

THE PATHOLOGY OF EXPERIMENTAL INFECTION
PRODUCED BY A FILTERABLE AGENT,
ISOLATED FROM A FIELD CASE OF MUCOSAL DISEASE
OF CATTLE

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by
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Signatures have been redacted for privacy

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INTRODUCTION*

Since the first report of mucosal disease in Iowa by Ramsey and Chivers in 1953, this disease has been studied extensively. Ramsey described the lesions in detail in 1956 and in 1960 Whiteman described changes of the adrenal gland and of the pituitary gland. Also in 1960, Trapp reported on the changes of the blood vascular and lymphatic systems. These reports all covered the disease as it was seen in Iowa and clearly defined the clinical and pathological effects of the disease.

Studies on the etiology of the condition have not been as clear cut. Ramsey attempted to pass the disease to experimental calves and concluded that these studies were inconclusive. In conjunction with a Department of Agriculture contract* Ramsey and co-workers (1959) carried on transmission studies over a period of several years.

During the course of these studies a filterable agent was isolated. This agent could be passed in calves and produced a definite clinical reaction in experimental calves. The experimental disease was mild and only slightly resembled mucosal disease as seen in the field. Yet, because the agent was isolated from an animal with typical mucosal disease, the possibility existed that it might be related to the condition. The present study was undertaken to accurately describe the

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disease produced by this agent.

There are no serological means of comparing mucosal disease and the disease caused by the Sanders agent. Therefore, it was hoped that the cellular changes seen in the two conditions could be compared, noting similarities and dissimilarities. This would also enable others to compare these cellular changes to those produced by other agents recovered from diseases having some features in common with mucosal disease.

In addition to studying the effect of the Sanders agent in experimental animals an attempt was made to grow or maintain it in tissue culture systems. If a tissue culture system could be developed in which the Sanders agent would have a cytopathic effect, it would permit the use of tissue cultures for viral titrations and immunologic work. At present this work is prohibitive because of the necessity to use calves as an indicator for the presence of infective material.

REVIEW OF LITERATURE

The voluminous literature of the mucosal diseases was thoroughly reviewed by Trapp (1960). The relationship of the many conditions reviewed by Trapp is still controversial and no conclusive comparison seems to be forthcoming. Therefore, for the purposes of this paper the term mucosal disease will be used in a narrow sense referring only to the condition described in Iowa by Ramsey and Chivers (1953) and Ramsey (1956). For purposes of later discussion the pathology of mucosal disease as documented by these authors will be summarized briefly.

They stated that erosions and ulcers occurred in all parts of the digestive tract from the muzzle and oral cavity to the anus. The specific distribution and severity of these lesions varied from one individual to the next. Lesions were commonly present on the muzzle and external nares as well as in the oral cavity, esophagus, small intestine, cecum and colon.

Lesions of the oral cavity and other areas of the digestive tract covered by stratified squamous epithelium were examined microscopically. Degeneration of all layers of the epithelium occurred. Commonly, the degenerative process began in the stratum spinosum where the intercellular bridges disappeared. The cells then enlarged with increased fluid and small vesicles formed as a result of cell death and liquefactive necrosis. Small vesicles coalesced with sloughing of surface epithelium followed by enlargement of the shallow

ulcer due to secondary bacterial infection. Inflammatory changes were not marked unless secondary bacterial infection was extensive.

Lesions such as these were especially common in the esophagus where they ranged from small slightly raised grayish white foci of necrosis to necrotic areas in which there was a loss of 80 to 90 per cent of the epithelium. The lesions tended to be irregular and linear in form.

These necrotic lesions were less common in the reticulum, omasum and rumen. The abomasum was found to contain multiple circumscribed lesions with raised borders measuring 1 to 15 mm. in diameter. They appeared to be ulcers surrounded by a pale halo and set off from the surrounding mucosa by a sharp ring of petechial hemorrhages. Histologically many of these lesions were atrophic cystic glands with intact but depressed epithelium. Other lesions were more advanced with erosion, ulceration, necrosis, inflammation and edema.

In the small intestine the initial reaction was acute catarrhal enteritis especially in the jejunum and ileum. This was most obvious over the areas of the Peyer's patches. As the severity increased there was necrosis and ulceration of the epithelium covering Peyer's patches with possible severe hemorrhage. Microscopic lesions were consistently found in the areas of Peyer's patches. These microscopic lesions ranged from complete disappearance of lymphoid nodules to varying degrees of necrosis. The overlying mucous glands were

often cystic; being filled with mucus, necrotic epithelium and leukocytes. This resulted in pressure atrophy on the glandular epithelium and led to necrosis and sloughing of the epithelium. Lesions of the colon were similar to those seen in the small intestine.

Blood vascular changes were observed in the lamina propria in association with the other changes of the digestive tract. These included hyperemia, hemorrhage, thrombosis, embolism and increased capillary permeability with edema and escape of fibrinogen into the surrounding tissues.

Lymph nodes often were either grossly normal or mildly edematous. At times there was a marked decrease in mononuclear cells of the cortex resulting in easy visualization of the stroma. This change was also observed in the spleen.

Additional studies on the pathology of mucosal disease as seen in Iowa were conducted by Whiteman (1960) and Trapp (1960). Whiteman described the histopathologic changes of the adenohypophysis and adrenal cortex. Trapp concentrated on the blood vascular and lymphatic systems and extended the studies which were begun by Ramsey.

Trapp stated that striking gross changes were not observed in the lymph nodes in most cases. In some instances all the nodes of the body contained excess fluid with hyperemia and hemorrhage being occasional observations. Microscopic lesions were variable but the variation was usually one of degree rather than type. Generally there was marked depletion

in the number of lymphocytes of the cortex with the germinal centers most severely affected. At other times the lymphocyte depletion was primarily focal. This change allowed the stroma to be readily seen. The mononuclear phagocytes remained even though other cell types disappeared in the cortex.

Most of the animals examined (56 of 64) were found to have accumulations of eosinophilic material in the germinal centers. This relatively acellular and amorphous to finely granular material was usually found in centers with few lymphocytes. Various vascular and cellular inflammatory changes were also present. The nodes most severely involved were the mesenteric and then the supra-pharyngeal. The Malpighian corpuscles of the spleen and the hemal nodes as well as the lymphoid nodules of the tonsils were also found to be involved in a process similar to that seen in the germinal centers of the lymph nodes.

Gross lesions of Peyer's patches described by Trapp (1960) closely correspond to those of Ramsey (1956). Microscopically severe lymphoid depletion, necrosis and inflammation were visible. The mucosal changes were the same as those described by Ramsey.

Hematological study revealed increased erythrocyte counts, hemoglobin concentrations, hematocrit readings and blood urea nitrogen levels. Most total leukocyte counts were normal or elevated and a relative lymphopenia and relative neutrophilia were observed on the differential examination.

Although the gross and microscopic lesions have been described in detail there is little concrete evidence as to the cause of mucosal disease. Ramsey (1956) carried on transmission studies but stated that the results were inconclusive. Results of later work by Ramsey and co-workers (1959) were also inconclusive. They were able to recover a filterable agent which could be passed in calves with a resulting clinical reaction. This agent was recovered from a confirmed case of mucosal disease but its relationship as an etiological agent is uncertain. This agent was recovered from an animal owned by Herbert A. Sanders of Route 2, Fayette, Iowa and has been designated the Sanders agent. The animal from which the agent was recovered was a 1 year old female Hereford Angus cross. It was sick for about 5 days with straining, diarrhea and depression. Post mortem examination revealed severe erosions and ulcers of all portions of the gastrointestinal tract with especially severe necrosis of Peyer's patches. These lesions were typical of a severe case of mucosal disease.

The Sanders agent was found to have the following characteristics:

1. It would serially pass in calves. Five serial passages were made.
2. It produced a distinct clinical reaction in calves which included leucopenia at 3 to 5 days post-inoculation, temperature rise to 106 to 108 degrees F. at 7 to 10 days post-inoculation, a transient diarrhea and depression.

3. It would pass a Seitz E-K filter.
4. It produced immunity up to 6 months after original infection.
5. Infection would spread from inoculated animal to susceptible animal by pen contact.

Filterable agents have also been recovered from cases of mucosal disease by Underdahl (1957) and by Noice and Schipper (1959). The clinical condition resulting from inoculation of calves with these agents is not known in detail.

Carlson et al. (1957) described an experimental disease of calves in their discussion of the pathology of virus diarrhea of cattle in Indiana. Because of the similarities of Indiana virus diarrhea to mucosal disease the experimental form of this disease will be reviewed for later comparison to the disease produced by the Sanders agent. Clinical examination revealed a 1.0 to 3.0 degree rise in temperature on the 3rd to the 5th day post-inoculation. This was accompanied by a drop in leukocyte count to an average of 4,000 cells per cmm. A second and more pronounced febrile response occurred on 7 to 8 days post-inoculation with an average of 105.5 degrees F. The leukocyte count remained low and depression was observed. Diarrhea occurred but varied in time of onset from 3 days to 7 to 8 days post-inoculation. Duration of diarrhea was 24 hours to 20 days and free blood with increased mucus was observed in the feces.

Three animals exhibited exacerbations of clinical signs

at two week intervals for 2 to 3 months at which time they were slaughtered. These animals were thin and unthrifty at that time.

The agent had an apparent affinity for the digestive tract and lesions were observed from the muzzle to the anus. The lesions (erosions and ulcers) of the upper digestive tract were similar in nature varying only in degree of involvement and severity. The foci often proceeded to necrosis and epithelial sloughing. Most lesions remained under 1.0 cm. in diameter.

Congestion, hemorrhages, edema and erosions were seen in the abomasum. Catarrhal enteritis with necrosis of tips of villi was observed in the small intestine. Peyer's patches were distinct because of edema and microscopically there was pronounced dilation of lymph spaces of the intestinal wall. Necrosis of surface epithelium was also observed in the cecum, colon and rectum.

Lymph nodes were enlarged in 50 per cent of the cases and Peyer's patches as well as the mesenteric nodes were enlarged and edematous in 90 per cent of the cases. There was lymphoid exhaustion of the nodes in 60 per cent of the acutely affected experimental animals and there was depletion of germinal centers in the spleen.

MATERIALS AND METHODS

Animals

Eighteen calves were inoculated with infectious material and were observed clinically. Nine calves developed a clinical reaction and were necropsied. Eight calves were examined histologically. In addition one of the clinically normal calves was necropsied. Comparable tissues were collected from 20 apparently healthy calves slaughtered at the Meats Laboratory of Iowa State University. These served as a study group to establish normal controls. Six rabbits were inoculated with infectious material to determine if they were susceptible.

The calves used for inoculations were from two sources. One group came from the State Mental Hospital at Independence, Iowa. They were all purebred Holstein male calves ranging from 3 months to 6 months of age. The remainder of the calves came from the state herd at Woodward, Iowa. They also were Holstein bull calves between 3 and 6 months of age. In both cases the herds were maintained as closed herds with little or no contact with outside animals. There was no obvious history of mucosal disease and all animals were clinically healthy and in good condition.

After each group of animals was purchased they were held in quarantine and kept on a uniform ration of alfalfa hay, cracked corn and oats. The pens, walls, ceilings and all utensils used in the quarantine area were scrubbed several times, treated with sodium hydroxide solution and washed with

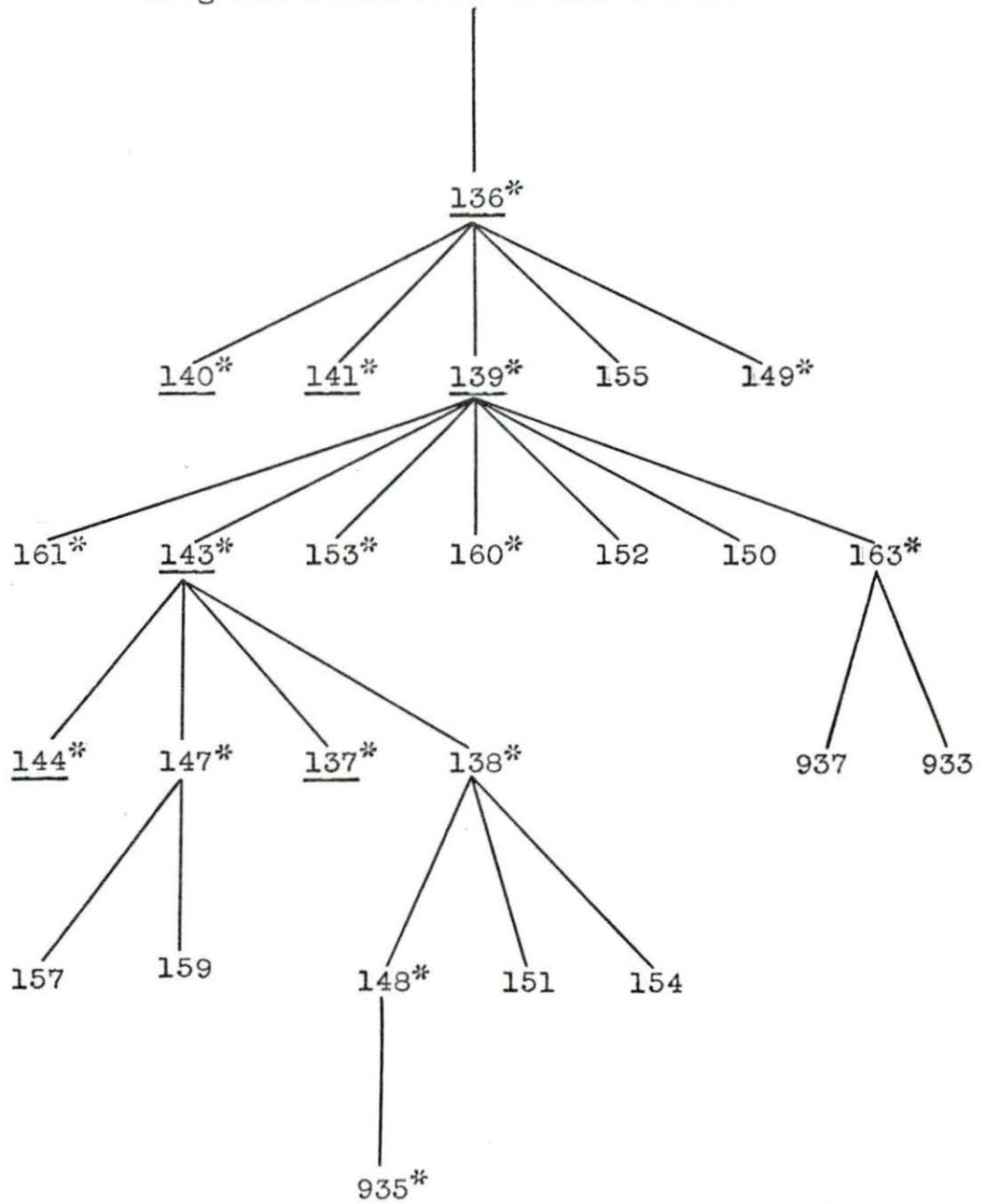
a quaternary ammonia compound before arrival of new animals. In spite of these precautions all incoming groups of animals went through a mild febrile period and several days of diarrhea one week after arrival. All animals were adjusted to the new environment, diet and routine of care for approximately two weeks before inoculation. Temperatures were obtained twice daily and blood samples were taken every other day. No animal was used until its temperature and white blood cell count were relatively stable. One or two animals at a time were then removed to separate isolation quarters for inoculation. Individual variations in handling and final disposition of each animal will be discussed in the section on results.

Infective Material

The Sanders agent was isolated from a typical case of mucosal disease and was originally prepared as a ground suspension of lymph node, abomasum, small intestine (Peyer's patches), liver, spleen and kidney. On subsequent passage it was determined that the agent was present in defibrinated blood obtained at the peak of the clinical reaction and for several days after. Material for this series of inoculations came from calves which had been inoculated in the original isolation of the agent. These passages were carried on in a sequence as illustrated in Figure 1. The specific animal and tissue used as a source of infective material will be stated in the detailed information on each calf. The inoculum was stored at minus 40 degrees F. for periods of one week to 9

Figure 1. Pattern of calf passage of the Sanders agent. Those calves which are underlined were inoculated during the initial isolation of the agent. The remainder of the animals were inoculated in this project. An asterisk denotes a positive clinical reaction.

Original Field Case of Mucosal Disease*



months. Defibrinated blood was thawed and used for inoculation without any further treatment.

The tissues were prepared for inoculation by cutting them into 1 to 2 mm. fragments and then grinding them in a mortar and pestle with sterile sand. An equal amount of Earl's balanced salt solution as modified by Madin et al. (1957) was added during the grinding process. The fluid was then poured off and centrifuged at 2000 r.p.m. for 30 minutes. The supernatant was then treated with 2000 units of penicillin per ml. and 1.0 milligram of streptomycin per ml. This material was then used for inoculum in those cases where ground tissues were used.

Tissue Culture Techniques

Unless specifically mentioned all cells were grown on a medium consisting of Earl's balanced salt solution as modified by Madin et al. (1957), 0.25 per cent lactalbumin hydrolysate (enzymatic) and 10 per cent bovine serum. Penicillin and streptomycin were added at levels of 200 units and 0.1 milligram per ml. respectively.

The complete medium was sterilized by filtration through Selas No. 02 filters using vacuum. The pH was then adjusted to 7.4 with a 7.5 per cent sodium bicarbonate solution which was sterilized by autoclaving.

Primary cultures of bovine kidney cells were obtained from animals 600 to 800 pounds in weight which were slaughtered at the Iowa State University Meats Laboratory. Porcine

kidney cells were obtained from W.P. Switzer of the Veterinary Medical Research Institute at Iowa State University. These cells were from pigs 1 to 8 days old. They were prepared as described by Switzer (1959). Guinea pig kidney cells were derived from young guinea pigs 1 to 3 months old. Serial passage cell lines were also obtained from W. P. Switzer.

Histopathology Techniques

The tissues were collected and fixed in 10 per cent buffered formalin (Armed Forces Institute of Pathology 1957) for 24 hours and then washed in running tap water for 30 minutes. They were then placed in fresh 10 per cent buffered formalin for storage until processing. Several 2 mm. thick adjacent sections were cut from the tissue. One section was stored for future reference and one was used for processing and sectioning. All tissues were dehydrated in a graded series of ethyl alcohol, cleared in xylene and infiltrated and embedded in paraffin. Sections were cut at 7 microns and stained with hematoxylin and eosin.

RESULTS

The results will be discussed in 4 sections. The first, entitled calf inoculations, will include the method and source of inoculation, clinical reaction and gross post mortem findings. Each animal will be discussed as an individual and then the group will be summarized. Clinical data on body temperature and total white blood cell count will be presented in figures 2 through 19. The second section, microscopic findings, will include the histopathological findings for each calf and then a summary of these findings. The third section will summarize the tissue culture work and the fourth section will summarize the rabbit inoculations.

Calf Inoculations

Calf no. 138 was inoculated intravenously with 5.0 ml. of defibrinated blood from calf 143. The material was passed through a Seitz E-K filter. The calf developed a leucopenia, a temperature rise, anorexia, depression, and intermittent diarrhea. Necropsy examination revealed the presence of numerous necrotic foci and linear erosions in the esophagus and edema of the small intestine in the areas of Peyer's patches.

Calf no. 147 was inoculated intravenously with material from calf 143. A leucopenia and temperature rise followed inoculation by 4 to 5 days. Blood tinged mucus appeared in the feces 3 days post-inoculation. Diarrhea was observed on days 7, 8, 9, and 10. Depression, partial anorexia and signs of abdominal distress were observed on days 7, 8 and 9.

Necropsy at 11 days after inoculation revealed the presence of 25 to 30 necrotic foci and or erosions in the esophagus and severe edema of the Peyer's patches throughout the small intestine.

Animal no. 148 was inoculated intravenously with 10 ml. of defibrinated blood collected from calf no. 138. Leucopenia developed on day 4 post-inoculation with a severe temperature rise, abdominal pain and anorexia on day 9. The animal was necropsied on day 11 and was found to have linear necrotic foci of the esophagus, hyperplasia of abomasal lymphoid nodules, severe edema of Peyer's patches, severe edema of the terminal portion of the ileum and enlargement of the lymph nodes which drain the terminal portion of the ileum. These nodes were 2 to 3 times their normal size.

Calf no. 149 was inoculated with material from calf no. 136. The material consisted of 1/3 defibrinated blood, 1/3 ground lymph node and 1/3 ground spleen. The animal was inoculated by injecting 3 ml. into a prescapular lymph node and 7 ml. into a jugular vein. There was a slight temperature rise and a definite leucopenia 5 days after inoculation. The animal was necropsied 15 days after inoculation and there was esophageal ulceration, hyperemia of the abomasum, catarrhal enteritis, and enlargement of Peyer's patches. The Peyer's patches were raised 2 to 3 mm. above the intestinal surface. There was excessive clear fluid in the peritoneal cavity.

Calf no. 150 was inoculated intravenously with 7 ml. of

material which consisted of 1/3 defibrinated blood, 1/3 ground lymph node and 1/3 ground spleen obtained from calf no. 139. There was no clinical reaction and the animal was not necropsied.

Calf no. 151 was inoculated intravenously with 10 ml. of defibrinated blood from calf no. 138 on one day and then received an additional 5.0 ml. of inoculum per day for two more days. There was no clinical reaction and the calf was not necropsied.

Calf no. 152 was inoculated intravenously with 5 ml. of material and into the prescapular lymph node with 2 ml. of material. This material was from calf no. 139 and was similar to that used to inoculate calf no. 150. A leucopenia occurred 7 days after inoculation. There was no other clinical response and there was no necropsy examination.

Calf no. 153 was inoculated with materials consisting of 1/3 defibrinated blood, 1/3 ground lymph node and 1/3 ground spleen obtained from calf no. 139. Five ml. of infective material were injected into a jugular vein and 3 ml. were injected into a prescapular lymph node. A severe leucopenia occurred on the 6th day post-inoculation and the animal was necropsied 11 days after inoculation. A few necrotic esophageal ulcers were present and there was catarrhal enteritis with excessive mucus and edema of the small intestine. Edematous mesenteric lymph nodes and Peyer's patches were very striking because of their enlargement. Petechial hemorrhages

occurred at the edges of the swollen lymphoid patches. There was excess fluid in the peritoneal cavity, pericardial sac and the joint cavities of the legs. This clear colorless fluid clotted on exposure to the air.

Calf no. 154 received 10 ml. of defibrinated blood from calf no. 138 by intravenous inoculation. There was no clinical reaction and there was no post mortem examination.

Calf no. 155 was inoculated with material from calf no. 136 by injecting 5 ml. intravenously and 2 ml. into a pre-scapular lymph node. The inoculum consisted of 1/3 defibrinated blood, 1/3 ground lymph node and 1/3 ground spleen. A leucopenia occurred 5 days after inoculation but there was no other clinical reaction. This animal was necropsied 13 days after inoculation. There were ulcers and linear erosions of the esophagus, as well as moderate catarrhal enteritis, edema of the mesenteric lymph nodes and enlargement of Peyer's patches. They were raised 3 to 4 mm. above the mucosal surface. There was hyperemia at their periphery and there was hyperemia on the abomasal folds. Clear fluid was found in the peritoneal cavity, pericardial sac and leg joints. Fluid was collected from the pericardial sac and several joints and taken to R. Ross at the Veterinary Medical Research Institute of Iowa State University. He made several attempts to grow pleuropneumonia-like organisms from this fluid but was not successful.

Calf no. 157 received 6 ml. of inoculum intravenously

and 6 ml. in a prescapular lymph node. The inoculum was defibrinated blood from calf no. 147. A mild leucopenia developed 6 days after inoculation and continued for a week. The reaction was considered inconclusive and there was no post mortem examination.

Calf no. 159 received 6 ml. of inoculum intravenously and 6 ml. in a prescapular lymph node. The inoculum was defibrinated blood from calf no. 147. There was no significant clinical reaction and the animal was not necropsied.

Calf no. 160 was inoculated with material consisting of 1/3 defibrinated blood, 1/3 ground lymph node and 1/3 ground spleen from calf no. 139. This mixture was given by injecting 5 ml. intravenously and 3 ml. into a prescapular lymph node. A moderate leucopenia developed but this was not severe. There was no post mortem examination.

Calf no. 161 was inoculated with ground clotted blood from calf no. 139. Ten ml. were injected intravenously and 2 ml. were injected into a prescapular lymph node. There was a mild leucopenia 3 days and 5 days post-inoculation with mild abdominal distress and depression during this period. Post mortem examination was conducted 12 days after inoculation. There was mild hyperplasia of lymphoid foci in the abomasum and enlargement of Peyer's patches. A mild catarrhal enteritis was present with excessive mucus and hyperemia. There was mild edema of many lymph nodes including the prefemoral nodes, popliteal nodes, mesenteric nodes and the tonsils. There was

excessive clear fluid in the abdominal cavity and in the joints of the legs.

Calf no. 163 was inoculated with ground clotted blood from calf no. 139. This was given by injecting 10 ml. intravenously and 2 ml. into a prescapular lymph node. There was a mild febrile reaction with leucopenia 5 days after inoculation accompanied by severe abdominal pain, mild diarrhea, inappetence and depression. Excessive mucus and blood clots were observed in the feces. The animal was necropsied 8 days after inoculation. There were small ulcers 2 to 4 mm. in diameter on the tongue. There was lymphatic congestion of the abomasal mucosa with lymphoid nodule hyperplasia. Peyer's patches were enlarged with some being hemorrhagic. The ileum was mildly edematous and there was cystic hyperplasia of the submucosal glands of the colon. All lymph nodes were edematous and the mesenteric nodes were twice normal size. There was clear fluid in the peritoneal cavity along with some clotted blood and fibrin.

Calf no. 933 was inoculated with a mixture of ground lymph node, ground liver and ground spleen obtained from calf no. 163. This was given by injecting 10 ml. intravenously and 10 ml. into a prescapular lymph node. The clinical reaction was indefinite and the animal was not necropsied.

Calf no. 935 was inoculated with a suspension of ground lymph node, ground liver and ground spleen from calf no. 148. Twenty ml. of this material were given intravenously and 10 ml.

were injected into a prescapular lymph node. There was a striking clinical reaction with a temperature rise to 107.6 on the 8th day after inoculation and a mild leucopenia on the 7th day. There was severe depression, abdominal pain and inappetence. This was followed by diarrhea with bloody feces on the 9th day. The calf was necropsied 10 days after inoculation. There was clear fluid in the peritoneal cavity, the pleural cavity, the pericardial sac and in the hock joints. This clotted on exposure to air. There were numerous erosions on the tongue, esophagus, and abomasum. The lymphatics of the abomasum were prominent. The ileum was dilated with a thickened wall and raised Peyer's patches which were reddened in spots. Excessive mucus was present in the intestinal tract. Cystic hyperplasia of the submucosal glands of the colon was present. The mesentery of the ileum was very edematous as were the mesenteric lymph nodes and ileum itself. The mesenteric nodes were 2 to 3 times their usual size.

Calf no. 937 was inoculated with ground lymph node, liver, and spleen from calf no. 163. Ten ml. were injected intravenously and 10 ml. were injected into a prescapular lymph node. There was no significant clinical reaction and the animal was necropsied 18 days after inoculation. There were no significant lesions.

Summary of Calf Inoculations

A total of 18 calves were inoculated. Nine of these developed a mild to severe clinical reaction. These were

necropsied and found to have significant lesions. Nine calves did not show any conclusive clinical response. Because of economic reasons only one of these (937) was posted. No gross lesions were observed in this animal.

Of the 9 calves with a clinical response, all 9 had a leucopenia, 6 had a febrile response, 5 developed inappetence, depression and signs of abdominal pain and only 4 had diarrhea. The reaction was very mild in most of the animals and it was of short duration. Even where diarrhea, depression and abdominal pain occurred it would have been missed under average farm conditions. The whole clinical reaction could best be described as mild, variable and transient.

The post mortem findings from those animals which were necropsied were remarkably uniform and definite in spite of the lack of clinical signs. All calves had definite and similar lesions. These consisted of small but definite linear esophageal ulcers, lymphatic edema of the abomasum, pinpoint ulcer-like lesions of the abomasum, severe enlargement of Peyer's patches and edema of the ileum.

The enlarged Peyer's patches were the most striking lesion, being raised above the surrounding tissue. These lymphoid patches were often reddened with small hemorrhagic foci. Catarrhal enteritis was also common and submucosal cystic hyperplasia of the colon occurred in several animals. The lymph nodes were edematous, especially the mesenteric nodes. The mesentery was also edematous in some cases. A very

striking and common lesion was the presence of clear fluid in all serous cavities. Fibrin was present in this fluid because it would clot on exposure to air. At times this fluid was also blood tinged.

Microscopic Findings

Tissues were collected from animal numbers 138, 147, 148, 149, 153, 155, 163, and 935. These were sectioned and examined microscopically. They will be reported individually and then will be summarized. Because of the small size of many of the gross lesions, especially the ulcers, it was not always possible to get a microscopic description of the grossly visible lesions. The tissues which were examined microscopically were tongue, cheek wall, esophagus, rumen, reticulum, omasum, fundic abomasum, pyloric abomasum, duodenum, ileum, jejunum, colon, cecum, major lymph nodes, trachea, lung, cardiac muscle, liver, pancreas, spleen, thymus, kidneys, urinary bladder, testis, adrenal glands, and thyroid glands.

Calf no. 138: There was a small area of necrosis of the superficial half of the epithelium of the esophagus. There was at least one area of necrosis and leukocytic infiltration in the epithelium of the omasum. Moderate lymphoid hyperplasia of Peyer's patches was present. Polymorphonuclear leukocytes were found infiltrating some small lymphoid nodules in the wall of the colon. The lymph nodes were normal except for some slight focal cortical congestion.

Calf no. 147: Several subepithelial inflammatory foci

Figure 2. Clinical data on calf no. 138. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.

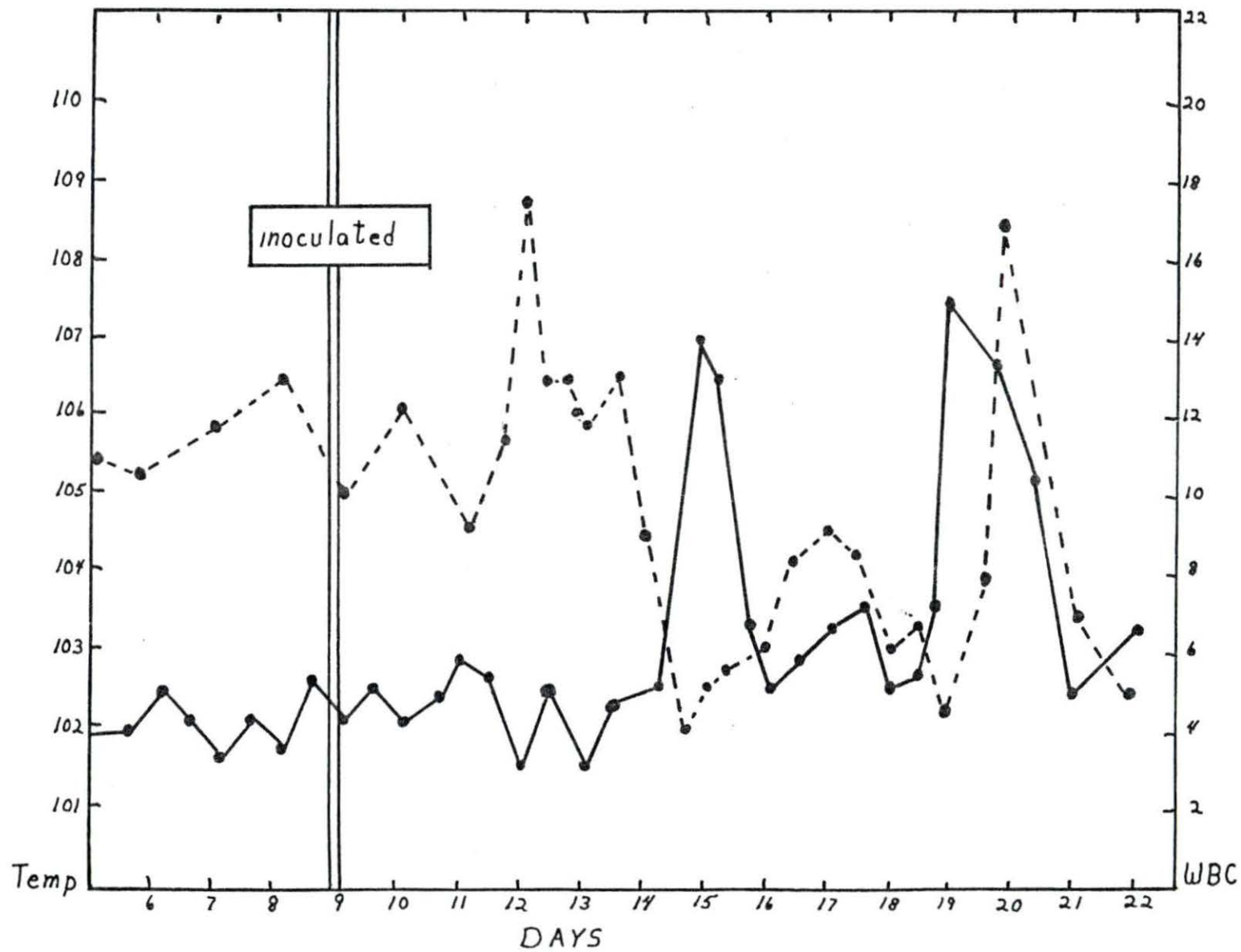


Figure 3. Clinical data on calf no. 147. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



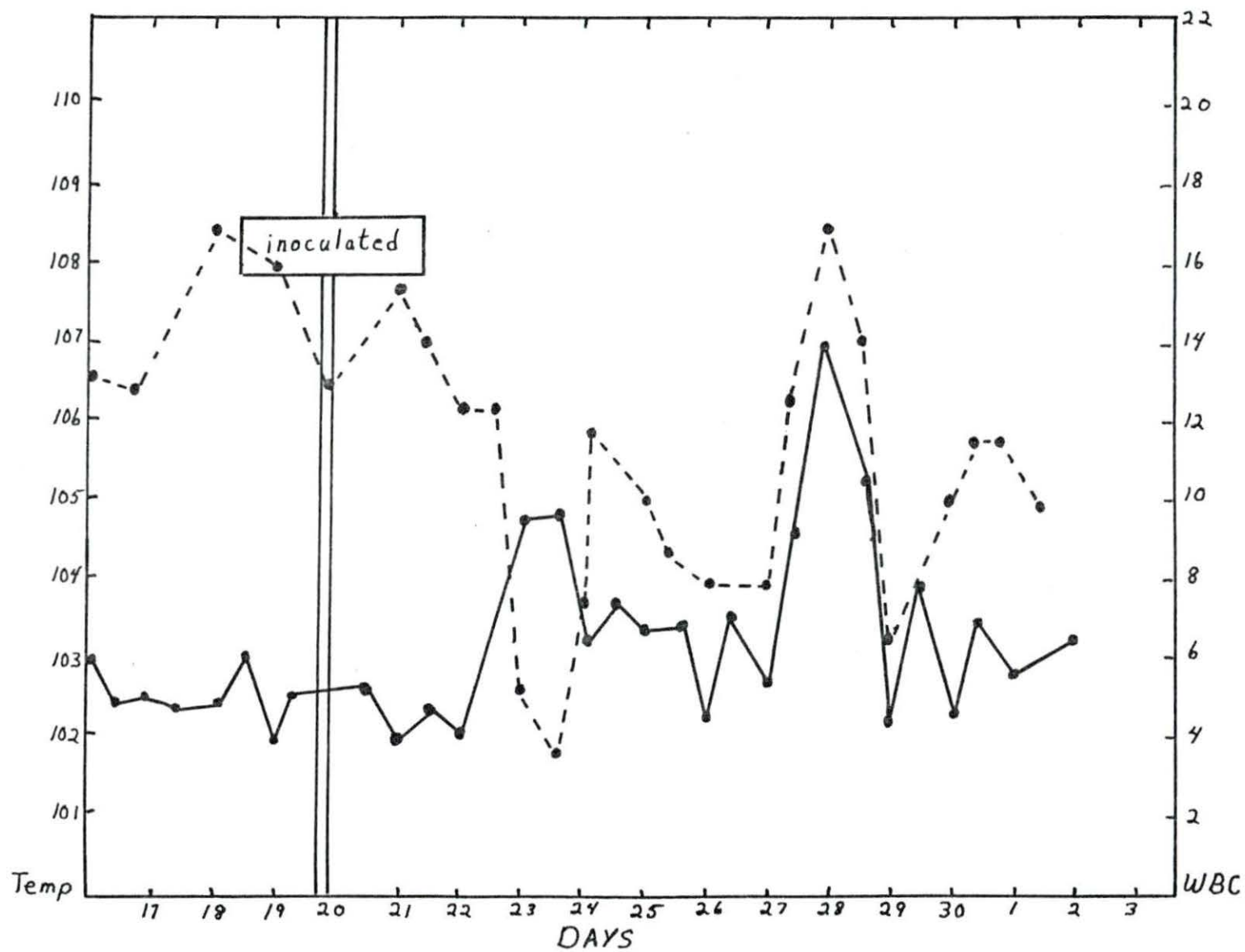


Figure 4. Clinical data on calf no. 148. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.

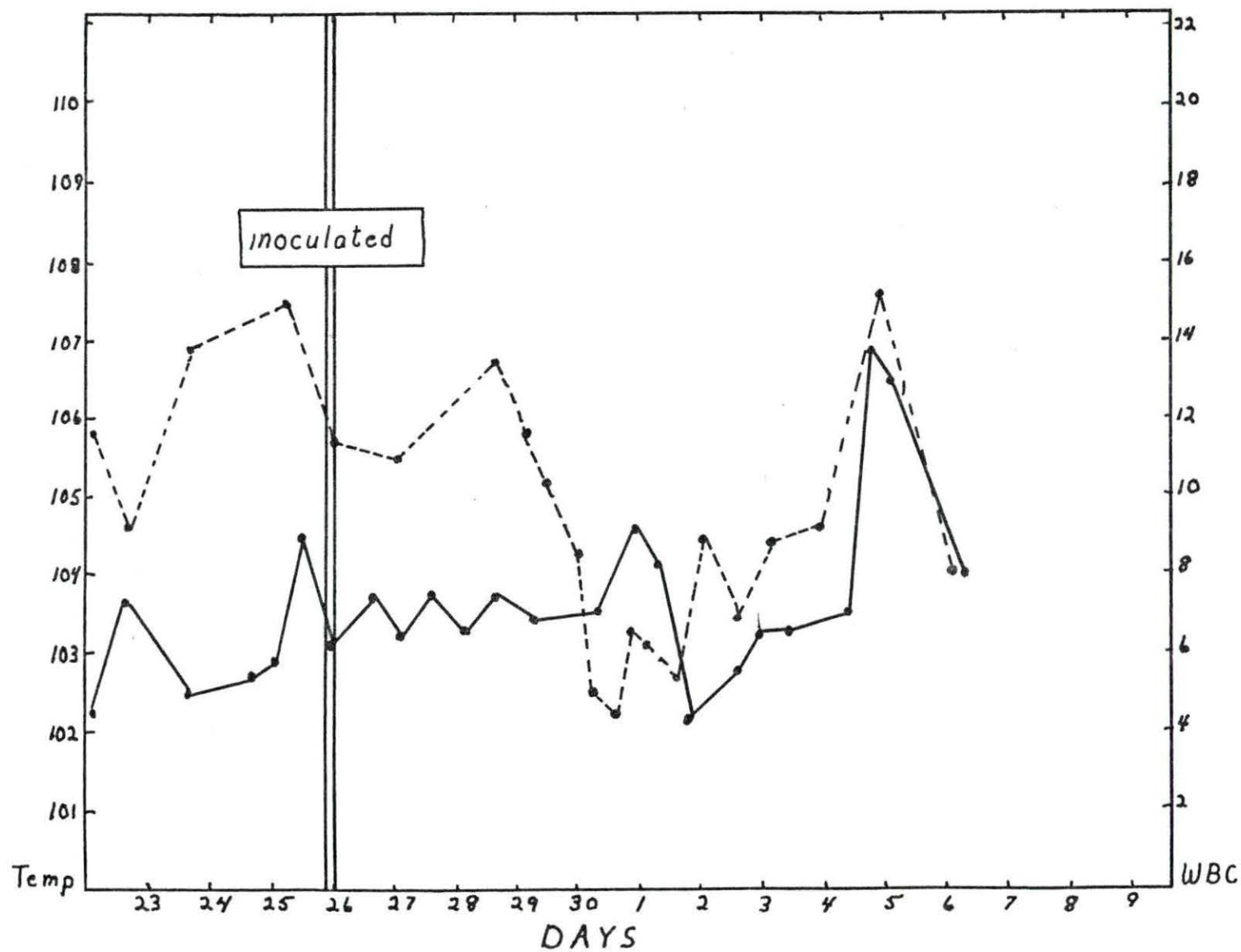


Figure 5. Clinical data on calf no. 149. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



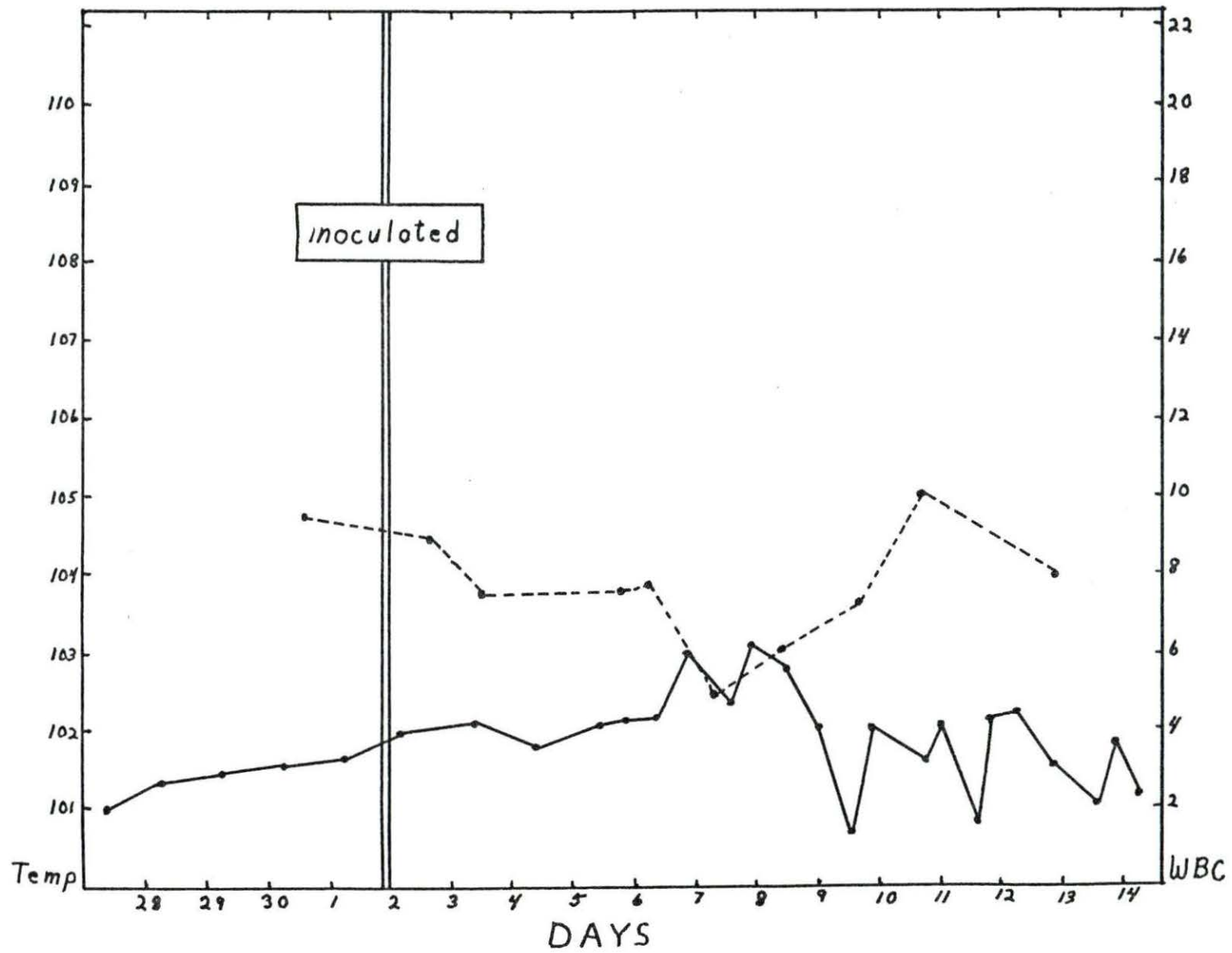
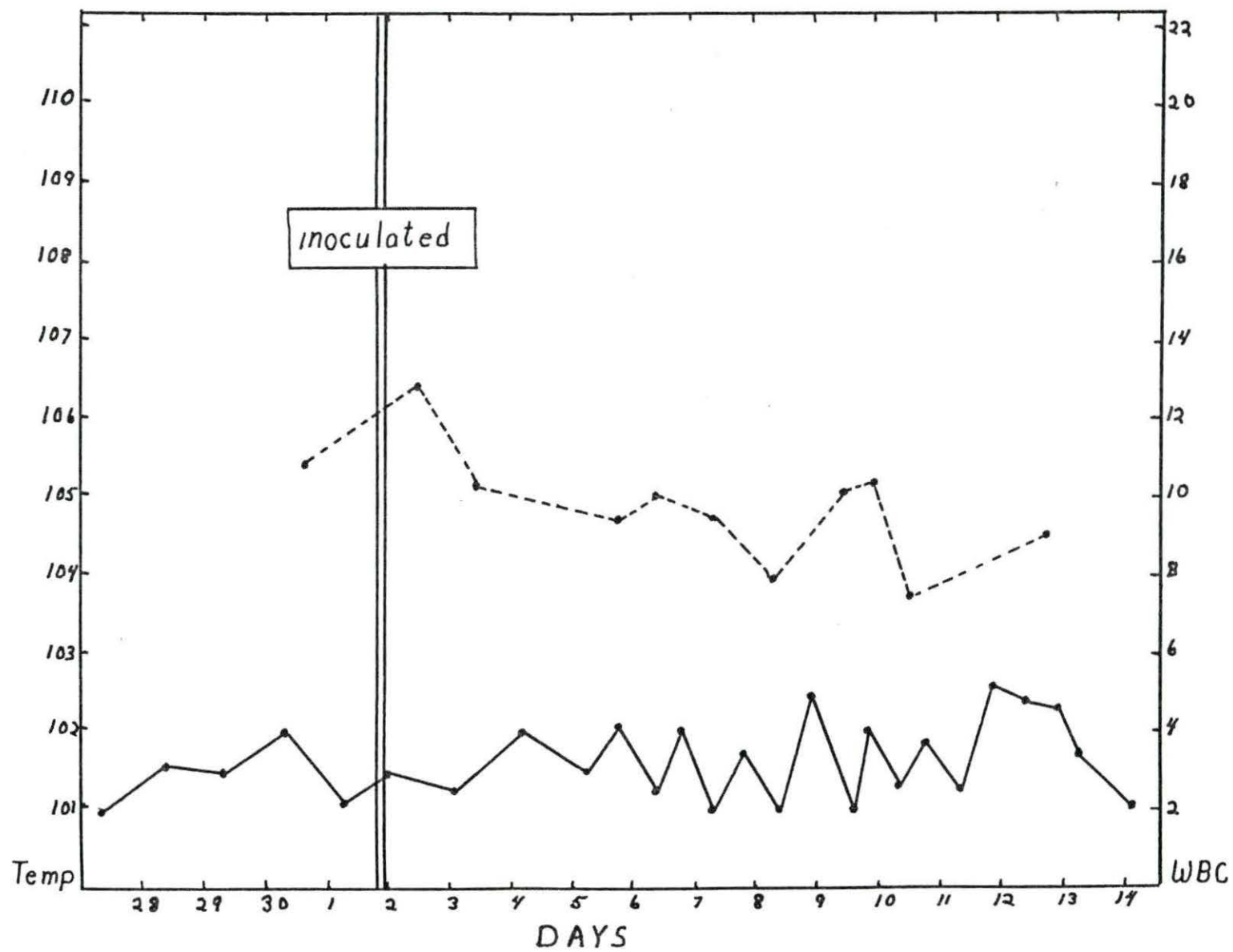


Figure 6. Clinical Data on calf no. 150. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



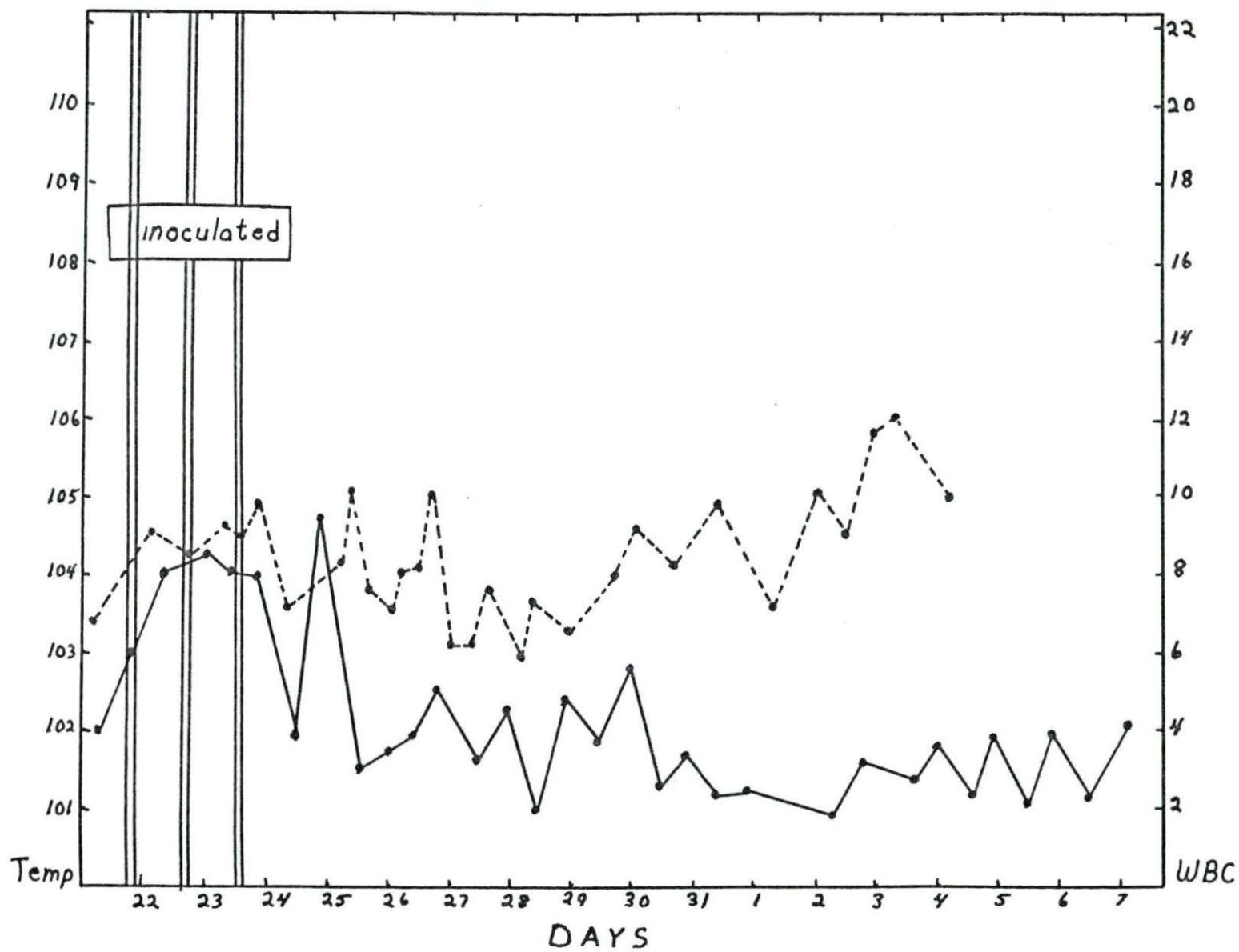


Figure 8. Clinical data on calf no. 152. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.

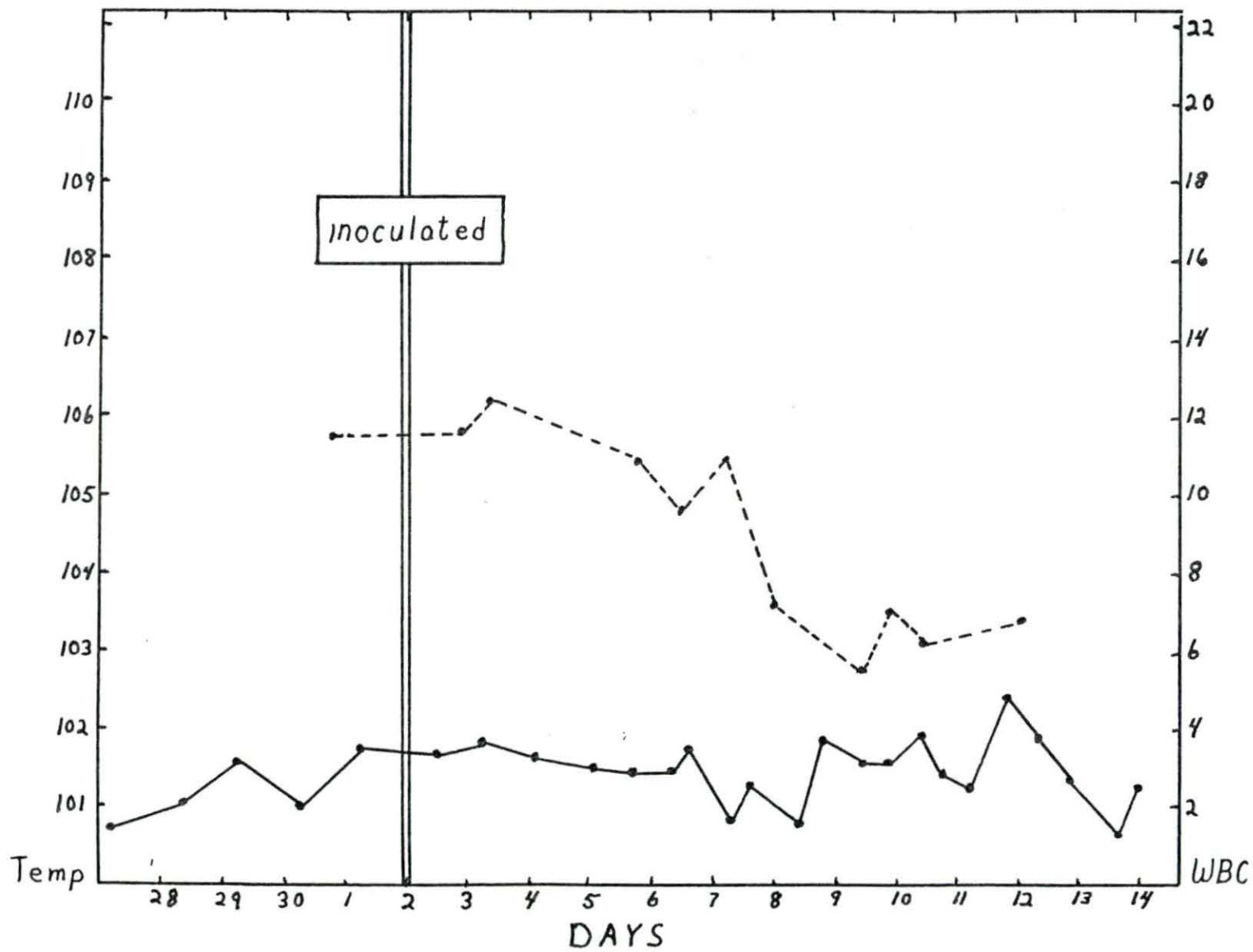
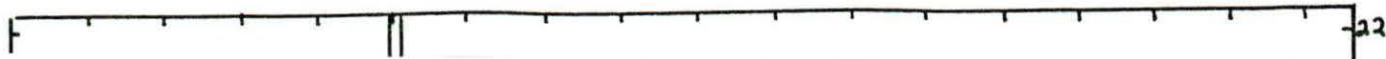


Figure 9. Clinical data on calf No. 153. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



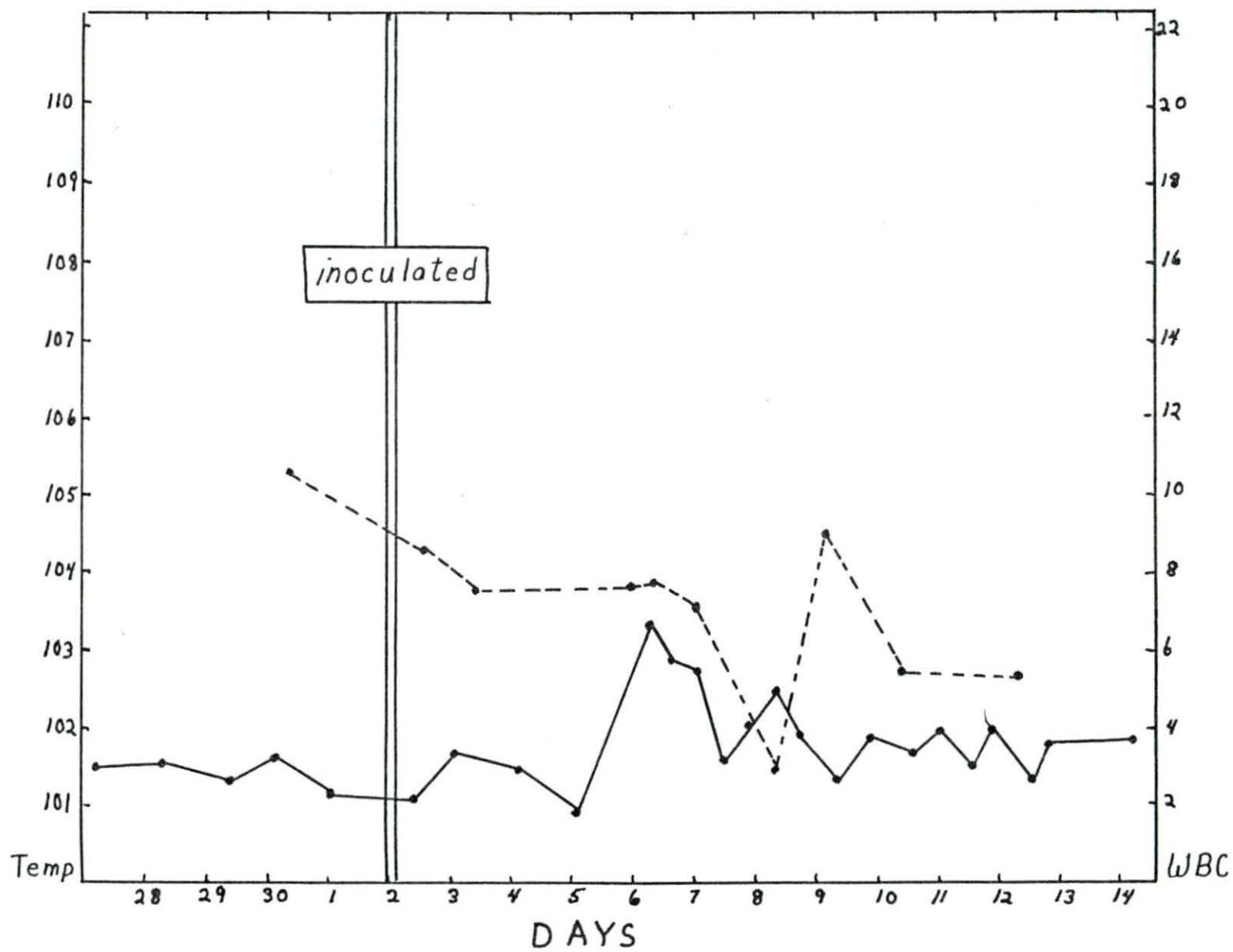


Figure 10. Clinical data on calf no. 154. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



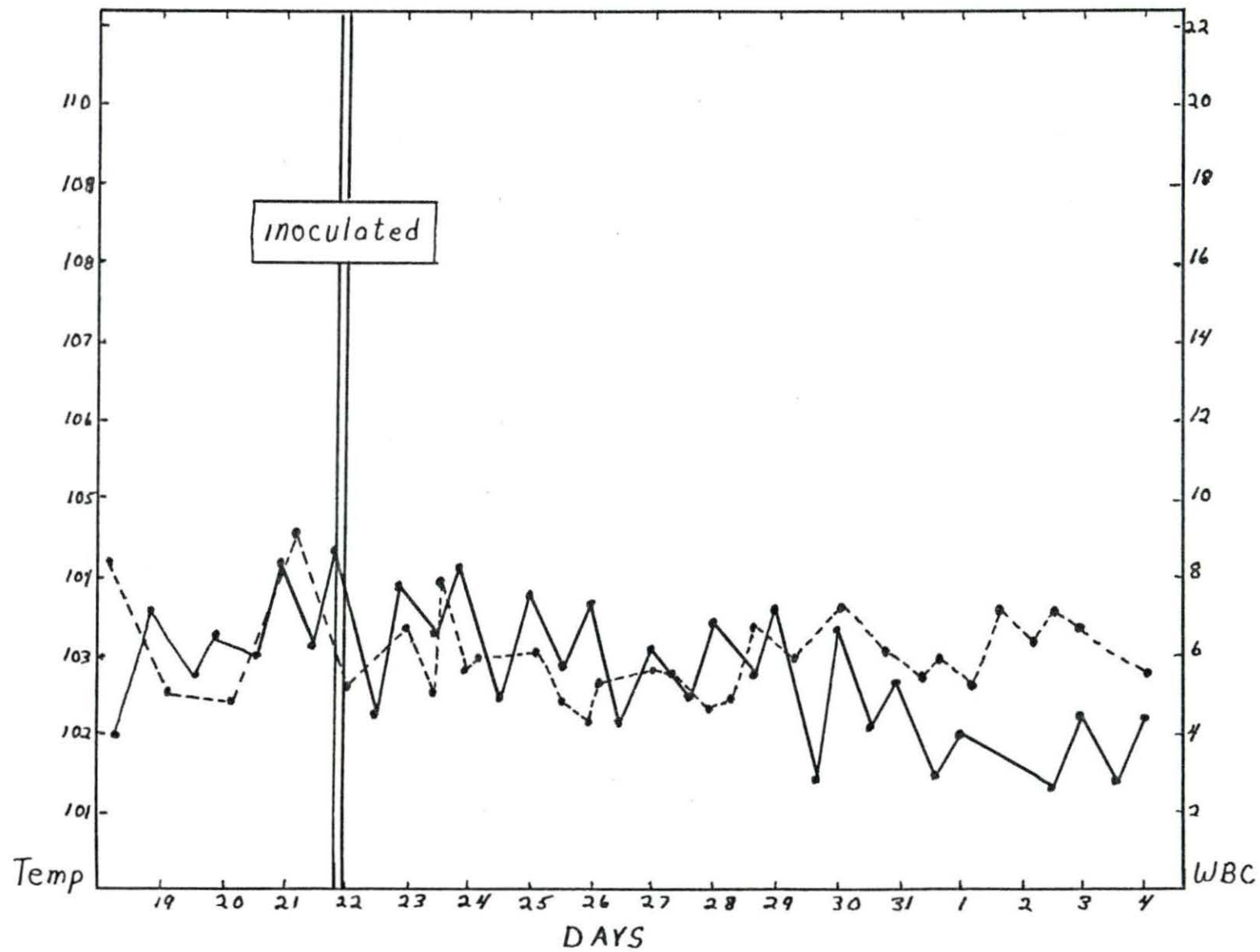


Figure 11. Clinical data on calf no. 155. The dotted line represents Leukocytes in thousands per cmm. The solid line represents body temperature.



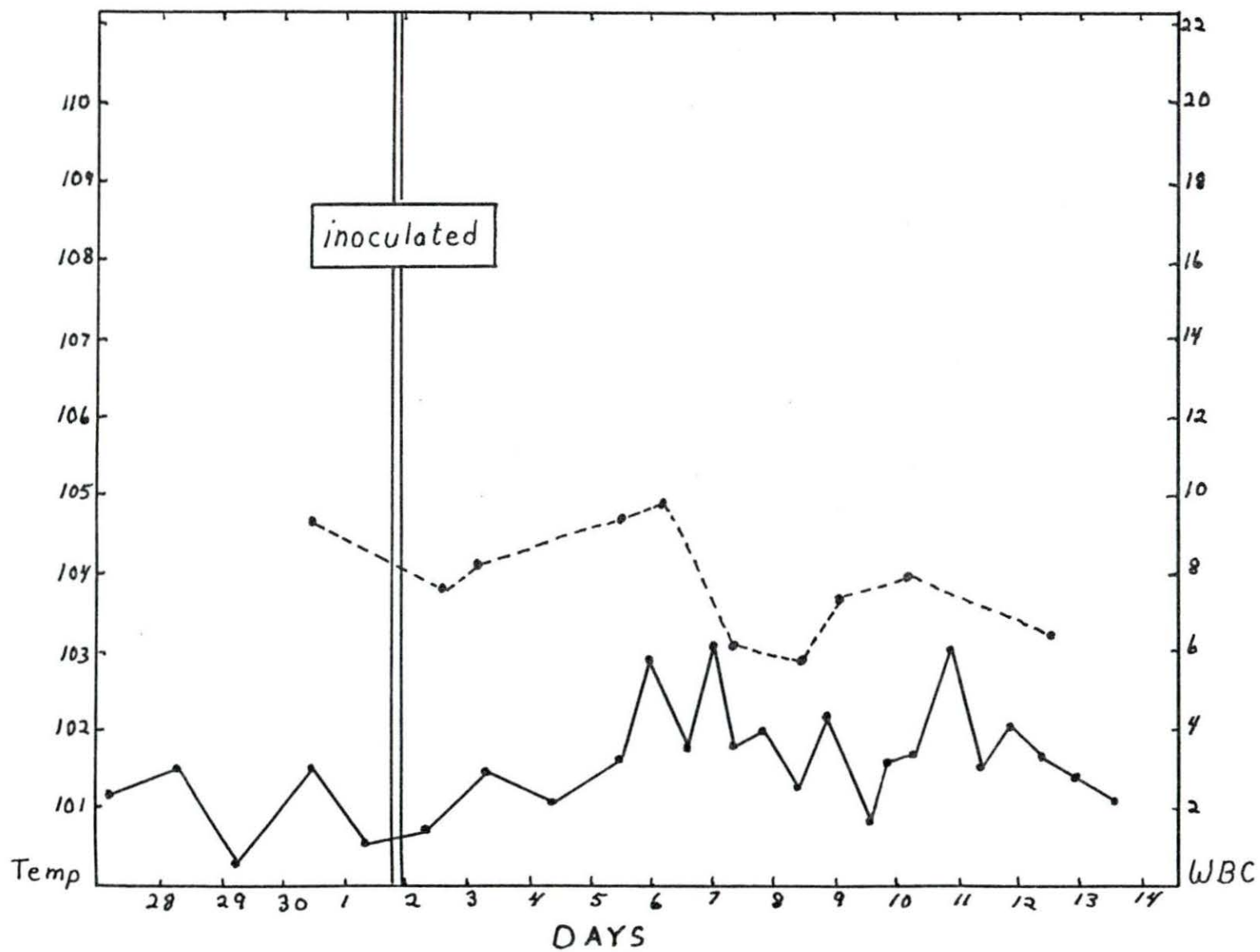


Figure 12. Clinical data on calf no. 157. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



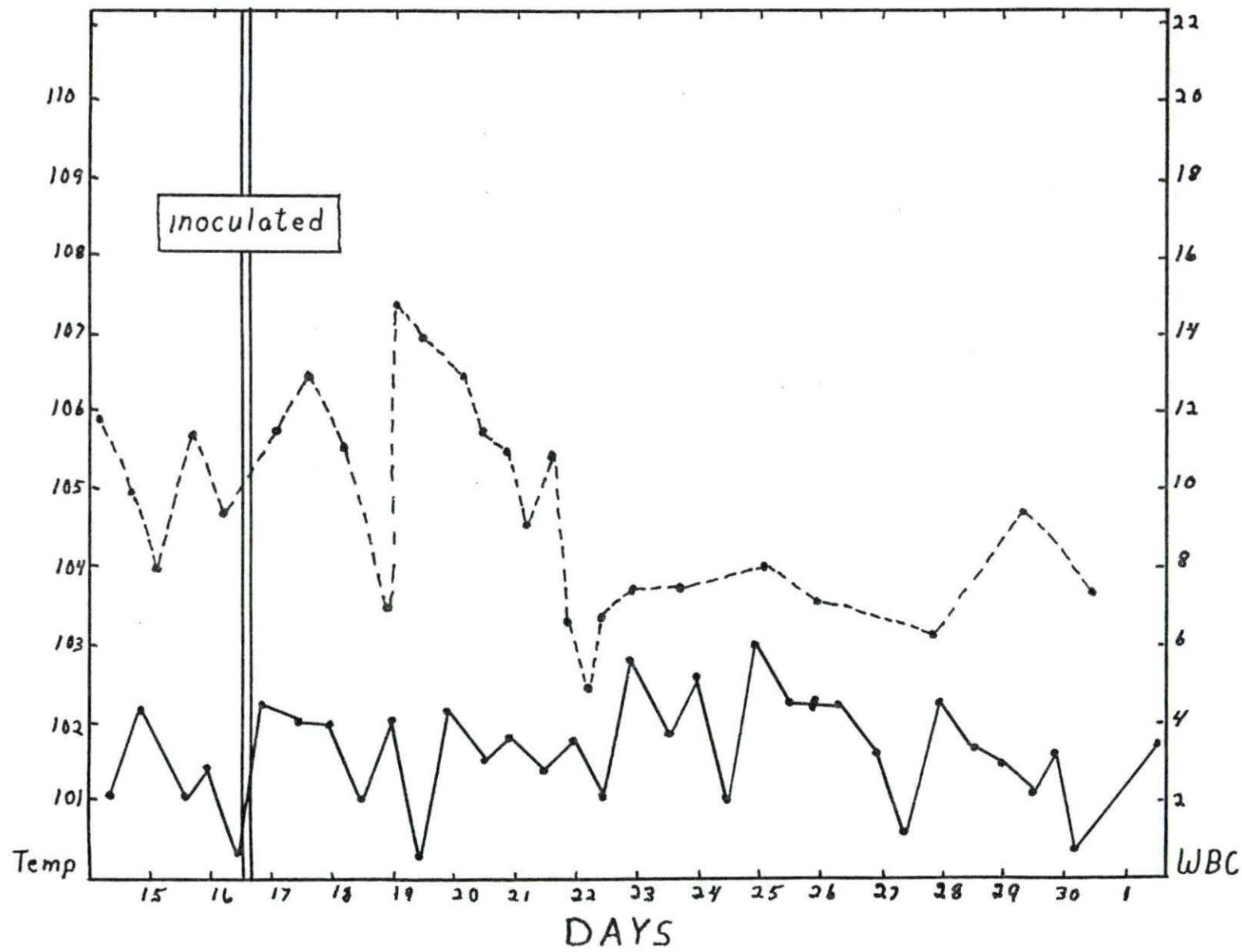


Figure 13. Clinical data on calf no. 159. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



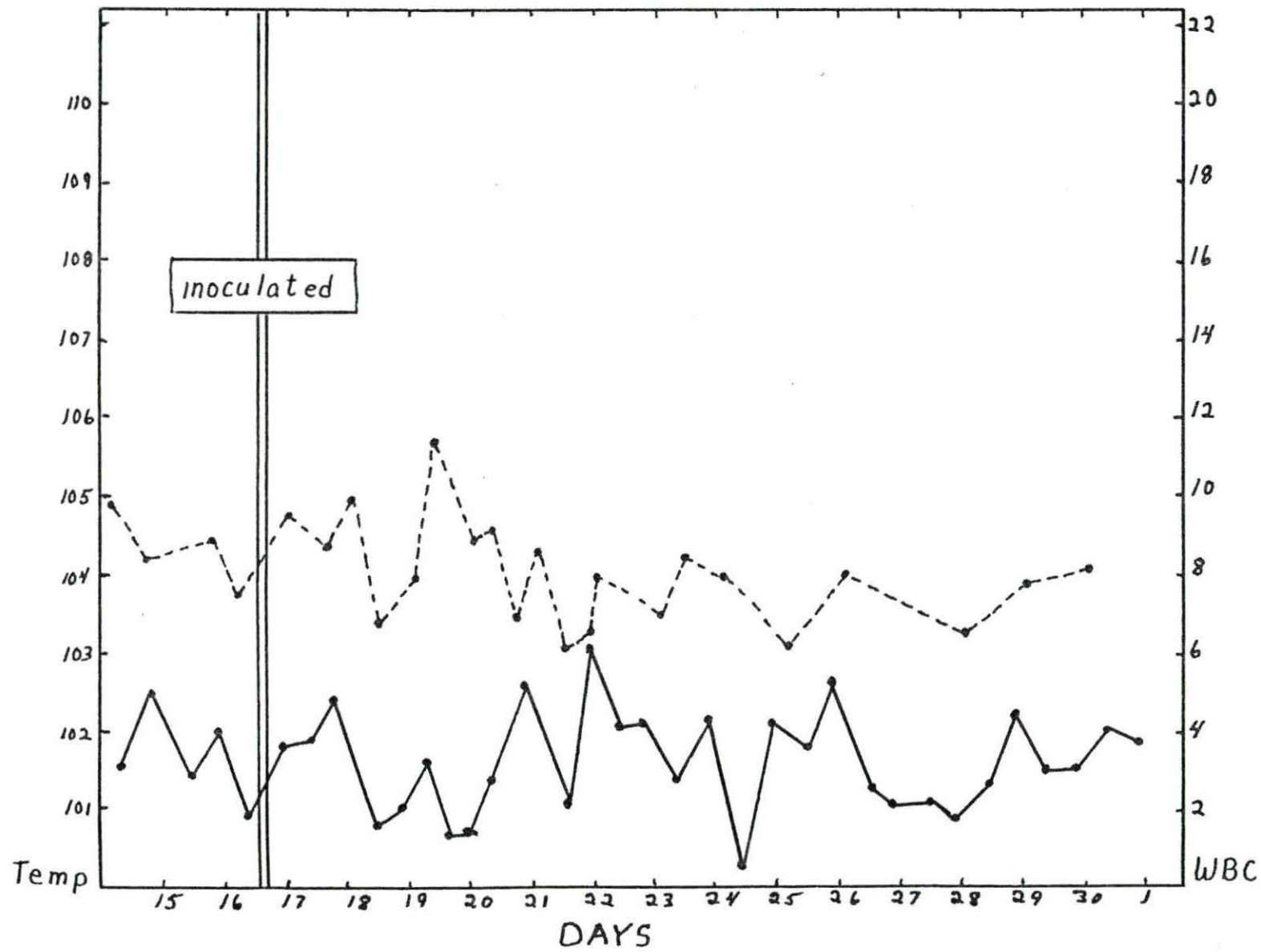


Figure 14. Clinical data on calf no. 160. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.

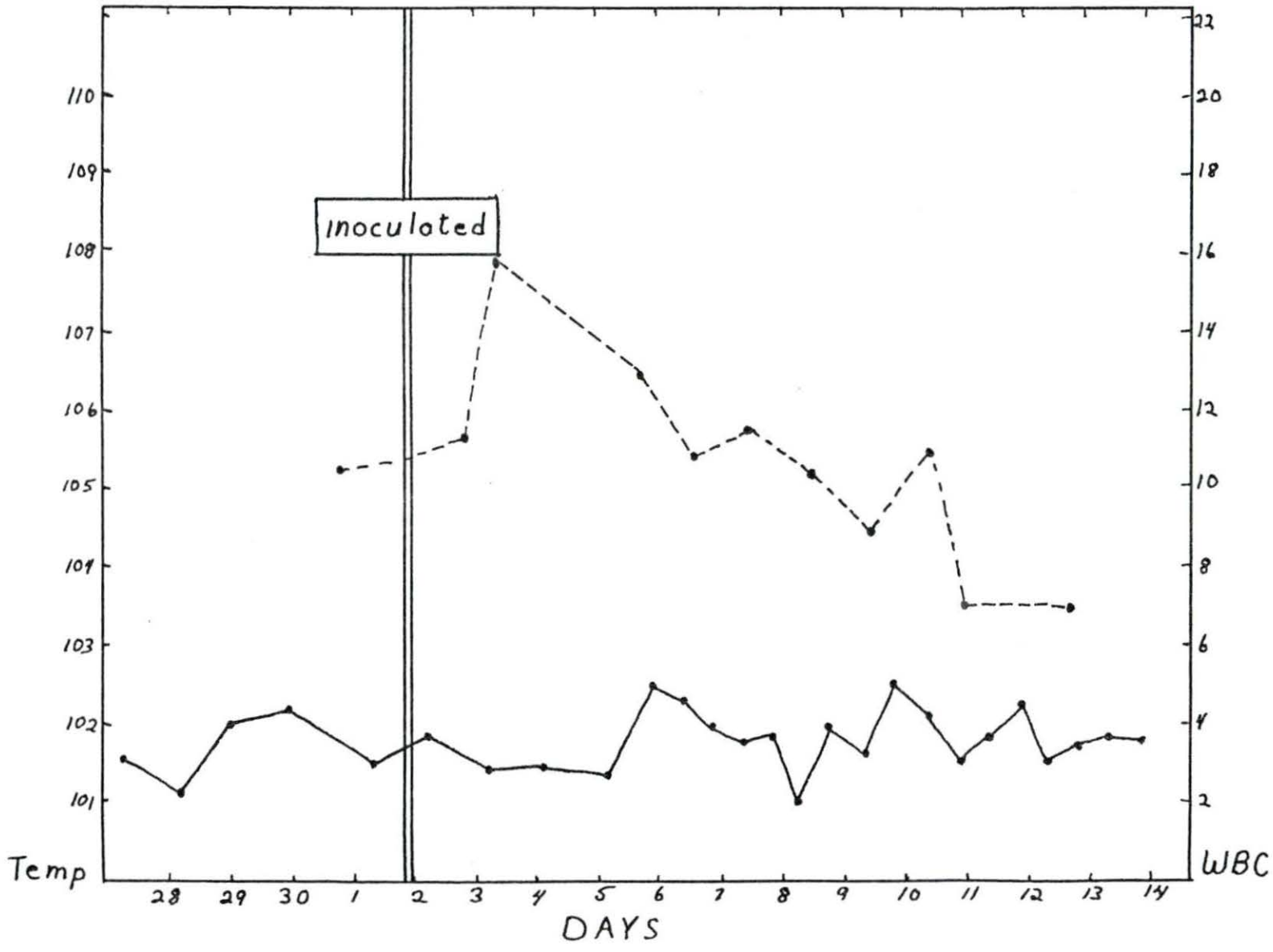


Figure 15. Clinical data on calf no. 161. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



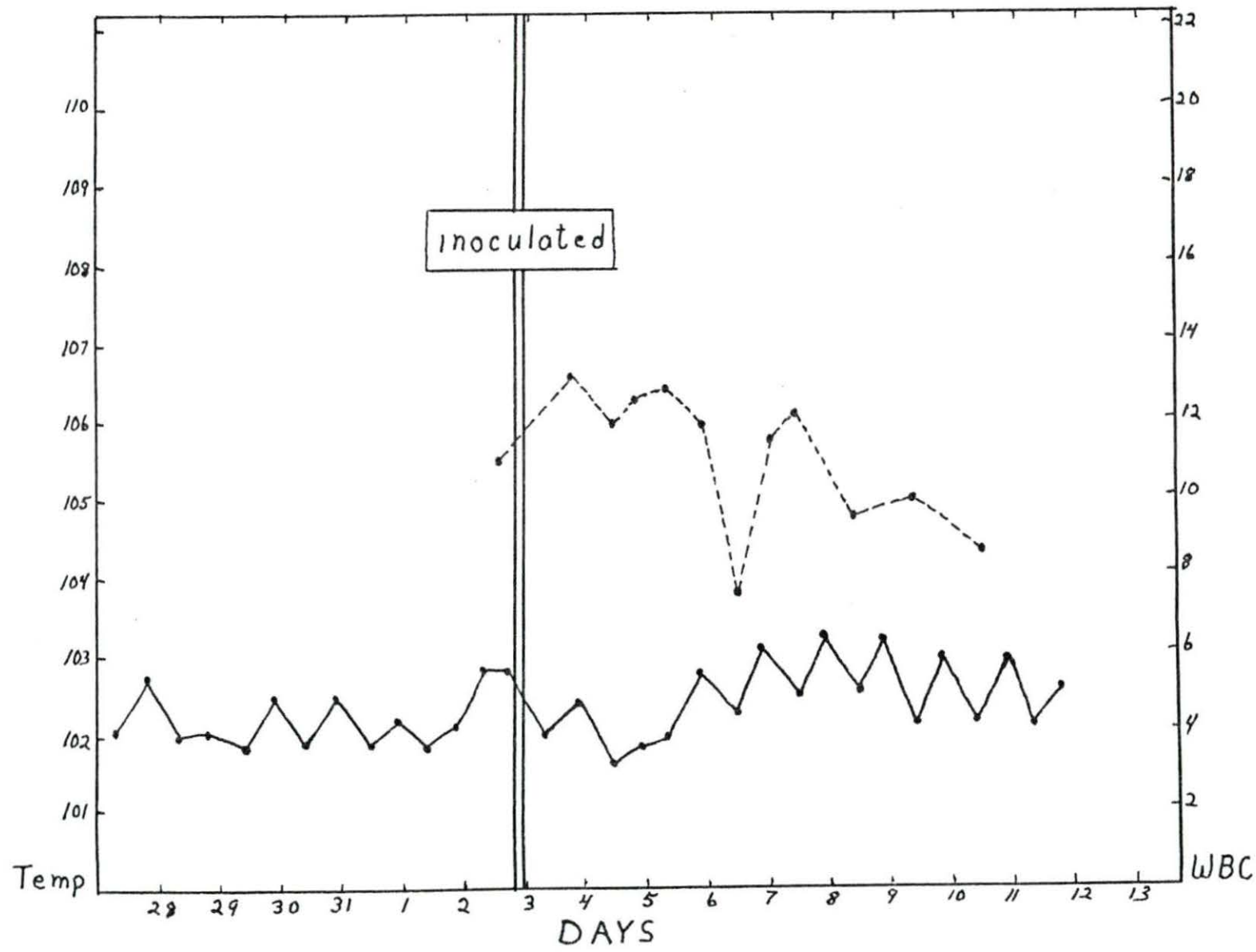


Figure 16. Clinical data on calf no. 163. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



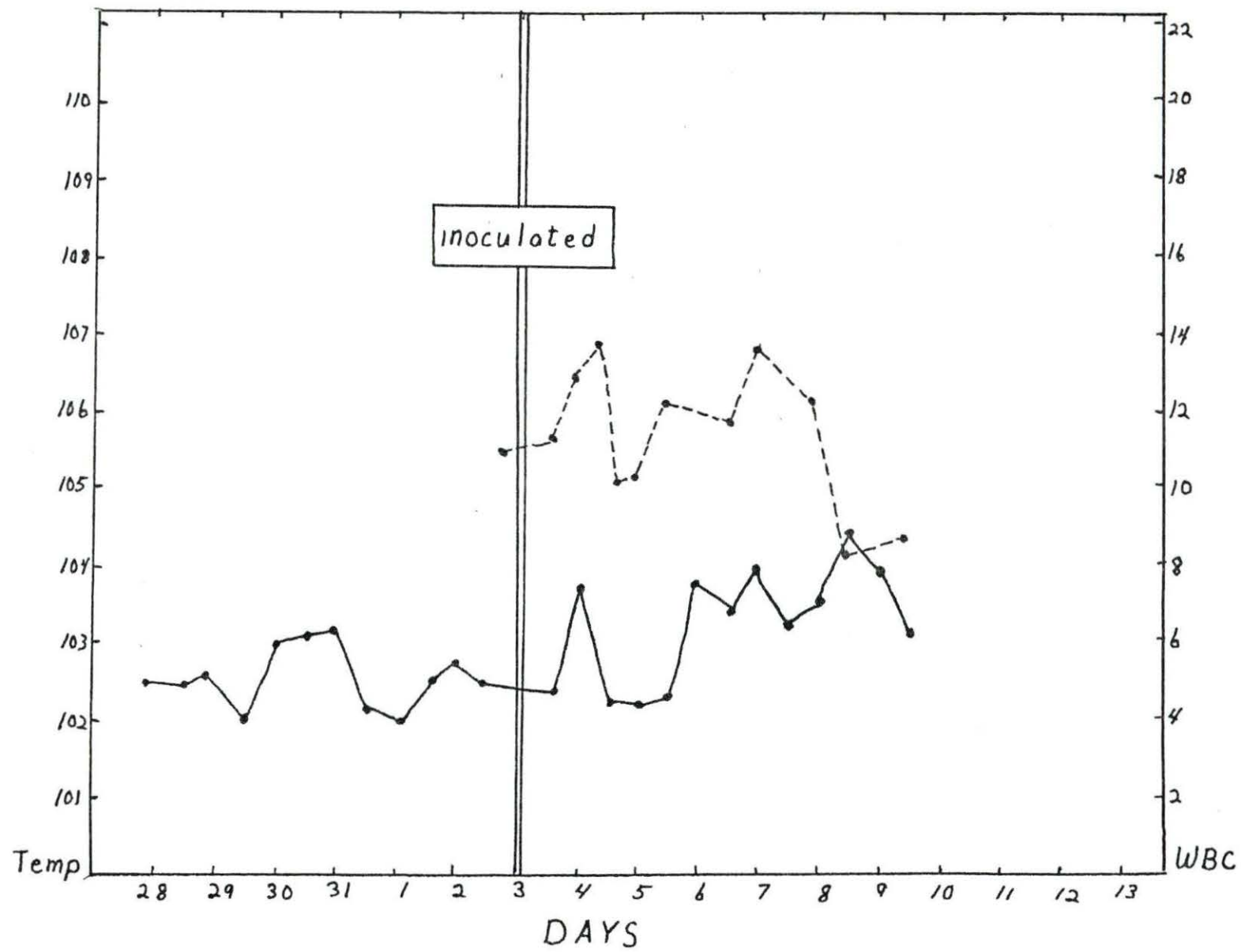


Figure 17. Clinical data on calf no. 933. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.

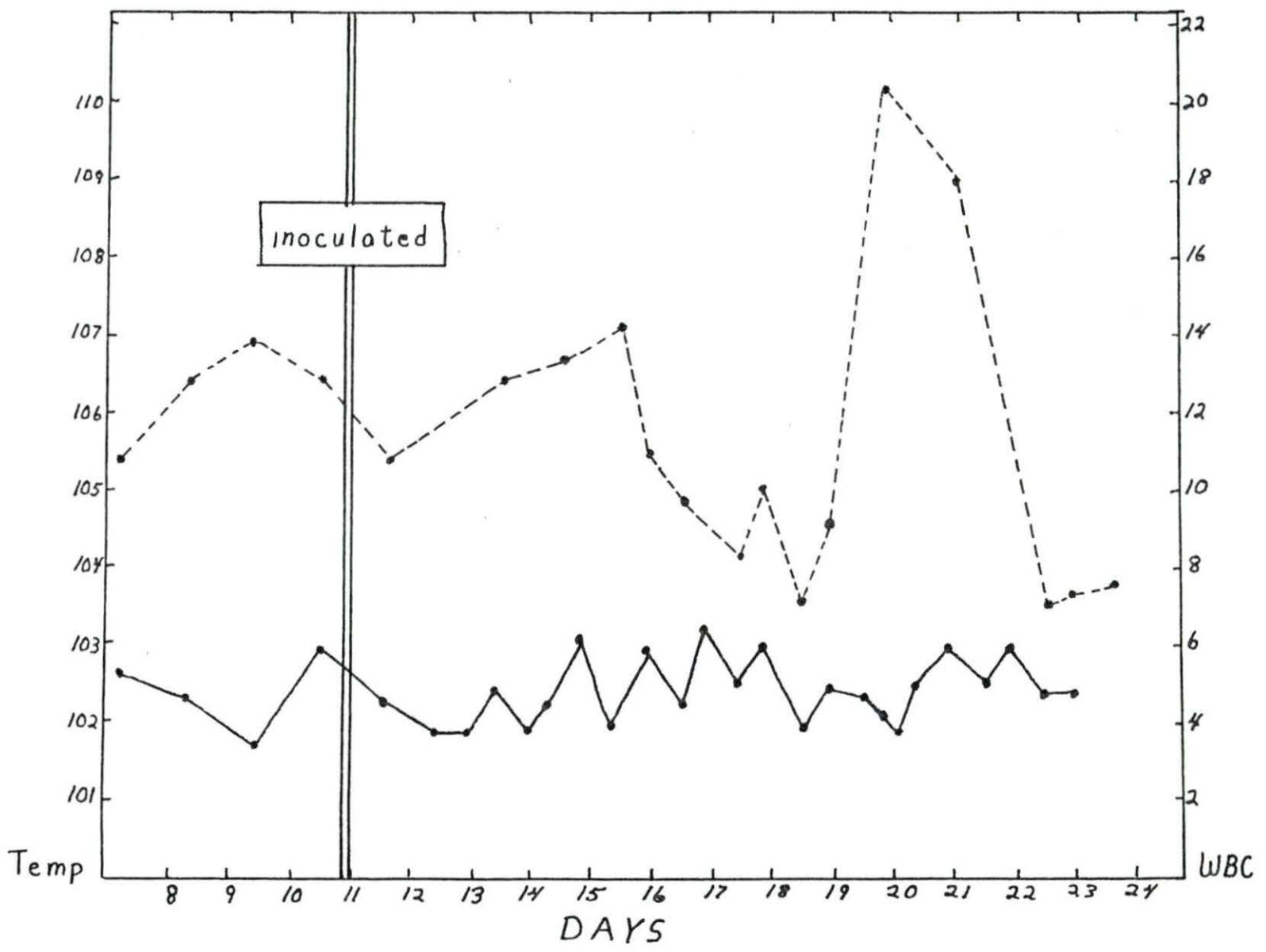


Figure 18. Clinical data on calf no. 935. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



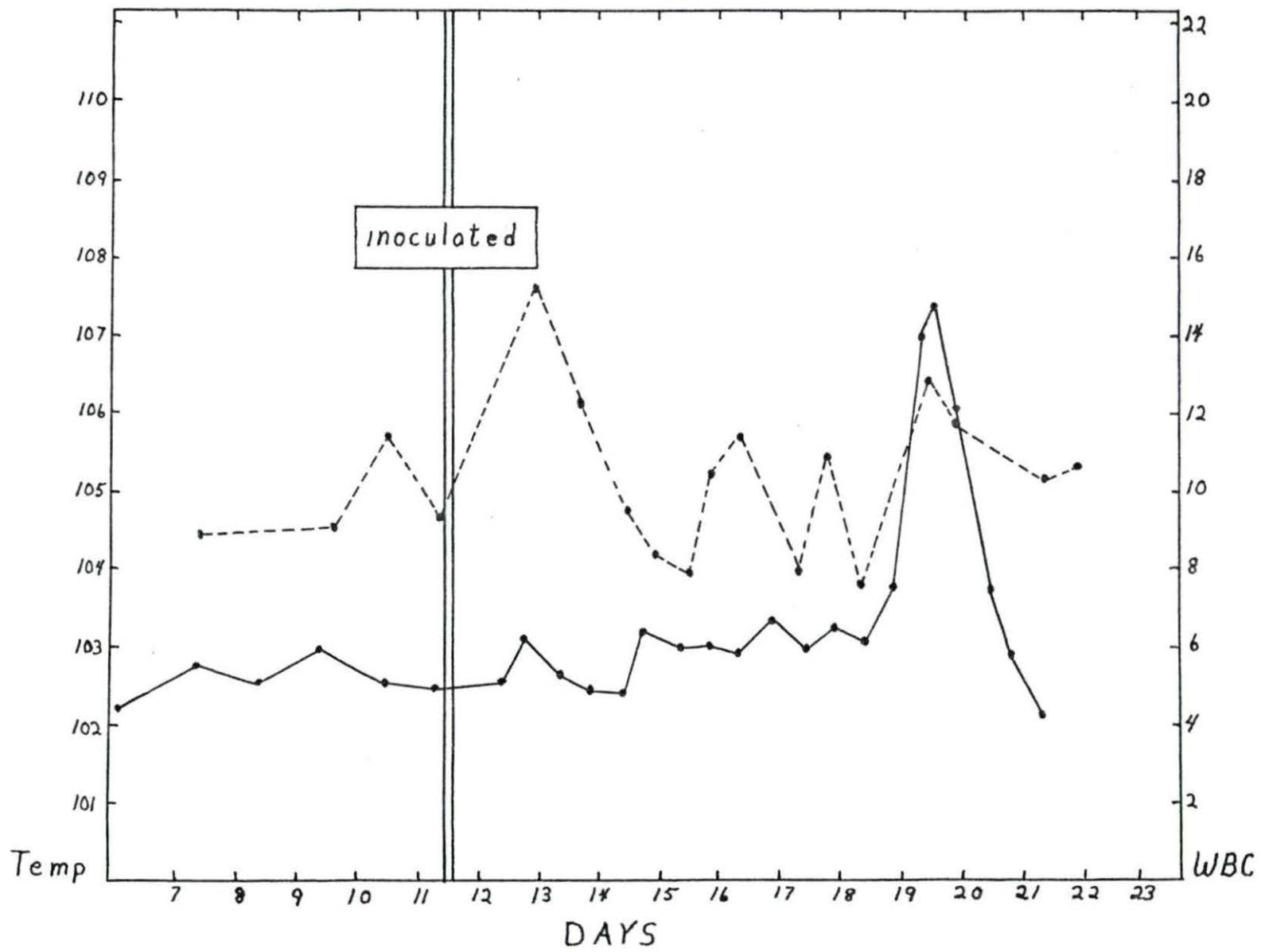
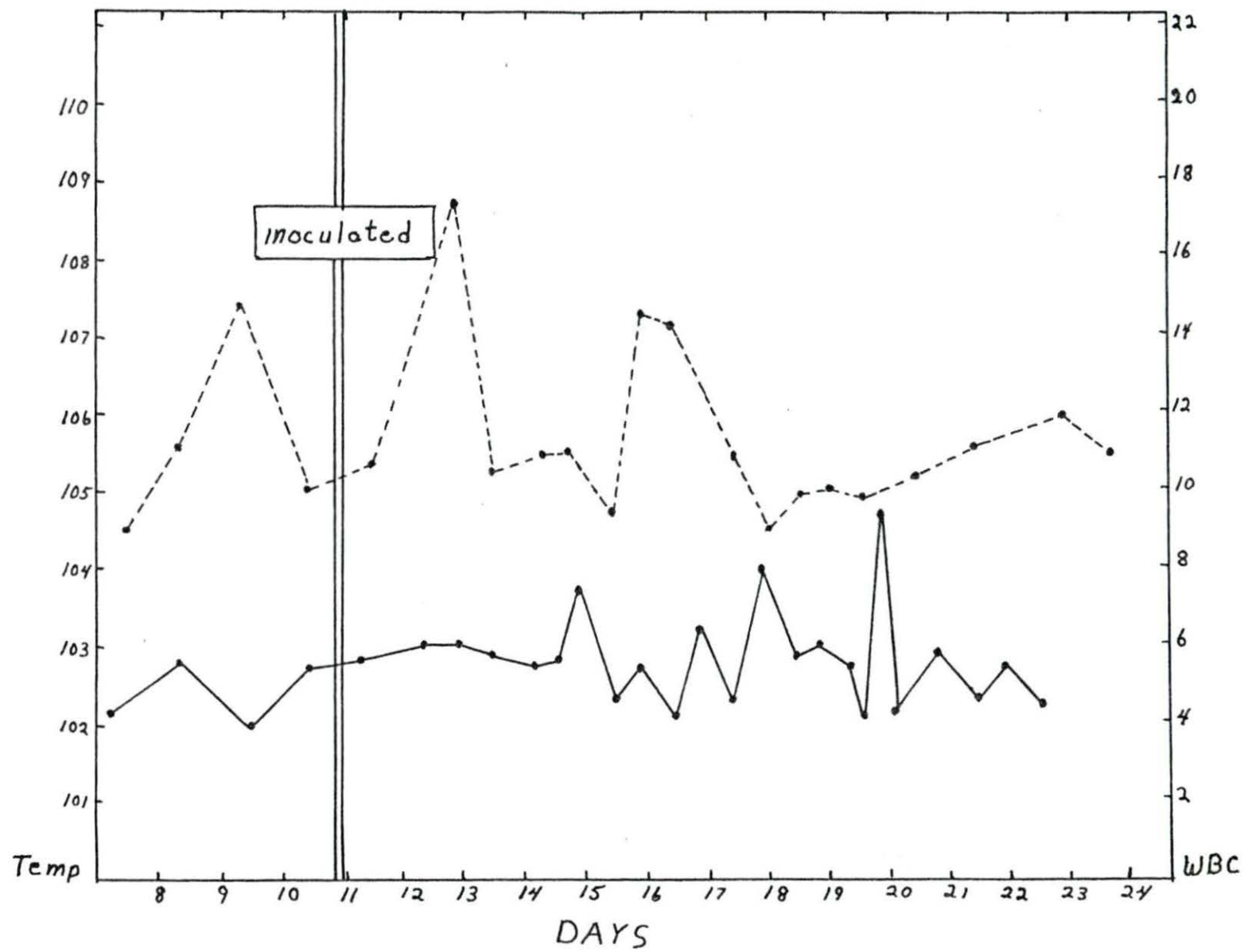


Figure 19. Clinical data on calf no. 937. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



and one small necrotic ulcer were present on the tongue. The omasum contained one small superficial necrotic focus but there were intact basal layers of the epithelium. The esophagus contained one necrotic ulcer filled with caseous debris well infiltrated with leukocytes. In the small intestine there was severe edema of the submucosa with numerous eosinophils being present in the lamina propria and even in the glandular lumina. Severe loss of lymphocytes in all nodules of Peyer's patches was existent and all that remained in these areas were scattered macrophages, granular leukocytes, a few immature lymphocytes and nuclear debris. There were traces of fibrin present in the lymphatics of the submucosa.

Calf no. 148: In this animal there was one focus of lymphocyte and macrophage infiltration under the epithelium of the omasum. The abomasum exhibits edema of the lamina propria especially at the tips of the folds. Numerous large lymphoid nodules were present in the lamina propria and some extended to the surface with no epithelium being present. In the small intestine many lymphatics of the Peyer's patch regions were filled with fibrin clots. These lymphatics were found beneath and separating the lymphoid nodules of Peyer's patches. Necrosis of the lymphoid nodules was present with amorphous eosinophilic ground substance, a few lymphocytes, and a few pyknotic nuclei occupying the areas. In the lymph nodes there was severe necrosis of germinal centers with only a few lacy eosinophilic fibers, cellular debris and a few scattered

lymphocytes remaining. There were some foci of congestion in the cortical areas. In the trachea there were nodules of lymphoid hyperplasia found under the epithelium.

Calf no. 149: There were several small cleavages in the esophageal epithelium extending down to the basement membrane. There was necrosis and cornification of the exposed epithelial surface but no inflammation of the surrounding tissue. One small focus of epithelial necrosis and cellular infiltration existed in the omasum. In the small intestine there was a thickening of Peyer's patches with diffuse collections of lymphoid tissue but few distinct nodules were present. Numerous eosinophils occurred in the lymphoid tissue. The lymph nodes contained many areas in which there was necrosis of lymphocytes in the lymphoid nodules. The central regions of these nodules were filled with a few lymphocytes, eosinophilic debris and acellular collagenous material. There was edema of the connective tissue at the hilus of these nodes especially of the mesenteric nodes. The spleen appeared normal although the lymphoid nodules were not as distinct and as large as usual.

Calf no. 153: There were fissures in the epithelium of the esophagus. These extended to the basement membrane and necrotic cells were present in the epithelial lesion. The surrounding tissue did not contain an inflammatory reaction. The abomasum contained many lymphoid nodules which were especially prominent although within normal limits.

Calf no. 155: There was a necrotic ulcer in the esophageal epithelium with no associated inflammatory reaction. The abomasum contained mild edema of the lamina propria and submucosa. There were lymphoid nodules present which extended to the surface of the epithelium. The small intestine had increased lymphoid tissue with much of the tissue severely depleted of lymphocytes. There was one large cystic submucosal gland filled with necrotic debris, cells and mucus. The lymphoid nodules of the lymph nodes exhibited moderate lymphoid depletion with some amorphous eosinophilic debris remaining. There was hemorrhage in the tissue around some of the nodules.

Calf no. 163: In this animal there was one area of necrosis of the esophageal epithelium down to the basement membrane. There was no inflammation but there was cornification of the exposed epithelial surfaces. The omasum contained one small area of superficial necrosis and leukocytic infiltration. The abomasum contained edema of the folds of the fundic and pyloric regions.

The lymphoid nodules in Peyer's patches were extremely enlarged and could be seen grossly on the slides. There was also severe lymphatic congestion with the lymphatics separating the lymphoid nodules being filled with fluid. There was edema of the lamina propria at the tips of the villi. The colon contained hyperplasia of the submucosal lymphoid tissue in the regions of the submucosal glands and in the lamina

propria.

The lymph nodes contained many lymphoid nodules in which there was severe lymphoid necrosis. There was perinodular hemorrhage of several of the lymph nodes and lymphatic congestion of the lymphatics in the medullary region of the mesenteric lymph nodes.

Calf no. 935: There was edema of the abomasal lamina propria especially in the folds of the mucosa. The lymphatics of the small intestine between the nodules in Peyer's patches were filled with fibrin and were distended. There was severe lymphoid necrosis of the nodules in Peyer's patches with eosinophilic debris remaining along with a few nuclear fragments and a few macrophages. In the colon there was a reduction of lymphocytes in the lamina propria but many macrophages were present. Edema of the wall of the colon and hypersecretion of mucus in the submucosal glands were present. The lymph nodes contained occasional cortical hemorrhages with some loss of lymphocytes in a few of the germinal centers. Acellular eosinophilic debris remained in those germinal centers affected.

Summary of Histological Changes

There were several changes which occurred quite frequently in these experimental animals. There were numerous small lesions of the epithelium of the esophagus. These lesions contained epithelial cells in various stages of necrosis (Fig. 20) often extending to the lamina propria. There were no vascular changes associated with the lesions. A number of

calves also had similar necrotic lesions of the stratified squamous epithelium of the omasum.

In almost all cases there was edema of the lamina propria of the abomasum. This varied in severity from case to case but in all cases the edema was most severe at the tips of the abomasal folds (Figs. 21 and 22). In some animals the lymphoid nodules in the lamina propria were enlarged and more numerous than normal.

Hyperplasia of the lymphoid nodules of Peyer's patches of the small intestine was observed in some cases. Even when within normal limits the amount of lymphoid tissue was abundant. Much of the grossly visible enlargement can be attributed to severe edema of the lymphoid tissue. There was severe congestion or dilatation of the lymphatics separating the lymphoid nodules (Fig. 23). In a number of cases there was actually fibrin present in the lymphatics, indicating severe damage to the vascular wall (Figs. 24, 25 and 27). In some of these severe reactions there was lymphocytic depletion in the lymphoid nodules with immature lymphocytes, macrophages and eosinophilic debris remaining (Figs. 26 and 27).

Depletion of lymphocytes was much more evident in the lymph nodes. In many of these there were clear circular punched out areas in the centers of lymphoid nodules (Figs. 29, 30, 31, 32 and 33). On close examination the centers contained a few fragments of cellular debris, a background of pale amorphous eosinophilic material and a few collagenous

Figure 20. Necrosis of esophageal epithelium. X 256.
Calf no. 138.

Figure 21. Mucosa of the abomasum illustrating the edema
of the lamina propria. X 100.
Calf no. 163

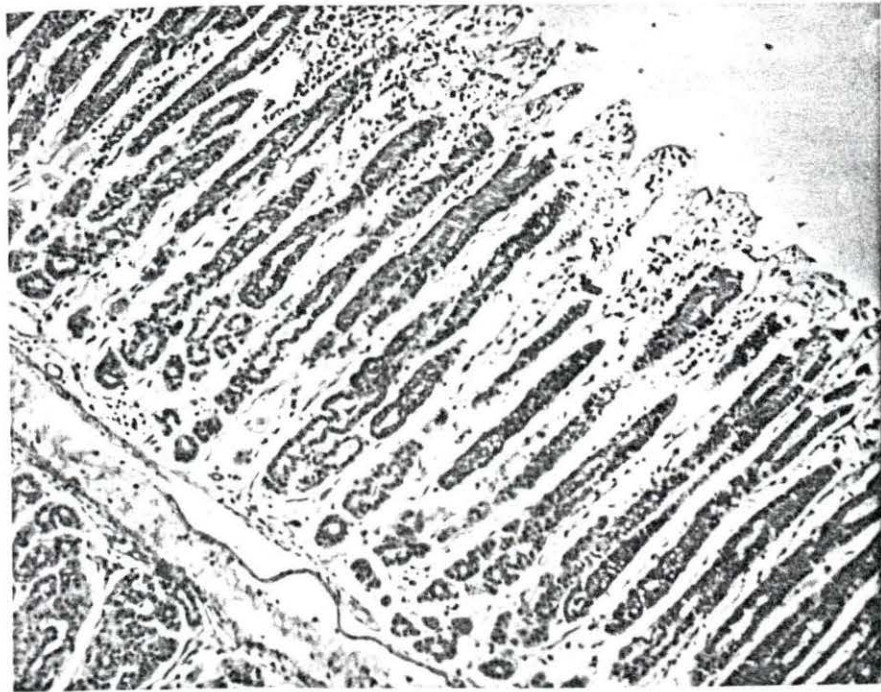


Figure 22. Severe edema at the tips of the folds of the abomasum. X 100.
Calf no. 163

Figure 23. Hyperplasia of the lymphoid nodules of Peyer's patches. Note the dilatation of the lymphatics between individual nodules. X 40.
Calf no. 163



Figure 24. Fibrin in the lymphatics of the Peyer's patch
region. X 220.
Calf no. 935

Figure 25. Another example of fibrin in the lymphatics
of the wall of the ileum. X 220.
Calf no. 148

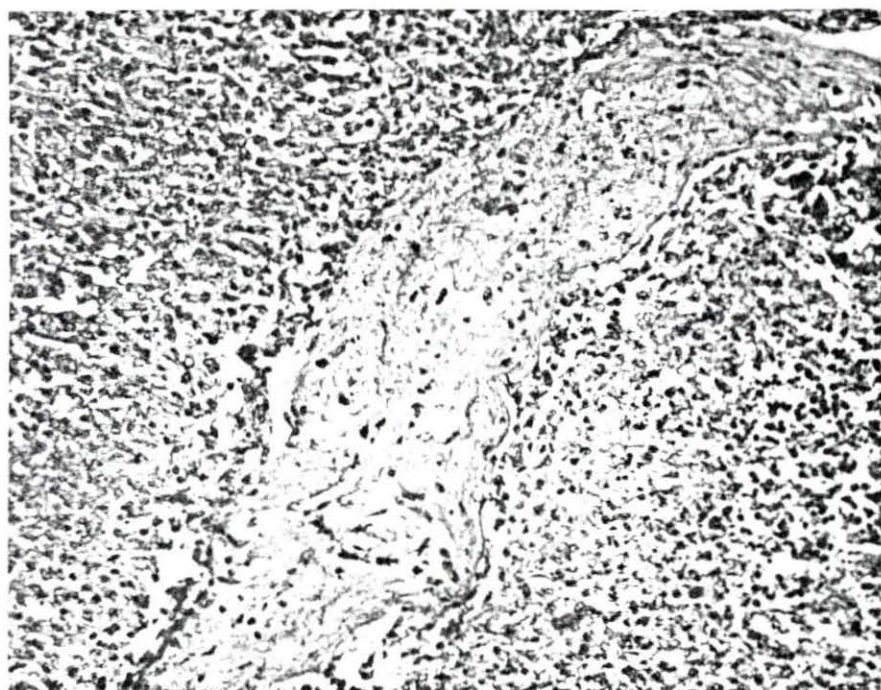
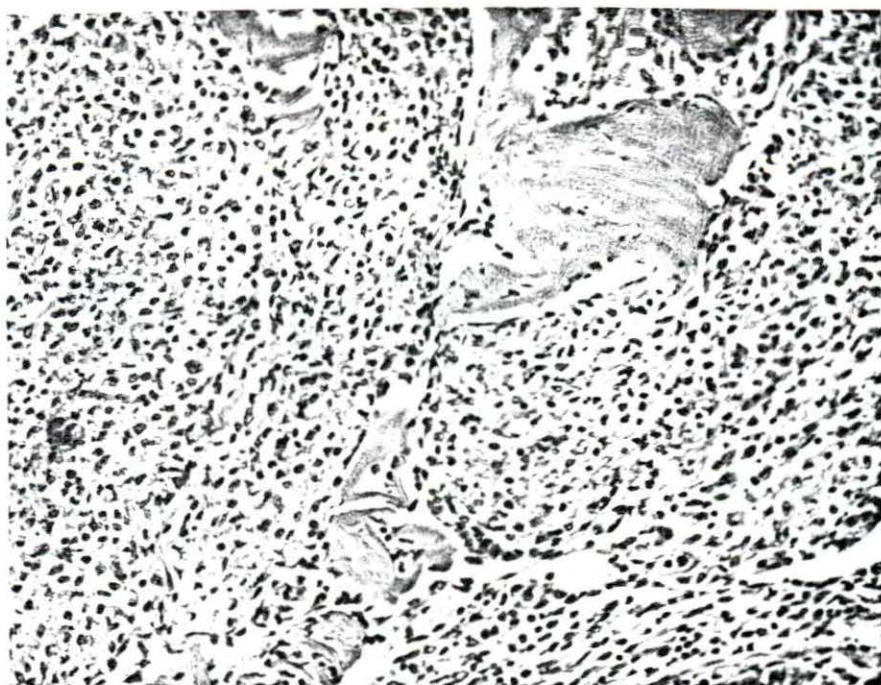


Figure 26. Depletion of Lymphocytes in a lymphoid nodule of Peyer's patch. Note the central core of debris. This is eosinophilic with hematoxylin and eosin staining. X 256.
Calf no. 935

Figure 27. Another example of lymphoid depletion in the Peyer's patch region. Note the gray acellular background material which is pink with hematoxylin and eosin stain. X 220.
Calf no. 148

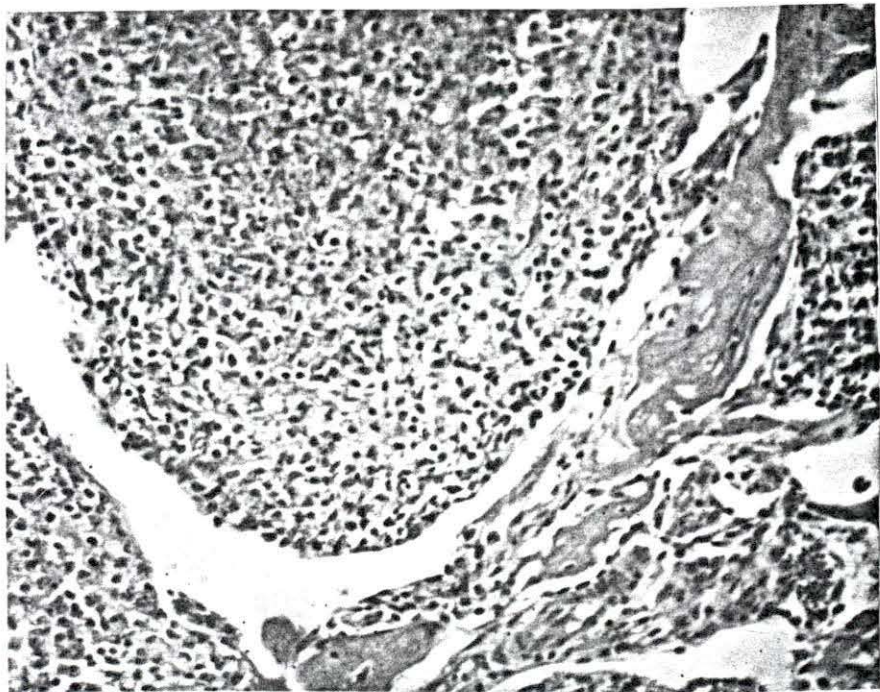
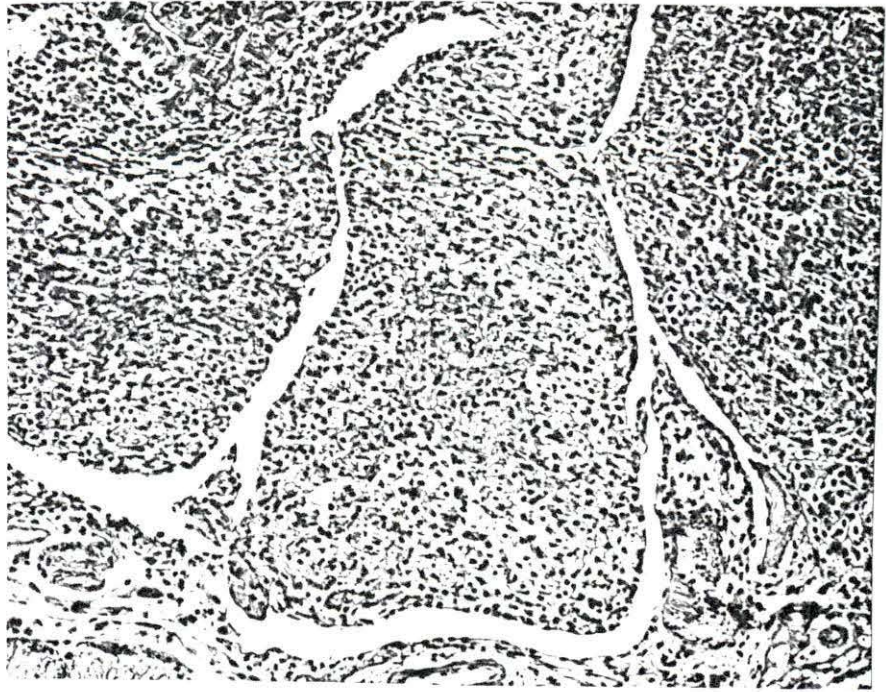


Figure 28. Hypersecretion of mucus in the submucosal glands of colon. X 165.
Calf no. 935

Figure 29. Necrosis of centers of lymphoid nodules in a lymph node. A few lymphocytes and acellular material remains. X 100.
Calf no. 148

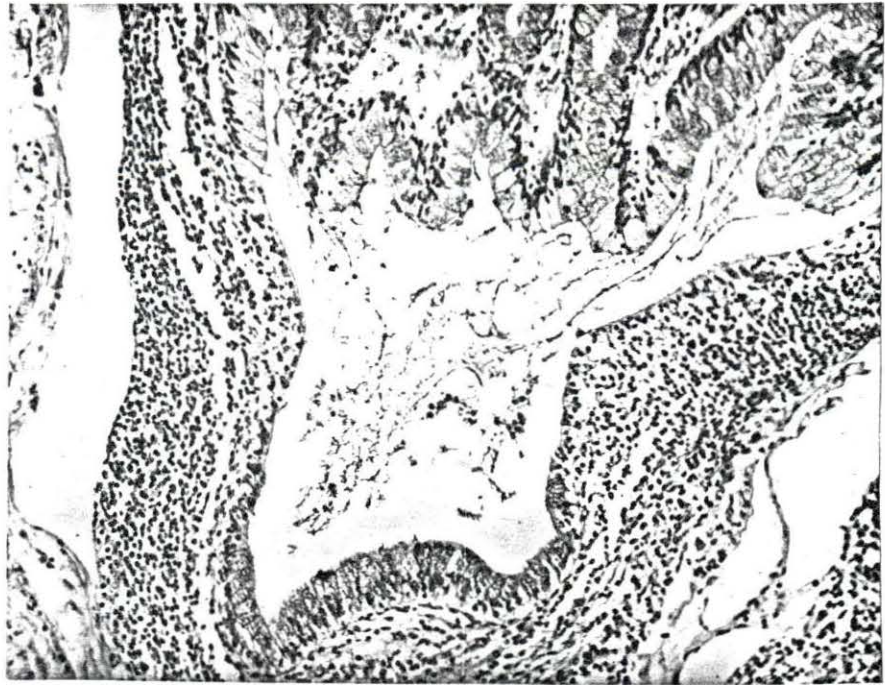
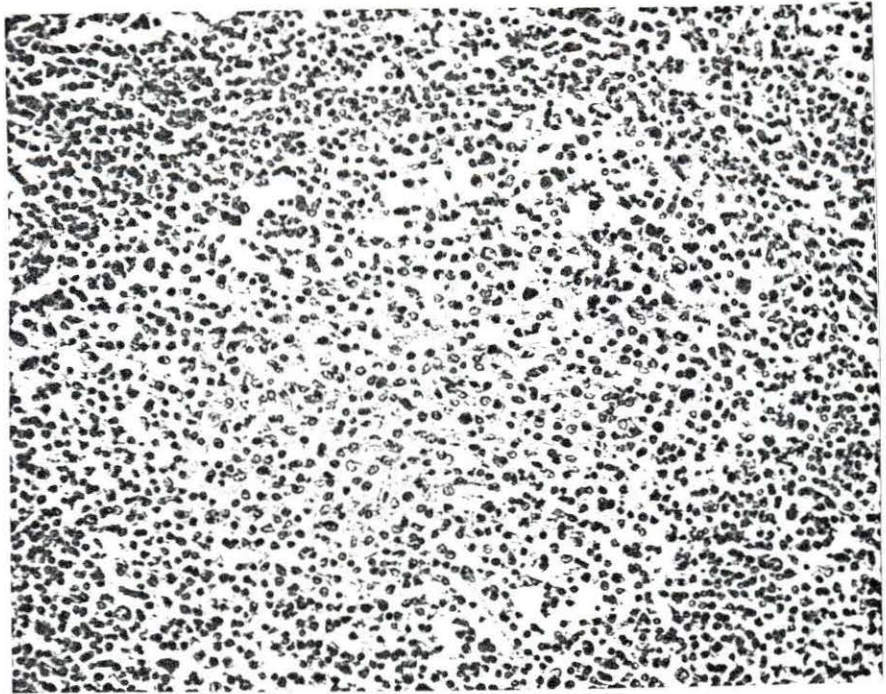


Figure 30. Moderate loss of lymphocytes in a lymphoid nodule. The normal reticular network is visible in the background. X 256.
Calf no. 155

Figure 31. More severe lymphoid depletion in a lymph node. X 256.
Calf no. 148

sible



node.

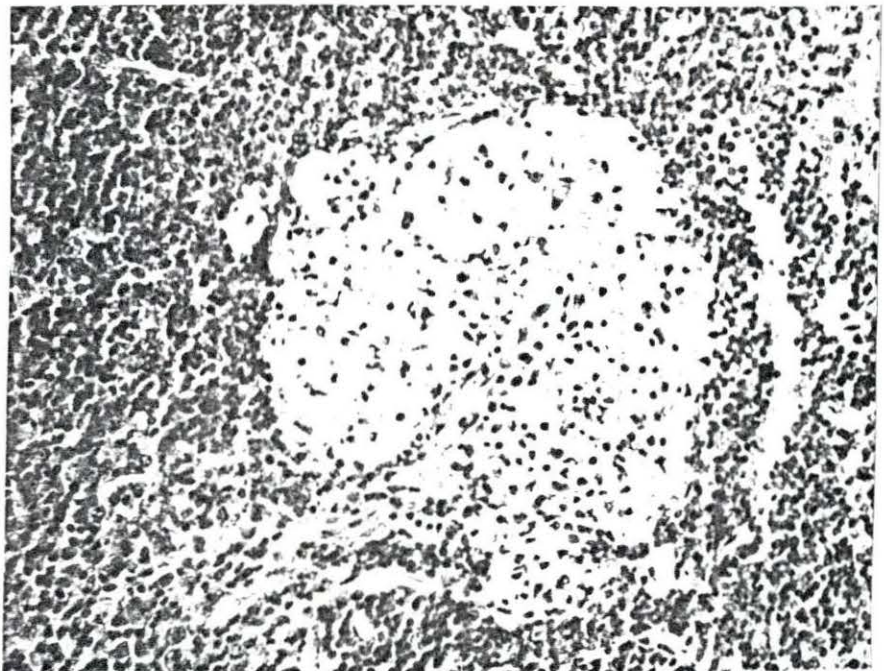
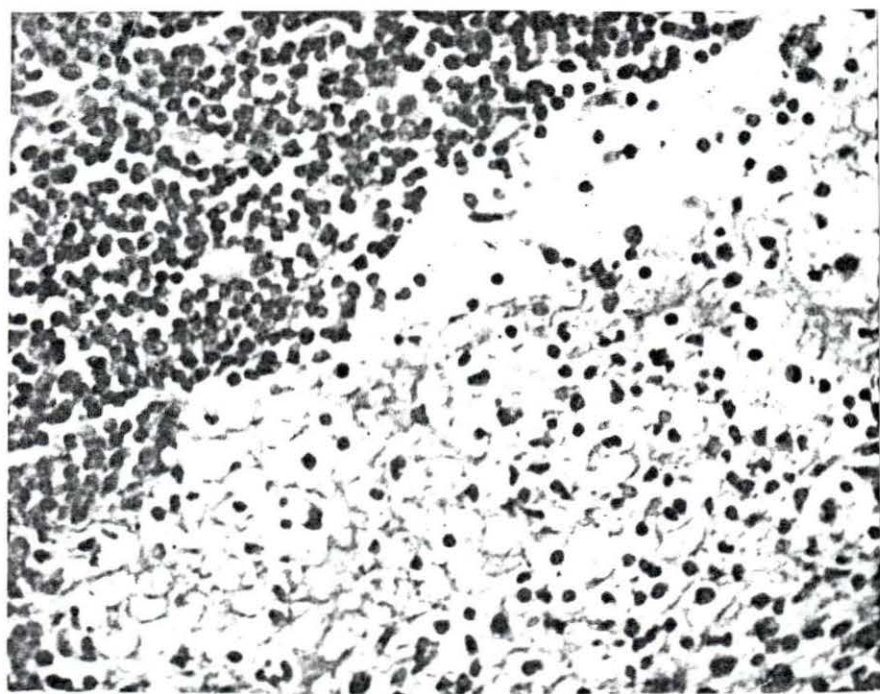


Figure 32. Severe lymphoid necrosis in a lymph node.
X 230.
Calf no. 148

Figure 33. A higher magnification of a portion of figure
32. Note the fibers in the background. X 460.
Calf no. 148



fibers. Presumably, there had been necrosis of the lymphocytes occupying this central portion of the lymphoid nodules and the normal ground substance plus degenerative tissue remained behind. The colon was not as severely affected although there was some edema and moderate lymphoid hyperplasia. In two cases there was hypersecretion of mucus in the submucosal glands (Figure 28).

Summary of Tissue Culture Work

An extensive attempt was made to grow the agent in primary bovine kidney cells. These cells were grown until a confluent sheet of cells formed in test tubes. A medium change was made and groups of 6 tubes were inoculated with 0.2 ml. and 0.4 ml. of infective material. The infective material was prepared in the same way as that used for the calf inoculations. The tubes were observed for 7 to 10 days and a blind passage was made at 6 to 7 days. At least three passages were made before discarding the material. The infective material was obtained from calves 135, 136 and 139. The inoculum from each of these animals consisted of individual samples of defibrinated blood, ground kidney, ground liver, ground spleen, ground abomasum, ground intestine and ground lymph node as well as feces. These tissues were used separately and as a mixture of all of them. Identical tissues from these calves prepared on the same day were inoculated into calves and found to be infective.

In order to determine if the tissue culture medium had any

influence on the ability to grow the agent in tissue culture a mixture of tissues from calf no. 136 was inoculated into tubes of primary porcine kidney cells maintained on the following maintenance media using media 199 of Morgan, Morton and Parker (1950).

1. 199 plus 10% lamb serum.
2. 199 plus 20% lamb serum.
3. 199 plus 10% bovine serum.
4. 199 plus 20% bovine serum.
5. 199 plus no serum.

These were negative for cytopathic effect after 3 passages. The same material was inoculated on guinea pig primary kidney cultures maintained on 199 plus 10% lamb serum.

Liver, kidney, lymph node and mixed tissues from calf no. 139 were checked individually on porcine kidney with 10% lamb serum. Three blind passages were made at 6 to 7 day intervals and the final tissue culture fluid was inoculated into a calf. The calf remained normal and was later proven to be susceptible to the Sanders agent by challenge.

Defibrinated blood from calf 147 was inoculated on 2 day old porcine kidney cells. These cells were just beginning to grow. No cytopathic effects were noted after two passages and the cells grew at a normal rate.

All of these attempts to produce a cytopathic effect in tissue culture with the Sanders agent were negative. The one trial in which 3 blind passages were made failed to maintain

the agent in detectable levels.

In addition to the attempts at growing the Sanders agent in tissue culture, tissues from 19 field cases of mucosal disease and 2 cases of nonspecific feed lot enteritis were inoculated on various tissue culture systems. Pleuropneumonia-like organisms were recovered from mixed ground tissues of 3 field cases of mucosal disease. These agents had a cytopathic effect on bovine kidney cells and one was carried in tissue culture for 5 passages on bovine kidney cells. These organisms would pass a Selas O2 filter. Pleuropneumonia-like organisms were cultured from the filtered tissue culture material and suspensions of the organism produced the same cytopathic effect as the original inoculum. The pleuropneumonia-like organisms were not studied in any more detail.

Filterable cytopathic agents presumed to be viral were recovered from nasal swabs of two animals with mucosal disease. These animals came from separate herds but were housed in the same pen while in the Iowa State University Clinic. Therefore the agents may have originated in one animal and spread to the other by pen contact. Both of these agents produced an intense vacuolization of the cytoplasm of serial passage bovine kidney cells. One agent was studied in detail and was carried through 12 serial passages in tissue culture. It was found to have the following characteristics.

1. It would serially pass in bovine kidney cell lines with cytopathic effect.

2. It did not produce visible effect on bovine conjunctiva cell lines or swine kidney cell lines.
3. It would pass a Selas O2 filter.
4. It did not grow on PPLO media or blood agar.
5. It withstood treatment with penicillin and streptomycin.
6. It did not produce visible effects on chicken embryos inoculated by various routes.
7. It did not produce clinical signs of disease in guinea pigs inoculated by intraperitoneal, intramuscular and intranasal routes.
8. It withstood freezing in sealed glass vials for 6 weeks at minus 45 degrees F.
9. It produced a clinical reaction in two calves. Calves 129 and 117 were inoculated with tissue culture media and in both cases there was a definite temperature rise associated with a slight drop in the white cell count (Figs. 34 and 35). The animals were depressed during the febrile period but returned to normal with the return of the temperature to normal. At this time the bovine kidney cell line was lost and it was impossible to grow the agent in any other cell system. The relationship of this agent to the Sanders agent and to mucosal disease is unknown. The clinical reaction in calves is similar to that seen in some of the calves inoculated with the Sanders agent but this is not a very specific

Figure 34. Clinical data on calf no. 129. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.

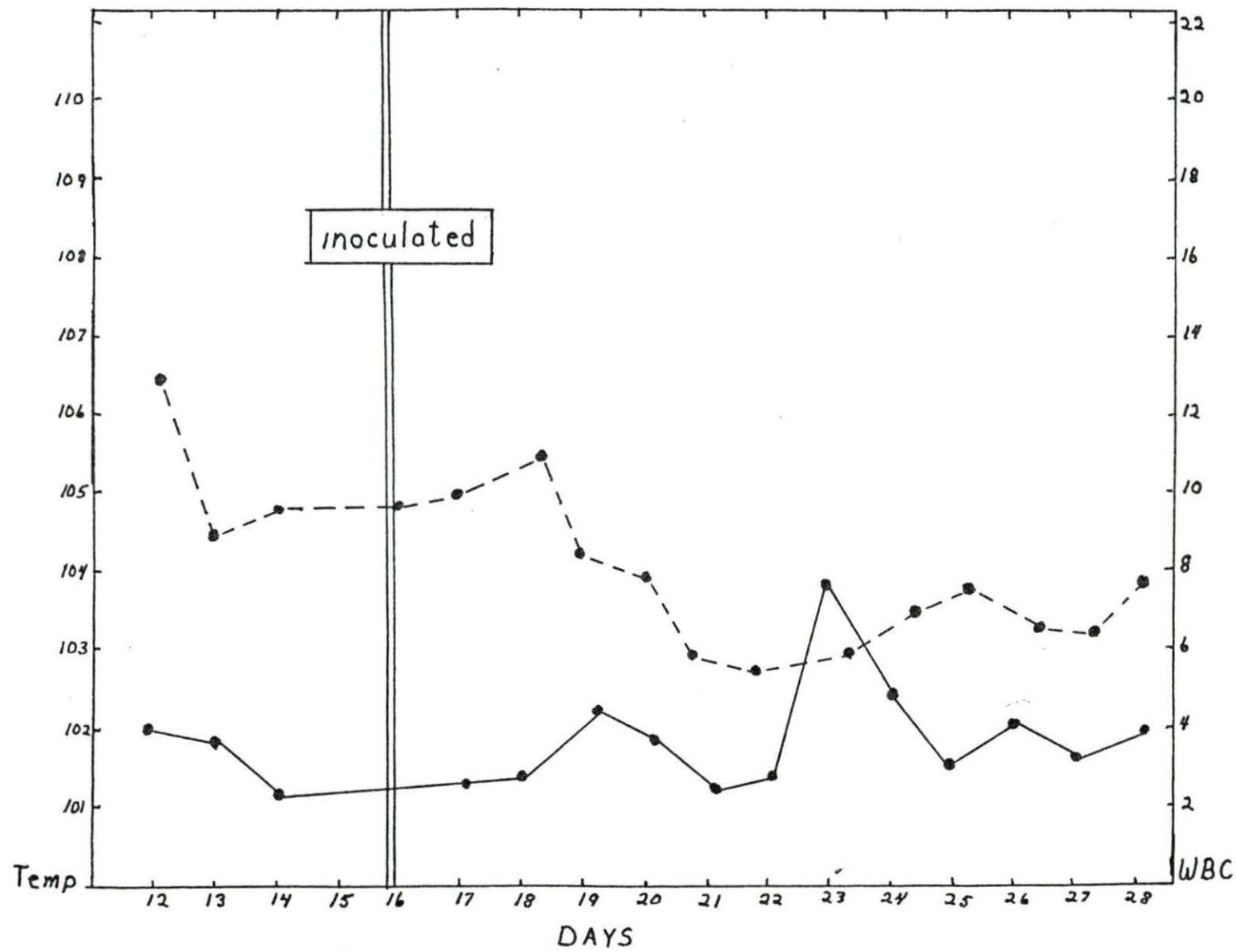
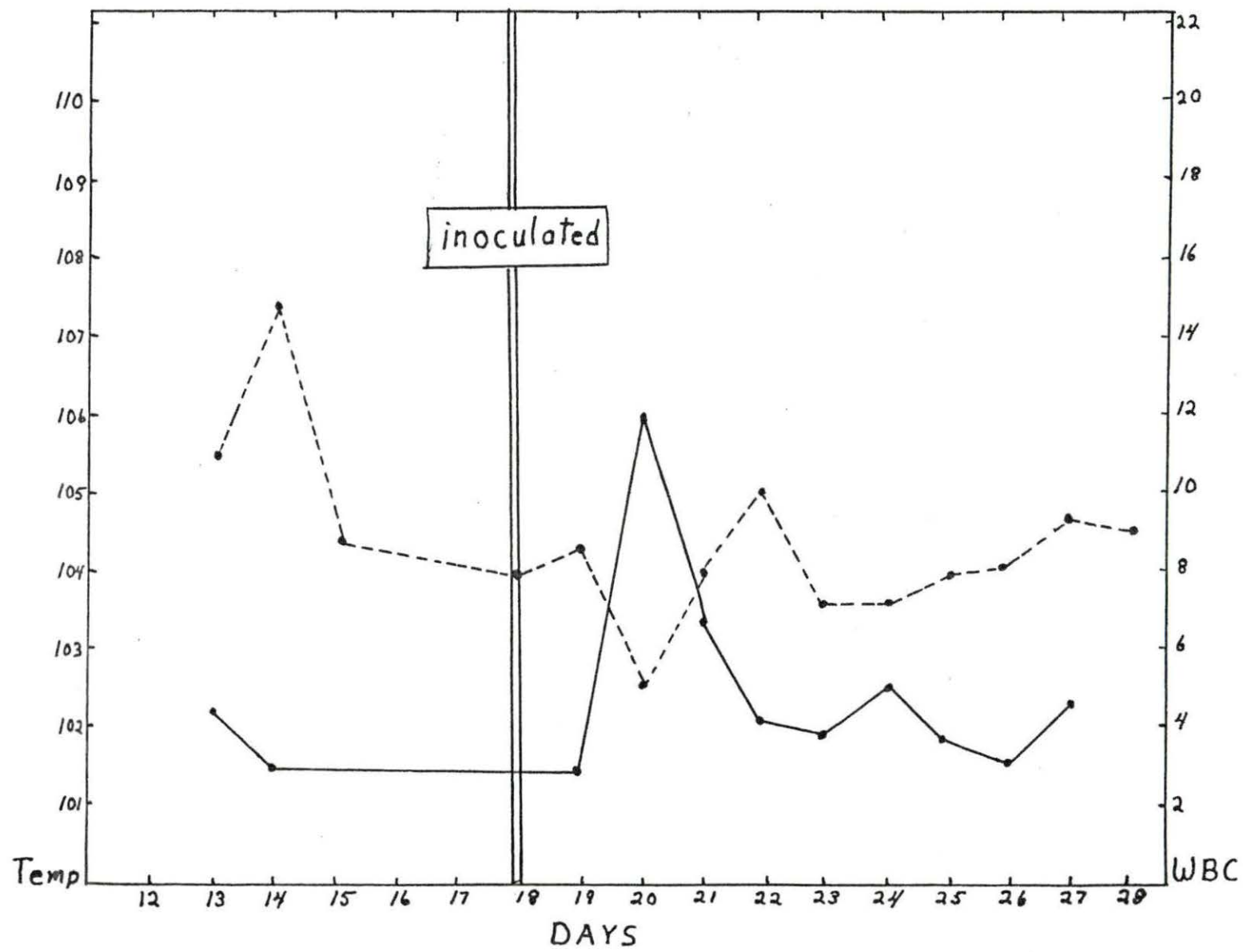


Figure 35. Clinical Data on Calf no. 117. The dotted line represents leukocytes in thousands per cmm. The solid line represents body temperature.



comparison.

Summary of Rabbit Inoculations

A total of six adult rabbits were inoculated with defibrinated blood from calf no. 138. One rabbit received 1.5 ml. into the ear vein, two rabbits received 3 ml. intraperitoneally, and three rabbits were inoculated by injection of 1 ml. into the wall of the sacculus rotundus and 3 ml. into the lumen of the sacculus rotundus. The animals were observed for one week with daily blood counts being taken. No unusual reactions were observed. All rabbits were necropsied after one week and histologic examination was made of the sacculus rotundus. There were no gross or microscopic changes. All of the injected material had disappeared from the wall of the sacculus rotundus.

DISCUSSION

In analyzing the clinical reaction of this group of calves the most obvious and the most important conclusion is that it was variable and mild. There are many possible reasons for the variation observed but there is no way of being certain of which is the case. There may have been a difference in natural or acquired immunity in the various groups of animals. Predisposing factors of the environment or infectivity of the agent could play a part in the severity of the infection. It is also possible that the clinical reaction did not vary as much as it appeared. In all cases the febrile period was of short duration and may have been missed because of insufficient observation.

The difficulty of detecting a clinical reaction places some severe limitations on the possibility of conducting immunologic studies with this agent. At the present time the only indicating system for the presence of viable Sanders agent is the living calf. Unless a group of calves is located which will respond to this agent in a definite and predictable manner this is not a very practical test for the presence of the agent.

There were some difficulties in connection with this experiment arising out of the indefinite clinical reaction. It was difficult to determine when to conduct a post mortem examination and if it was even necessary to do so. Because of economic reasons, some of the first calves inoculated were not

examined when there was no definite clinical reaction. At a later date some calves with a mild clinical reaction were examined and found to have lesions. This suggests that there may have been lesions present in the first animals also. The result is that some valuable information may have been missed. If this condition existed in the field as a naturally occurring disease it would probably be missed in its present form. None of the animals were sick enough to be noticed by casual observation under farm conditions. However, these animals may have displayed more severe symptoms had they been exposed to the more severe environmental stresses found on the farm.

In contrast to the clinical picture the post mortem observations were definite and followed a distinct pattern as summarized earlier. The most constant and striking change involved the lymphoid tissue and especially the Peyer's patches. In some cases the changes were proliferative and in some cases degenerative. These variations are probably a part of a sequence of events which was interrupted at different periods of time. The degenerative and necrotic lesions of the lymphoid tissue are strikingly similar to those described by Trapp (1960) in field cases of mucosal disease. It is also interesting to note that Carlson et al. (1957) found enlargement of Peyer's patches and the mesenteric lymph nodes with lymphoid exhaustion of these nodes in acutely affected animals. Also Maurer et al. (1955) found similar changes in the lymphoid tissues of animals affected with rinderpest. They

observed severe depletion of lymphocytes with a fibrillar, eosinophilic acellular matrix remaining in the lymphoid nodules. This is a change which they felt was degenerative rather than inflammatory.

In both rinderpest and mucosal disease the changes of lymphoid tissue have been found in all lymphoid areas of the body although it is most severe in those areas associated with the digestive tract. In infection by the Sanders agent the degeneration has not been observed in other than the Peyer's patches and the lymph nodes. In particular it has not been observed in the spleen although the Malpighian corpuscles were not especially prominent. There could easily have been mild lymphoid depletion but there were no distinct centers of degeneration. These observations do not establish a connection between these diseases but the same reaction occurs to a lesser degree in Sanders infection.

Lymphoid necrosis and proliferation of a mild degree occur in other diseases. Conway (1937) thought that a cycle of nodular proliferation and exhaustion or decay are part of the normal cycle of events in the lymph node in response to many irritating agents. The author has observed similar focal necrosis of lymphoid nodules in the early stages of canine distemper. However, the reaction in response to the Sanders agent is more extensive than usually observed in domestic animals and certainly the severe damage to lymphoid tissue in mucosal disease and rinderpest is far beyond the usual cycle of

lymphoid response.

The digestive tract lesions are not nearly as extensive as those seen in field cases of mucosal disease or those reported by Carlson et al. (1957) in experimental virus diarrhea. No lesions of the muzzle or oral regions were observed. There were numerous linear ulcers of the esophagus but these were not nearly as extensive as those seen in field cases of mucosal disease. They did extend all of the way to the basement membrane of the epithelium and were degenerative and necrotic in nature. The ulceration of esophageal epithelium in mucosal disease is the result of a degenerative lesion.

The rest of the intestinal glands were primarily affected with edema and some cystic hyperplasia of the submucous glands. The edema was most severe in the ileum in the region of Peyer's patches. Carlson et al. (1957) found similar intestinal edema in their calves which were infected with Indiana viral diarrhea agent. The small spots on the surface of the abomasum which appeared as ulcers turned out to be small nodules of proliferating lymphoid tissue which projected to the surface of the epithelium. Because these enlarged nodules could be seen grossly as small white spots they appeared as ulcers. Microscopic examination did not reveal any ulcers associated with this lymphoid tissue.

The presence of large amounts of fluid in all serous cavities was interesting. Associated with this and probably related to it was the lymphatic congestion in the lymphatics

around the lymphoid nodules of Peyer's patches. Often these channels were filled with fibrin and fibrin was occasionally found in the peritoneal cavity. This collection of fluid is not observed in mucosal disease but it should be noted that by the time most mucosal disease animals are examined there is severe dehydration as a result of the diarrhea. The presence of fluid in all serous cavities is similar to the lesions seen in other species as a result of PPLO infection. This is especially true in relation to the excessive joint fluid. Because of this observation an attempt was made to recover PPLO from the fluid. These trials were not successful. However, one cannot draw the conclusion that PPLO were not present from this for the culture methods may not have been adequate.

Another interesting observation in connection with the findings of Carlson et al. (1957) in virus diarrhea is that virus diarrhea experimental infection can become chronic but this does not seem to be so in Sanders infection. Diarrhea never lasted for more than 2 to 3 days in any of this series of calves, while infection with virus diarrhea resulted in diarrhea being prolonged for weeks in some cases.

This study has shown some interesting similarities between Sanders infection and mucosal disease. They tend to involve the same general tissues of the body but one is a very severe disease with dramatic changes and the other condition is a mild disease. This study does not prove that there is any connection between infection by the Sanders agent and

mucosal disease but it does not rule out the possibility and at least suggests that they could be two different forms of the same disease. If this were so the experimental condition must lack some factor which is necessary for the production of a field case of mucosal disease. It could very easily be possible that only a small percentage of the animals react in the manner as seen in mucosal disease and that the Sanders agent is widespread in the cattle population. This mild infection could go unnoticed and provide immunity for the recovering animals. This would explain in part the epidemiology of mucosal disease as seen in Iowa. However, all of this is conjecture and in no way is there any proof of such a situation.

Efforts must be made to develop serological techniques for the comparison of this whole group of intestinal viral diseases. If an experimental animal could be found to grow this agent and develop a distinct disease the problem would be simplified. Attempts at adaptation of this and similar agents to laboratory animals should be made.

SUMMARY

1. A group of 18 calves were inoculated with the Sanders agent, a filterable agent isolated from a field case of mucosal disease.
2. Nine of these animals developed a clinical reaction characterized by varying degrees of leucopenia, fever, depression, anorexia and abdominal pain.
3. Ten animals were necropsied and 8 of them were found to have grossly visible lesions. The major gross lesions were esophageal ulcers, abomasal edema, edema and hyperplasia of Peyer's patches, catarrhal enteritis, edema of the ileum, edema of the colon, edema of lymph nodes, and the presence of fluid plus fibrin in all serous cavities.
4. Tissues from 8 animals were examined microscopically. The findings substantiated the gross observations. In addition there was lymphoid depletion and necrosis of lymphoid nodules of Peyer's patches and many lymph nodes. Fibrin was observed filling many lymphatics of the wall of the ileum.
5. These lesions resemble mucosal disease in many ways. The same tissues are affected in both diseases. However, this similarity only suggests and does not prove a relationship between mucosal disease and the Sanders agent infection. Mucosal disease is a severe disease but Sanders infection remains mild and of brief duration.
6. An extensive attempt was made to adapt the Sanders agent

to tissue cultures using several cell types and several media. This was not successful.

7. An attempt was made to isolate a viral agent from 19 field cases of mucosal disease by the use of tissue cultures. Two pleuropneumonia-like organisms plus one viral agent were isolated from these animals. The viral agent produced a clinical disease in two calves. The reaction was similar to that seen in Sanders infection. The relationship of these two agents is unknown.
8. Six rabbits were inoculated with Sanders agent in an attempt to produce disease. The rabbits did not develop clinical illness or apparent gross lesions.

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