. Studies included clinical, hematological, immunological, serological, and pathological observations on experimentally inoculated cattle, sheep, and rabbits.

The experimental infection in cattle was characterized by an acute monophasic febrile reaction combined with a severe leukopenia and heteropenia. Clinical signs of the experimental infection in cattle included partial anorexia, nervousness, tachycardia, increased respiration, ocular discharge, depression, and constipation. The experimental infection lasted from six to ten days and resulted in immunity measurable by serum-neutralizing antibodies and resistance to challenge 14 days after the initial inoculation.

The major gross and histopathological lesions produced by the North Dakota mucosal disease virus were confined to the adrenal glands, the gastrointestinal tract, the lymphatic tissues, the kidneys, the liver, and the bone marrow. They consisted of inflammatory and necrotizing changes of the

epithelial tissues of the gastrointestinal tract and the parenchymatous organs. Inflammatory, regressive, and necrotizing changes were noted in the lymphatic system. Regressive changes were also present in the bone marrow.

Contact transmission did not occur. Experimental cattle inoculated with fresh blood and organ emulsions of infected animals failed to contract the infection. Three or more inoculations of calves with organ emulsions resulted in immunity to challenge with the tissue-culture-grown virus.

Virus reisolation attempts failed to reveal the presence of the virus in fresh blood, nasal and ocular swabs, mucosal scrapings, and fecal specimens of infected cattle. The virus was recovered from the spleen, kidneys, and lungs of two calves six and nine days, respectively, after virus inoculation.

The experimental North Dakota virus infection in cattle did not resemble in all respects the classical descriptions of Iowa mucosal disease, malignant catarrhal fever, Ume disease, parainfluenza-3 respiratory infection, virus diarrhea, or infectious bovine rhinotracheitis.

The experimental disease in sheep and rabbits was similar but milder in degree and of shorter duration.

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APPENDICES

Fig. 1.--Swollen jejuno-ileal lymph node plus edema and hyperplasia of Peyer's patches as seen from serosal surface.

Fig. 2.--Swollen and edematous mesenteric lymph node with cortical and subcapsular ecchymoses.

Fig. 3.--Linear demarkation hemorrhage along the lateral edge of a jejunal Peyer's patch as seen from the serosal surface.

Fig. 4.--Subcapsular and cortical ecchymotic hemorrhages in a posterior jejunal lymph node.

Fig. 5.--Cortical ecchymotic hemorrhages in a congested and edematous ileocecal lymph node.

Fig. 6--Cortical and parenchymal hemorrhages in edematous and congested mesenteric lymph nodes.

Fig. 7.--Hemorrhagic marbling in a Peyer's patch of the jejunum.

Fig. 8.--Ecchymotic hemorrhages in a hyperplastic jejunal Peyer's patch.

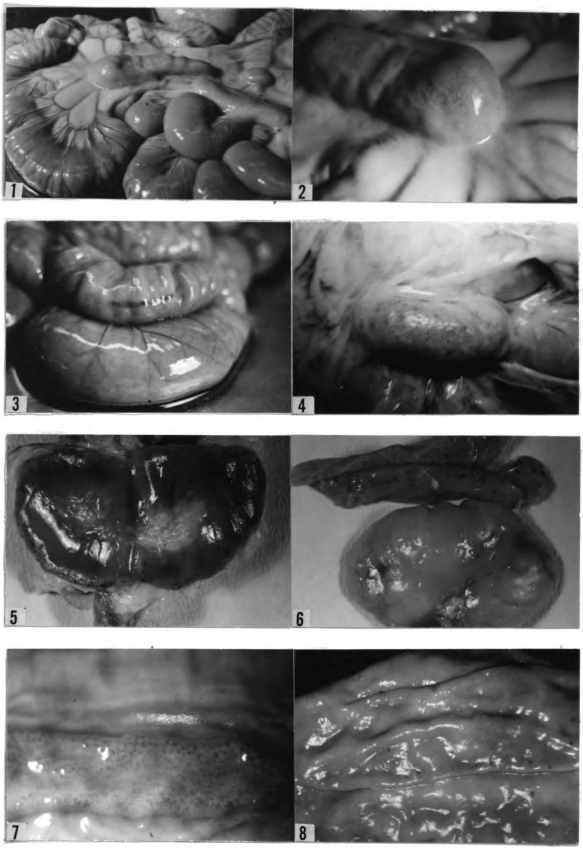
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Fig. 9.--Severe catarrhal to hemorrhagic enteritis of the posterior jejunum, and ileum (above). Less affected cecum and colon below.

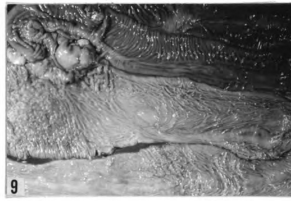
Fig. 10.--Hemorrhagonecrotic seam around the ileocecal ostium.

Fig. 11.--Patchy hemorrhagic congestion of the ileum; petechial hemorrhages in the mucosa of the ileocecal ostium and surrounding cecum.

Fig. 12.--Ecchymotic hemorrhages and cystic degeneration in the mucosa of the cecocolic junction.

Fig. 13.--Petechial hemorrhages in the cortex of a kidney.

Fig. 14.--Subcapsular suffusion in the adrenal gland.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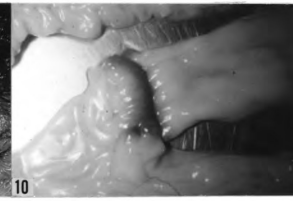
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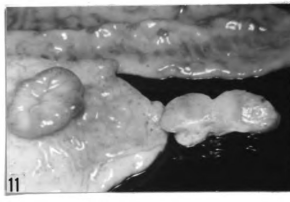
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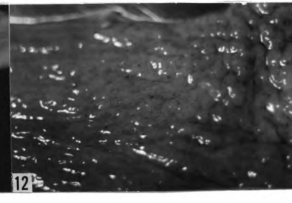
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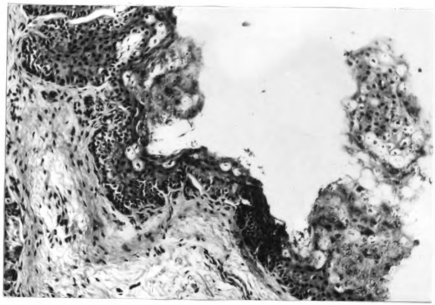

Fig. 15.--Rumen: focal hyperkeratotic and proliferative changes in the mucosa. H. & E. x 200.

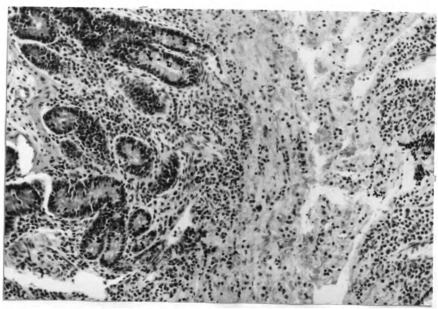

Fig. 16.--Jejunum: catarrhal enteritis with necrosis and denudation of surface epithelium. H. & E. x 200.

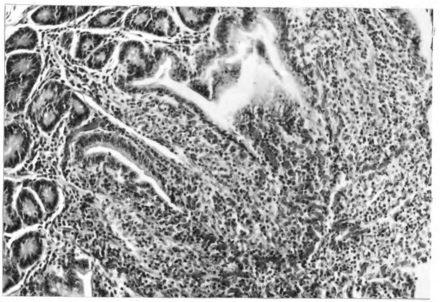

Fig. 17.--Posterior jejunum: catarrhal enteritis with inflammatory hyperemia of capillaries in denuded villi. H. & E. x 200.

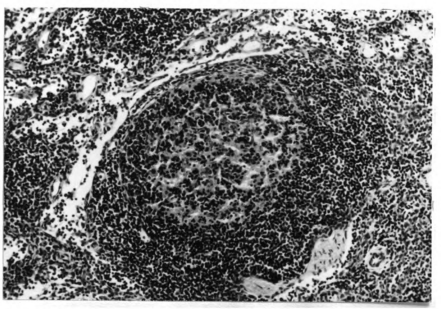

Fig. 18.--Lymph node: Hemorrhage in reaction center showing lymphocytic depletion. H. & E. x 200.

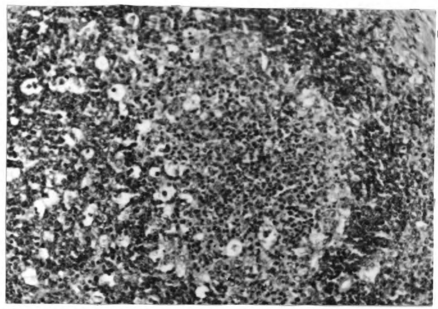

Fig. 19.--Lymph node: necrosis of lymphoid cell elements in an exhausted reaction center. H. & E. x 200.

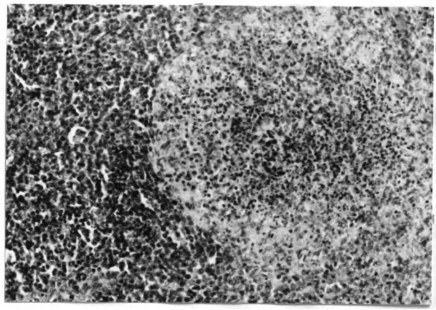

Fig. 20.--Spleen: necrotic focus in reaction center with heterophilic cell infiltration. H. & E. x 200.

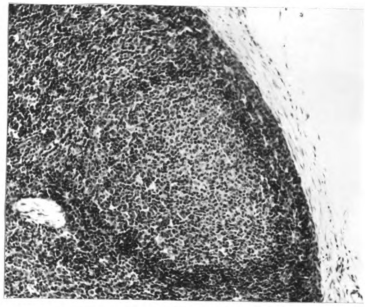

Fig. 21.--**Lymph node:** Tertiary lymph nodule: marginal sinus congested with lymphoid and reticulo-histiocytic cell elements. Capsule shows thinning and distention. H. & E. x 200.

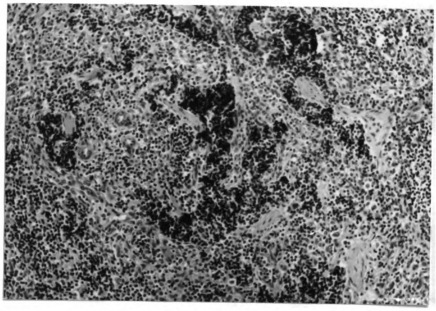

Fig. 22.--**Lymph node:** conglomerated lymphoid and reticulohistiocytic cells. H. & E. x 200.

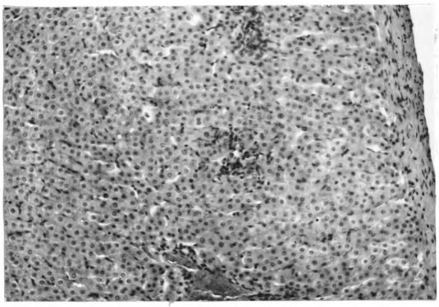

Fig. 23.--Liver: focal areas of reticuloendothelial and lymphocytic infiltrations in the vicinity of pyknotic parenchymal cells. H. & E. x 200.

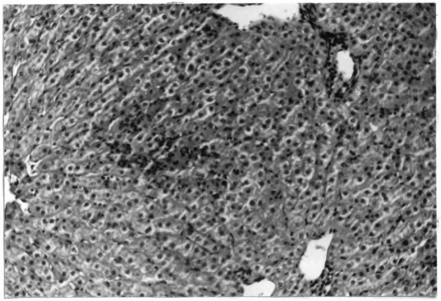

Fig. 24.--Liver: focal area of parenchymal necrosis with reactive reticuloendothelial cell proliferation. Sinusoidal spaces show distention. H. & E. x 200.

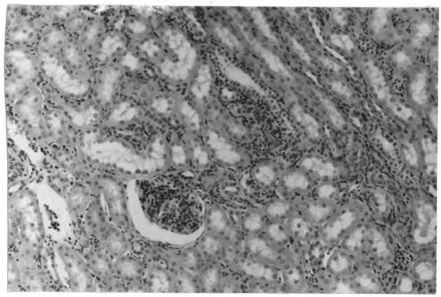

Fig. 25.--Kidney: shrunken glomerular tuft and distended Bowman's capsule. Focal interstitial lymphocytic infiltration. H. & E. x 200.

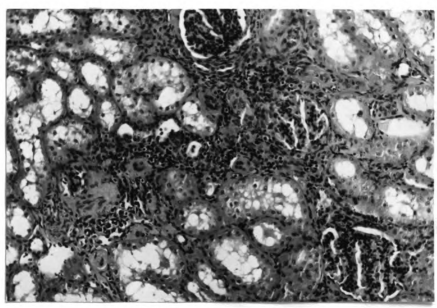

Fig. 26.--Kidney: thickened Bowman's capsule and focal lymphocytic infiltration. H. & E. x 200.

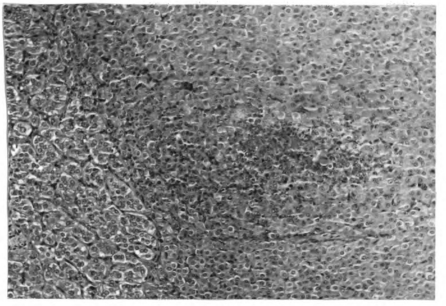

Fig. 27.--Adrenal: small necrotic focus in the central zona fasciculata. H. & E. x 200.

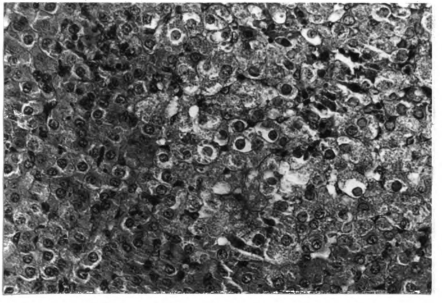

Fig. 28.--Adrenal: pattern of vacuolar cell degeneration in the peripheral zona fasciculata. H. & E. x 400.

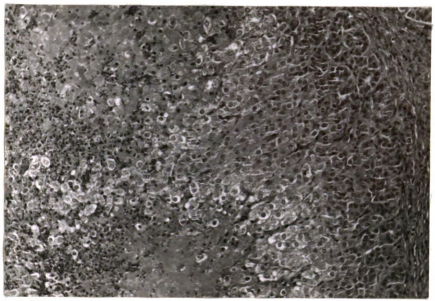

Fig. 29.--Adrenal: foci of coagulation necrosis in the zona fasciculata with reactive lymphocytic and heterophilic infiltrations. H. & E. x 200.

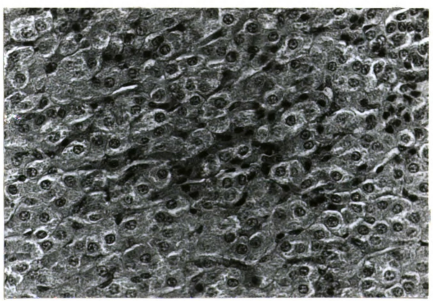

Fig. 30.--Adrenal: strands of darkly staining cells in the zona fasciculata with homogeneous pyknotic nuclei. H. & E. x 400.