EVALUATION OF THE INTRAVENOUS TUBERCULIN TEST IN CATTLE

by

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INTRODUCTION

In bovine tuberculosis eradication, one of the serious problems that is encountered is the presence of tuberculous cattle which are anergic to the intradermal tuberculin test. These anergic cattle fail to react to the intradermal test and remain in the herd as a potential source of infection for nontuberculous cattle, new additions to the herd, and to human beings who work and care for these cattle. Therefore, a test is needed which will detect these anergic cattle and at the same time not cause false reactions in cattle infected with nonpathogenic acid-fast organisms. Such a test has been developed and field trials have been described by Larsen and Kopecky (1965).

This test is an intravenous thermal tuberculin test utilizing Agricultural Research Service (ARS) contract tuberculin. It is a modification of the standard subcutaneous tuberculin test utilized for about 40 years in the diagnosis, control and eradication of tuberculosis. Field studies reported by Larsen and Kopecky indicate that this test will detect tuberculous animals that fail to react to the intradermal tuberculin test. It did not detect all tuberculous cattle; however, in this field study, no tuberculous animals were missed by the combined use of the intradermal test and the intravenous test.

The object of this project is to expand on the field studies and provide technical guidelines for a more extensive field evaluation of the intravenous tuberculin test. Controlled studies will be done to determine whether normal cattle will react, whether cattle experimentally infected with atypical nonpathogenic mycobacteria will react, whether cattle with paratuberculosis will react and how these nonspecific reactions compare with reactions in cattle that have been experimentally infected with Mycobacterium bovis.

REVIEW OF THE LITERATURE

The first use of tuberculin for the diagnosis of tuberculosis was by Guttman in Dopart, Russia, in 1891 (Myers, 1940). In March, 1892, Pearson used subcutaneous tuberculin in Pennsylvania with very dramatic results. This test was also used as a method of checking dairies which were supplying milk to Philadelphia (anonymous, 1892). The University of Pennsylvania (1891-1892) began the first controlled studies on tuberculin in the United States, they concluded that it was a suitable diagnostic agent but possessed no curative value. For several years following the initial demonstration, there was very little written in the literature on the tuberculin test. It was accepted without any doubt. In 1895, Cooper Curtice of the United States Bureau of Animal Industry (BAI) wrote the first lengthy article on the subcutaneous tuberculin test. With some modern refinements and a change in the route of injection, much of this information still holds true for the type of test employed in this thesis.

Acceptance of the test as a perfect diagnostic tool is seen in the statement of A. D. Melvin (1907), "there is no more reliable diagnostic agent than properly prepared tuberculin in the hands of the careful observer." Other types of tests began to come into use for human beings and cattle (see Edwards and Edwards, 1960, page 2). In the 1911 BAI Report, the statement is found that "the subcutaneous injection of

ordinary old tuberculin is by far the most reliable manner in which tuberculin can be used as a diagnostic agent for cattle tuberculosis." Many other statements concerning the reliability of the statement can be found in the 13th chapter of Myers (1940) book on tuberculosis. This all adds up to the fact that the early investigator believed tuberculin to be infallible.

Moussa (Myers, 1940) first used the intradermal tuberculin test in about 1908. Montana was the first state to officially use this test in tuberculosis eradication. It was adopted by the BAI in March 1920 and approved by the United States Livestock Sanitary Association at its 24th meeting in December 1920. At the same time there were advocates of the combination test using both the subcutaneous and intradermal test (Hart and Traum, 1916-1917; Reynolds, 1918; Fretz, 1920; Ernest, 1920). Following the introduction of the intracutaneous test the use of the subcutaneous test decreased until it was practically discontinued.

The dosage of tuberculin used by the early workers in tuberculosis is not known. The standards used tell very little. Herzog (1910) claimed that a good tuberculin should kill in a minimal dose of 0.5 cc. a guinea pig infected with tuberculosis for 3-4 weeks. Schroeder and Brett (1918-1919) described the standard set by the BAI. Boerner and Barnes (1920) described their modification of this standardization test. Moore (1913) gave the dose used in the subcutaneous test

as 0.25 cc. which was diluted up to 2 cc., but he went on to say that the exact dose to give the best reaction had not been determined. Cary (1921) listed the amount of tuberculin used in large cattle as 4-6 cc. and 2-4 cc. in small or young cattle. Linch (1917) did not believe the size of the dose to be important. He claimed that at least 2 cc. must be used and had used as much as 4 to 20 cc. Wills (1916) cautioned that insufficient dosage may be the cause of some inaccuracies in tuberculin testing. But in 1918 Wills also said that increased dosage of tuberculin has no ill effects in the animal.

In recent literature, there have been very few references to the subcutaneous test in cattle. Maunder (1948) first described the short 8-hour test to be used as a supplement to the intradermal. Gregory (1949) discussed this test and compared its accuracy with the intradermal test.

The earliest report of an intravenous tuberculin (IV) test was reported by Rivera (1937). He injected 25 cattle intravenously with tuberculin. In a positive reaction, the temperature begins to rise in 1-4 hours and reaches a peak after 5-9 hours. Ten animals reacted to both the skin and the IV tests and eight were negative to both tests. In the remaining seven, three were positive to the IV test and negative to the skin test while the reverse was the case in the other four. Postmortem examinations carried out on 14 animals agreed with both tests in seven positive and five negative cases. There was disagreement in two animals. Rivera con-

cluded that the intravenous test was valuable as it enables a rapid diagnosis to be made.

Ukrainezyk-Laborie and R. Laborie in France began to use the intravenous tuberculin test experimentally in 1943 in cattle. In the summary of a paper written by these investigators in 1952, they said that the fever begins 30 minutes after injection and lasts for four hours, and that the IV test can be repeated at short intervals and produce no side effects.

In 1956, Kovats, in Hungary, ran a short study on the use of intravenous tuberculin. He found when he tested 49 skin reactors that he found only 18 animals that reacted to the IV test. He did not perform postmortem examinations so did not know if he was actually dealing with tuberculosis.

Thorpe in 1957 used intravenous tuberculin in dogs. He found that by using 0.1 ml. of a 1:10,000 tuberculin he could only come to a conclusion that this diagnostic test was unreliable in dogs.

Kutleša and Marič (1961) studied the value of the subcutaneous and intravenous tuberculin test for the purpose of distinguishing between specific and nonspecific sensitization in cattle. They found that the use of either test in connection with other diagnostic methods was a significant step toward differentiating between specific and nonspecific sensitization. In their investigations they used 4-6 ml. of original bovine PPD tuberculin in the intravenous test. In animals that were infected with the bovine type, there was a

temperature rise of from 2° F. to 4.15° F. The reaction appeared between 1 and 8 hours after infection, most frequently at 3-4 hours, and lasted only 1-2 hours. In animals that were infected with the avian type, none reacted to the IV tuberculin test. The peak temperature was 103.4° F.

Hasting, Beach, and Thompson (1930) first studied the subject of nonpathogenic Mycobacterium as the cause of tuberculin reactions in cattle. These workers inoculated a group of cattle with various acid-fast organisms isolated from no gross lesion reactors. These organisms caused a sensitization in the cattle but produced no lesions. They concluded from this that other acid-fast organisms could sensitize cattle to tuberculin.

In 1959, Runyon published the now classical classification of the anonymous or atypical Mycobacterium. Runyon set up the first useable procedure for dividing the atypical acid-fast organism into groups so various interested workers could now talk about the same organism with less confusion. Berman, Tervola and Erdmann (1959) reported isolating organisms resembling Myco. avium from tuberculin reactor cattle. The most important group of the four described by Runyon is Group III. Mallman et al. (1962) summarized it this way, "at the present time there is no way to differentiate bovine, avian and group III infections by sensitivity reactions. There is no way to evaluate the extent of each type except by bacteriological procedures." Mallman et al. (1963) further reported that most

of the isolates from no gross lesion reactors were classified as Runyon Group III Mycobacterium. In 1964, Ellis and Yoder reported on a two-year survey of tuberculosis lesions submitted by the Meat Inspection Division of the U.S. Department of Agriculture. These workers found that 24% of the atypical isolates belonged to Group III, 66% were classified as Runyon Group IV, and the remaining were Runyon Group II.

The organism, Myco. paratuberculosis, which causes Johne's disease in cattle has some antigens which are common to the mammalian tubercle bacillus. As a consequence, animals which have Johne's disease may react to the routine skin tuberculin test. In 1956, the European Productivity Agency warned against the use of vaccines in the control of Johne's disease, particularly when the tuberculin test is used for the eradication of bovine tuberculosis.

Johnin has been used for the diagnosis of Johne's disease for many years. Beach and Hastings (1922) first used intravenous johnin to diagnose Johne's disease. Until recently, the intravenous Johne's test has been accepted without any real doubt. Recently, Larsen and Kopecky (1965a) reported on the uses of intravenous johnin in a group of 99 animals. These workers found the test was satisfactory only in animals that were heavily infected with Johne's disease.

MATERIALS AND METHODS

Plan of Research

In this project there were six different groups of animals utilized in six experiments. Each of these experiments will be briefly explained. Experiment I was preliminary to the major work since it was necessary to determine the effects of intravenous tuberculin on normal cattle and normal pregnant cattle in a herd that did not have tuberculosis. A second part of Experiment I was designed to determine whether intravenous tuberculin would sensitize cattle to a future intravenous tuberculin test.

In the second experiment, pregnant cattle were sensitized with killed Myco. bovis in order to determine two things: (1) whether artificially sensitized cattle would react to the intravenous test, and (2) the effect of intravenous tuberculin in sensitized cattle in the late stages of pregnancy.

The third experiment was performed on cattle that were infected with Myco. paratuberculosis. The purpose of this experiment and the fourth experiment which was done on cattle infected with an organism classified as a Runyon Group III, was to determine whether cattle infected with organisms closely related to Myco. bovis would react to the intravenous test.

Experiment V is a summary of data collected from the field testing of cattle in "Red Flag"[1] herds as reported by Larsen and Kopecky (1965). This data was not published in their article, but was extracted from information about individual animals in which a tuberculous infection was shown to be present either histopathologically or bacteriologically.

The sixth experiment was threefold in purpose. The main object was to study the use of intravenous tuberculin in cattle experimentally infected with a virulent strain of Myco. bovis. The second object was to study the use of various forms of tuberculin on the experimentally infected cattle. Thirdly, comparisons of observations made in previous experiments on the Group III and Johne's infected animals and the field cases of tuberculosis were made against these experimentally infected animals.

Materials Common to All Experiments

Tuberculin

Agricultural Research Service (ARS) contract tuberculin[2] was used throughout the project. It was used directly from

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[1]A herd with tuberculin reactors showing lesions of tuberculosis on repeated test. Personal communication, A. F. Ranney, Chief Staff Officer for Tuberculosis Eradication, AnHD, ARS, USDA.

[2]Kindly provided by Dr. A. F. Ranney, Chief Staff Officer for Tuberculosis Eradication, Animal Health Division, ARS, USDA.

the bottle in which it was originally dispensed except in Experiment VI. The alteration of the tuberculin and utilization of a different preparation of tuberculin in that experiment will be discussed later. When diluted tuberculin was used, sterile physiological saline was the diluent. The quantity of tuberculin--3/4 ml., 1-1/2 ml. or 3 ml.--was diluted with saline q.s. 10 ml. Diluted tuberculin was prepared the afternoon preceding the date of the test in order that an equilibrium in the distribution of the tuberculin would be established.

Johnin

This was produced according to the method described by Dorset and Henley (1934). Briefly, it is prepared by growing the Myco. paratuberculosis in place of Myco. tuberculosis on Dorset and Henley synthetic medium for 12 weeks. The culture is then sterilized and filtered. Glycerol is added to the filtrate and this is evaporated to 1/5 its original volume. A 1% phenol solution is added to bring the volume back to 40% the original volume.

Syringes and needles

Ten ml. disposable syringes were used with 18 ga., 1-1/2 inch disposable needles. These were used once and discarded.

Thermometer

Five inch heavy duty veterinary stubby thermometers were used throughout the project.

Cattle

All the cattle except the steers in Experiment IV and the cattle from Red Flag herds in Experiment V were from the closed Holstein herd at the National Animal Disease Laboratory. There has never been any tuberculosis in this herd.

Materials and Methods Peculiar to Individual Experiments

Experiment I (normal cattle)

Procedure The 115 cattle in the NADL herd were each injected into the jugular vein with a 30% solution of ARS tuberculin. The rectal temperature of each animal was taken just previous to injection and then at 3, 4-1/4, 5-1/2, 6-3/4 and 8 hours postinjection. In the second part of this experiment, 15 animals that had been tested in the first part of the experiment were retested with tuberculin given intravenously (IV tuberculin); 5 were retested 30 days following the initial test; 5 more including the 5 tested at 30 days were retested at 60 days and the complete group of 15 animals were retested at 90 and 120 days.

Experiment II (cattle injected with killed Myco. bovis)

Cattle Four cattle that were obtained from the NADL herd and in the last trimester of their pregnancy were artificially sensitized with killed Mycobacterium bovis.

Procedure for sensitization of cattle Cultures of Myco. bovis (Ravenal) grown for 6 weeks on Sauton's liquid medium were heat-killed by autoclaving at 120° C., for one hour. The organisms were then collected by filtration. One hundred milligrams of the killed Myco. bovis was resuspended in 20 ml. of Freund's incomplete adjuvant. Each animal received 5 ml. distributed in three different sites in the dewlap. All four animals received the same dose on the same day.

Procedure for testing Five weeks following inoculation, the four animals were tested with IV tuberculin. The dose of tuberculin ranged from 3/4 to 6 ml. The temperature of each animal was taken just prior to the injection of IV tuberculin and at 1-1/2 hour intervals up to 7-1/2 hours.

Experiment III (cattle infected with Myco. paratuberculosis)

Cattle The cattle in this experiment were from the NADL Holstein herd.

Infective material Inoculum for each animal was prepared in the following manner. Fifty grams of intestinal mucosa from a cow that had clinical Johne's disease was added to 450 ml. of 1% trypsin and ground up in a TenBroeck glass tissue grinder until it was of a uniform consistency. The tissue was then digested for 30 minutes at 37° C. at a pH of 9 to 9.5 on a magnetic stirrer. It was decontaminated with a 1% solution of sodium hydroxide for 30 minutes at 37° C. on

a magnetic stirrer. Sufficient concentrated hydrochloride acid was then added to bring the solution to pH 7.4. The resulting suspension was centrifuged at 2,200 r.p.m. for 1 hour. The supernatant was decanted and the pellet was resuspended in 100 ml. saline for intravenous inoculation.

Procedure The rectal temperature of the animal was taken just previous to injection of the tuberculin or johnin and the temperature was then taken at various intervals over an eight-hour period following the administration of the test antigen.

Experiment IV (cattle infected with Runyon Group III atypical Mycobacterium)

Cattle Two groups of cattle were used. The first group consisted of 3 Holstein calves about 3 months of age that were obtained from the Iowa State University dairy farm. The second group contained 4 Holstein calves about 4 months of age which were obtained from a Grade A dairy in Story County, Iowa. All calves were negative to the cervical tuberculin skin test.

Procedure of culture for infection A strain[1] of Mycobacterium sp. classified as a Runyon Group III atypical Mycobacterium by the Mycobacteriology Laboratory of the Animal Health Division, ARS, USDA, at the National Animal Disease

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[1]Courtesy of Dr. Wayne Yoder, USDA, ARS, AnHD, NADL, Ames, Iowa.

Laboratory was used. It was grown on Dubos-Tween albumin medium for 4 weeks. Six flasks of grown culture were pooled and the material was measured for the concentration of organisms. By means of a Hopkins tube (Kubica, 1959) it was determined that approximately 240 mg. wet weight of organisms was present. The pooled cultures were centrifuged at 2,000 r.p.m. for 30 minutes. The sediment was resuspended in sterile saline, washed and centrifuged again at 2,000 r.p.m. for 30 minutes. The sediment was then resuspended in 24 ml. of saline for a resulting concentration of 10 mg./ml. The following day each calf was injected with 5 ml. of the suspension in the dewlap. Therefore, each animal received approximately 50 mg. of a Group III atypical Mycobacterium.

Procedure for testing Group I was initially tested one month after infection and Group II was tested about 7 weeks after injection. The dose of tuberculin used was 1-1/2 ml. and 4 ml. of johnin. The temperature was taken just previous to the injection and at various intervals over an 8-hour period. The time interval between tests was variable.

Experiment V (naturally infected cattle)

Cattle The cattle which were utilized in this experiment were all from herds in which tuberculosis was a chronic problem. The data are from animals in which tuberculosis was diagnosed either bacteriologically or histopathologically at postmortem.

Procedure The procedure in this experiment has been described previously (Larsen and Kopecky, 1965). It is briefly outlined here. The cattle were initially skin tested by ARS regulatory veterinarians. Seventy-two hours following the skin test, the intravenous tuberculin test was applied by the authors. The temperatures were taken just previous to the IV test and at various intervals over a 7-1/2 to 9-hour period. The dose varied from 3/8 to 1-1/2 ml. of ARS tuberculin. The animals were all slaughtered within 13 days of the test and tissues were submitted to the Diagnostic Service, Animal Health Division, National Animal Disease Laboratory for histopathological and bacteriological examination.

Experiment VI (cattle artificially infected with Myco. bovis)

Cattle Six steers used in this experiment were from the NADL Holstein herd.

Culture and infection The Mycobacterium bovis used in this experiment was designated 310B-2.[1] This strain was originally isolated from a thoraic lymph node of a bovine tuberculin reactor. The culture was stored on Lowenstein's slants for nine months at 3° C. It was shipped to NADL in Dubos broth base 0.5% dextrose, 150 mg. in 1.5 ml. of medium.

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[1]Kindly provided by Virginia H. Mallmann, Ph.D., Department of Microbiology and Public Health, College of Veterinary Medicine, Michigan University, East Lansing, Michigan and the description is taken from that which was provided by Dr. Mallmann.

This strain had been found to be highly infectious for calves, guinea pigs and rabbits.

In infecting the six steers, the material as received was diluted with Dubos broth base 5% dextrose liquid medium to a concentration of 75 mg./ml. One-tenth ml. of this suspension was injected over the left shoulder region into the intradermal layer of the skin of each animal.

Antigens

(a) Diluted tuberculin The saline used to prepare the diluted tuberculin was made with triple distilled water; the diluted tuberculin was then made by adding 1-1/2 ml. of ARS tuberculin to 8-1/2 ml. of saline.

(b) Dialyzed tuberculin Fifty ml. of ARS tuberculin was placed in a dialysis bag and placed in 1 liter of distilled water containing 0.1% phenol. The water was changed at 5, 10, 22, 56 and 72 hours. A precipitate that had formed in the tuberculin was redissolved by adjusting the pH 59 7.4. The tuberculin was concentrated back to 50 ml. by pervaporation at 90° C. for 2 hours. It was brought to isotonicity by the addition of 0.425 gm. of sodium chloride. The preparation was sterilized by filtration through a 0.3 mμ Millipore filter, bottled in 6-ml. lots and stored at 3° C. until used.

(c) PPD Tuberculin purified protein derivative[1] was

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[1]Bio. 489, Parke, Davis and Co., Detroit, Michigan.

used. Seventy-five thousand tuberculin units which is the number of TU's in 1-1/2 ml. of ARS tuberculin was made up by dissolving thirty 0.05 mg. tablets (2,500 TU each) to 10 ml. of buffered diluent that was supplied with the tablets. This preparation was made up about 16 hours previous to the beginning of the test.

Procedure The cattle were randomized by use of the following Latin square:

Cow	Period					
	1	2	3	4	5	6
1	A	B	C	D	E	F
2	B	C	D	E	F	A
3	C	D	E	F	D	B
4	D	E	F	A	B	C
5	E	F	A	B	C	D
6	F	A	B	C	D	E

Two weeks elapse between tests. The letters refer to the following antigen: A - no treatment or antigen

B - 10 ml. saline

C - 10 ml. diluted tuberculin

D - 1.5 ml. dialyzed tuberculin

E - 1.5 ml. ARS tuberculin

F - PPD

Temperatures were taken just previous to injection and every hour following injection for eight hours.

The six animals were put through this procedure before infection, then the animals were infected as described above and two months later the procedure was repeated in an identical manner.

Two control animals from the same original group that the six principals came from were put through two rounds of testing. The difference in the controls and the principals was that the controls were not infected during the interval between treatments.

The following setup was used.

Cow	Period					
	1	2	3	4	5	6
7	A	B	C	D	E	F
8	D	E	F	A	B	C

RESULTS

Presentation of Data

The results for this project are listed by experiment. A summary of the time the peak temperature is reached, the change in temperature and the time the temperature returned to normal are summarized at the end of the section. The complete set of results can be found in the Appendix.

Experiment I (normal cattle)

In no case did any animal show a temperature increase significant enough to be considered a reactor under the criterion set up for the field study, Larsen and Kopecky (1965). In only 13 of the 115 animals tested was the peak temperature higher than the initial preinjection temperature.

Forty-one cows were pregnant at the time of the test. There were no abortions or any other disturbances that in anyway terminated or affected the state of pregnancy.

There were no reactions to the IV tuberculin test in any animal at 30, 60, 90 or 120 days following the initial IV tuberculin test.

Experiment II (cattle injected with killed Myco. bovis)

In this experiment, the artificially sensitized pregnant cattle all reacted with a temperature rise regardless of the dose. The temperature peaked mainly at 6 to 7-1/2 hours and ranged from a 2^{o} to a 6.2^{o} rise in temperature. The only exception to this was cow 4778, which during the second test,

had an elevated temperature at the beginning which remained elevated during the entire test period. No ill effects on the pregnant state of the animals were noted.

Experiment III (cattle infected with Myco. paratuberculosis)

In this experiment using animals infected with Johne's bacillus, the most significant finding was that in most cases when the homologous antigen was used--johnin--the peak temperature was reached at a later time than when tuberculin was used. The change in temperature (ΔT) was greater in these cases when johnin was administered. When tuberculin was administered, the peak temperature was generally reached earlier and was not as high. The average peak temperature with johnin was 104.9° F. compared to 102.75° F. using tuberculin. The temperatures did not return to normal within the 8-hour test period when johnin was the test antigen, but generally returned to normal within 4 hours when tuberculin was the test antigen. All cattle had lesions of paratuberculosis.

Experiment IV (cattle infected with Runyon Group III atypical Mycobacterium)

In this experiment, using two different groups of cattle and the same organisms, two different sets of results were obtained. In the group of 3 cattle 5278, 5279, 5280, tuberculin caused an early reaction with an early return to normal and the temperatures change varied from 1.4° to 2.6° F. On the other hand, when johnin was used the temperatures peaked at a —

later time and did not return to normal within the test period. The change in temperature was also greater when johnin was the test antigen.

In the second group of cattle, a reverse situation was seen. Tuberculin gave a reaction in 3-1/2 to 5 hours, but returned to normal at 6-1/2 hours. After the peak was reached the temperature quickly returned to normal. When johnin was used, there was no reaction at all.

Experiment V (naturally infected cattle)

All of these animals had tuberculosis and all reacted to the intravenous test. It was observed that the temperature response was independent of dose. From the data it can be seen that there were five groups of animals, each group represented a different herd. The peak temperature in each group was either the same or fell within a narrow range. In the first four groups, the time of the peak was late--from 4-1/2 to 9 hours--while in the last group the peak was very early. The change in temperature varied from 1.2° F. to 6.6° F. In the first four groups, the temperature did not return to normal within the 8-hour test period except in one case. However, in the last group, 5 of the 7 animals returned to normal within the test period.

Experiment VI (cattle artificially infected with Myco. bovis)

When the cattle received no antigen, the temperature varied and the peak time also varied, both pre- and post-

infection. The same holds true when the cattle received only saline. Only in one animal did the temperature remain elevated at the end of the test period, that was in animal number 3 pre-infection.

When diluted tuberculin was used, the peak time was between 4 and 7 hours averaging about 5-1/2 hours. In all cases, pre-infection, except in animal 2, the temperature returned to the normal range if it had been elevated during the test period. However, in all cases, the peak temperature was higher post-infection, ranging from a pre- and post-infection difference of 0.2° F. to 3.4° F. with the peak post-infection temperature of 106.8° F. occurring in animal 1.

When dialyzed tuberculin was used as the antigen, the time of the peak temperature varied in both pre-infection and post-infection treatment. The average peak temperature elevation was 102.5° F. pre-infection and 104.6° F. post-infection. In all tests conducted during the post-infection period, there was a significant rise in temperature which remained elevated throughout the test period.

The results from the use of undiluted, undialyzed tuberculin were the most consistent in the experiment. In all cases, the time of the peak temperature post-infection was between 5 and 6 hours while it fluctuated more during the pre-infection testing. The average peak temperature pre-infection was 102.8° F. while the average post-infection peak temperature was just below 105° F. The post-infection, peak temperature remained

elevated during the test period except in one case where it returned to the normal range at 8 hours.

Tuberculin PPD also gave consistent results in that it did not cause a reaction. In 4 out of the 6 animals, the pre-infection peak temperature was greater than the post-infection peak temperature. The time of the peak temperature varied in both pre- and post-infection tests.

Statistical treatment of the data from Experiment VI is summarized in the following paragraphs. It should be noted that the animals were extremely consistent across time in that they all responded very similarly to the treatment.

The difference between no treatment and saline treatment versus the use of diluted, dialyzed and regular tuberculin was highly significant; that is to say, prior to infection both gave similar responses, whereas after infection a temperature rise was noted in the groups receiving tuberculin.

The difference between the no treatment and the 10-ml. saline groups were compared and no significant difference was found between pre-infection or post-infection tests.

The use of diluted and dialyzed tuberculin versus regular tuberculin were compared and no significant difference was found. Therefore prior to infection, there was no difference in response, but after infection both groups gave a significant rise in temperature.

The use of dialyzed tuberculin was compared with the use of diluted tuberculin. Here again, the same as in the previous

comparison was found.

A comparison of the average of the results using diluted, dialyzed and regular ARS tuberculin versus the use of tuberculin PPD showed that prior to infection there was no significant difference in the response across time, whereas, after infection the temperature rise was much higher in the use of ARS tuberculin than with PPD. At necropsy all animals exhibited lesions of tuberculosis. No reactions were observed in the control animals.

Summary of Data

The following symbols are used in this summary:

P = hour after injection peak temperature was observed

Δt = change in temperature from beginning of test to peak temperature rise

T = peak temperature (first two numerals are omitted)

N = hour after injection that temperature returned to normal. If blank, temperature did not return to normal within 8-hour test period. An asterisk indicates temperature never left normal range.

J = johnin

TBC = regular ARS tuberculin

Experiment I (normal cattle)

No reactions were observed, therefore the data does not fit the format of this summary.

Experiment II (cattle injected with killed Myco. bovis)

Animal no.	Antigen	Dose	P	$\frac{\Delta t}{T}$	N
4998	TBC	3/4	7-1/2	$\frac{5.4}{6}$	.....
	TBC	6	6	$\frac{3.8}{4.8}$	.....
5004	TBC	1-1/2	6	$\frac{2.6}{4.8}$	.....
	TBC	3	4-1/2	$\frac{2.2}{3.6}$	7-1/2
4978	TBC	3	7-1/2	$\frac{6.2}{7.8}$	.....
	TBC	1-1/2	3	$\frac{0.6}{3.8}$	.....
4987	TBC	6	6	$\frac{5.0}{6.4}$	.....
	TBC	3/4	6	$\frac{2.0}{3.6}$	.....

Experiment III (cattle infected with Myco. paratuberculosis)

Animal no.	Doses	P	$\frac{\Delta t}{T}$	N
	Antigen - johnin			
5114	3	4	$\frac{3.6}{5.4}$	.....
	3	8	$\frac{4.2}{6.2}$	.....
5122	3	6	$\frac{5.0}{6.6}$	.....
	3	8	$\frac{5.0}{6.2}$	.....

Animal no.	Doses	P	$\frac{\Delta t}{T}$	N
5123	3	2	$\frac{1.0}{3.2}$	4
	3	8	$\frac{2.5}{3.8}$	.....
5127	3	6	$\frac{2.3}{3.8}$	.....
	3	8	$\frac{1.4}{3.4}$	.....
	5	4	$\frac{4.6}{6.6}$	.....
	5	6	$\frac{3.4}{5.2}$	.....
5170	5	6	$\frac{3.2}{5.0}$	.....
	5	8	$\frac{1.6}{3.2}$	.....
	5	no reaction		
	Antigen - tuberculin			
5114	3	2	$\frac{0.6}{2.6}$	4
5122	3	3	$\frac{0.8}{3.0}$	4
5123	3	3	$\frac{1.6}{2.6}$	4
5127	3	3	$\frac{1.0}{3.0}$	4
	1-1/2	2	$\frac{1.0}{3.2}$	8
	3/4	8	$\frac{1.2}{3.0}$	.....
	1-1/2	8	$\frac{1.4}{2.6}$	.....

Animal no.	Doses	P	$\frac{\Delta t}{T}$	N
5170	3/4	2	$\frac{0.6}{2.2}$	3
	1-1/2	6	$\frac{0.8}{2.6}$	8

Experiment IV (cattle infected with Runyon Group III atypical Mycobacterium)

	Antigen - tuberculin			
5278	1-1/2	3	$\frac{1.4}{2.8}$	4
	1-1/2	no reaction		
5279	1-1/2	3	$\frac{2.6}{3.6}$	4
	1-1/2	4	$\frac{2.6}{3.4}$	6
5280	1-1/2	3	$\frac{2.0}{3.2}$	6
	1-1/2	3	$\frac{2.4}{2.8}$	6
5337	1-1/2	3-1/2	$\frac{3.0}{4.2}$	6-1/2
5338	1-1/2	5	$\frac{2.0}{3.4}$	6-1/2
5339	1-1/2	5	$\frac{4.4}{4.8}$	6-1/2
5340	1-1/2	5	$\frac{4.2}{4.6}$	6-1/2
	Antigen - johnin			
5278	4	8	$\frac{3.2}{3.2}$	.....
5279	4	4	$\frac{3.8}{3.8}$	.....

Animal no.	Doses	P	$\frac{\Delta t}{T}$	N
5280	4	6	$\frac{2.6}{3.8}$	.....
5337	4	no reaction		
5338	4	no reaction		
5339	4	no reaction		
5340	4	no reaction		

Experiment V (naturally infected cattle)

All received tuberculin.

5108	1-1/2	5	$\frac{2.2}{5.6}$	.....
5172	1-1/2	5	$\frac{2.4}{3.8}$	.....
5115	1-1/2	5	$\frac{6.4}{6.8}$	.....
5216	1-1/2	5	$\frac{3.2}{4.4}$	.....
6777	3/4	8	$\frac{6.6}{7.0}$	.....
6783	3/8	8	$\frac{6.3}{6.6}$	.....
6780	3/4	8	$\frac{3.1}{5.0}$	.....
R92648	3/4	9	$\frac{4.8}{4.6}$	.....
13300	3/4	9	$\frac{2.8}{3.8}$	.....
13280	3/4	9	$\frac{1.2}{3.2}$	.....
13280	3/4	9	$\frac{1.2}{3.2}$	.....

Animal no.	Doses	P	$\frac{\Delta t}{T}$	N
6512	3/4	4-1/2	$\frac{3.4}{3.6}$	8
6511	1-1/2	7-1/2	$\frac{6.2}{7.4}$	.....
6510	3/4	5	$\frac{5.3}{6.7}$	.....
6502	1-1/2	7-1/2	$\frac{3.9}{5.4}$	.....
4585	1-1/2	3-1/2	$\frac{2.6}{4.2}$	8
0985	1-1/2	3-1/2	$\frac{3.4}{4.8}$	.....
4584	1-1/2	3-1/2	$\frac{3.2}{5.0}$	8
6602	1-1/2	3-1/2	$\frac{3.8}{5.2}$	.....
6778	1-1/2	2	$\frac{3.8}{5.2}$	8
6277	1-1/2	2	$\frac{1.2}{3.4}$	6-1/2
4583	1-1/2	2	$\frac{3.2}{5.2}$	6-1/2

Experiment VI (cattle artificially infected with Myco. bovis)

Animal no.	Before			After		
	P	$\frac{\Delta t}{T}$	N	P	$\frac{\Delta t}{T}$	N
		No treatment				
1	5-7	$\frac{0.6}{2.0}$	*	4	$\frac{0.6}{1.6}$	*
2	2	$\frac{1.4}{2.6}$	*	3	$\frac{1.0}{2.2}$	*

Animal no.	Before			After		
	P	$\frac{\Delta t}{T}$	N	P	$\frac{\Delta t}{T}$	N
3	6	$\frac{2.6}{3.6}$	8	6	$\frac{1.0}{2.6}$	8
4	6	$\frac{1.6}{2.6}$	7	6-8	$\frac{1.0}{1.6}$	*
5	6	$\frac{1.4}{2.6}$	*	6	$\frac{0.8}{1.4}$	*
6	2	$\frac{0.4}{2.2}$	*	8	$\frac{0.6}{2.6}$	*
		Saline				
1	6	$\frac{1.2}{2.2}$	*	5	$\frac{0.4}{1.8}$	*
2	2	$\frac{1.4}{2.8}$	*	6	$\frac{1.0}{2.4}$	*
3	7	$\frac{2.2}{3.6}$	.....	2	$\frac{2.4}{2.0}$	*
4	6	$\frac{1.6}{3.4}$	7	7	$\frac{0}{2.6}$	*
5	3	$\frac{1.8}{2.8}$	4	8	$\frac{0.6}{1.6}$	*
6	6	$\frac{1.2}{2.2}$	*	5	$\frac{1.4}{3.0}$	6
		Diluted tuberculin				
1	6	$\frac{3.2}{4.6}$	8	6	$\frac{6.2}{6.8}$	...
2	6	$\frac{2.4}{3.4}$	.....	5	$\frac{2.0}{4.2}$	...
3	5	$\frac{2.4}{3.2}$	7	5	$\frac{1.6}{3.4}$	...
4	7	$\frac{1.6}{2.4}$	*	7	$\frac{1.4}{3.0}$	...

Animal no.	Before			After		
	P	$\frac{\Delta t}{T}$	N	P	$\frac{\Delta t}{T}$	N
5	6	$\frac{1.8}{3.4}$	8	7	$\frac{4.0}{5.2}$	....
6	5	$\frac{2.0}{2.6}$	*	4	$\frac{4.6}{6.0}$	....
		Dialyzed tuberculin				
1	6	$\frac{1.0}{2.2}$	*	7-8	$\frac{3.0}{5.4}$	....
2	1	$\frac{1.0}{2.6}$	8	8	$\frac{4.6}{5.8}$	....
3	6	$\frac{2.6}{3.6}$	8	1	$\frac{1.4}{3.0}$	....
4	0	$\frac{0}{1.3}$	*	6	$\frac{4.8}{6.4}$	....
5	7	$\frac{1.6}{3.0}$	8	6-8	$\frac{1.4}{2.8}$	*
6	6	$\frac{1.4}{2.4}$	*	8	$\frac{1.4}{3.4}$	....
		Regular tuberculin				
1	5	$\frac{2.2}{3.2}$	8	5	$\frac{4.6}{6.0}$	....
2	3	$\frac{1.8}{2.4}$	*	6	$\frac{2.2}{4.8}$	....
3	3	$\frac{2.2}{2.6}$	*	5	$\frac{2.8}{4.0}$	8
4	2	$\frac{1.6}{3.2}$	8	6	$\frac{4.2}{6.6}$	....
5	6	$\frac{0.8}{2.4}$	*	5	$\frac{4.2}{5.0}$	....
6	5-7	$\frac{2.4}{3.0}$	8	5-8	$\frac{2.4}{3.4}$	....

Animal no.	Before			After		
	P	$\frac{\Delta t}{T}$	N	P	$\frac{\Delta t}{T}$	N
		PPD tuberculin				
1	5	$\frac{1.8}{2.3}$	*	3	$\frac{1.0}{2.2}$	*
2	6	$\frac{0.8}{2.6}$	*	4 and 8	$\frac{1.4}{2.4}$	*
3	5-6	$\frac{2.8}{3.4}$	.....	0-1	$\frac{0}{2.2}$	*
4	1	$\frac{1.8}{3.4}$	7	1	$\frac{0.4}{2.0}$	*
5	2	$\frac{1.2}{2.8}$	*	7	$\frac{2.4}{3.0}$	
6	6	$\frac{0.6}{2.6}$	*	8	$\frac{2.0}{3.0}$	....

DISCUSSION

When this project was initiated little was known about the intravenous tuberculin test. A search of the English language literature and abstracting journals revealed very few published reports of an intravenous tuberculin test, and the few that were found provided no technical guidelines. The information on the subcutaneous tuberculin test could not be utilized for this test since guidelines and standards varied from time to time. Another problem was the lack of uniform standards for tuberculin. Different countries use various standards for tuberculin and the published reports were vague and usually incomplete. In essence, there were no guidelines whatsoever and it was necessary to first establish guidelines to be used in this research.

The early work in this project with non-infected animals and artificially sensitized pregnant animals was done to establish methodology for the research on infected animals and for the field work reported by Larsen and Kopecky (1965). It also had to be established whether normal animals would develop a systemic response to the intravenous test. It was very fortunate at this time that no systemic reactions were observed in the normal cattle. This point will be enlarged upon later in the discussion.

Normal animals in various stages of pregnancy were tested with no apparent effect on the pregnant state. Four pregnant

animals which were sensitized with killed Myco. bovis showed no ill effects on their pregnant state as a result of testing. This was a very hopeful sign because the test, if it were to be used in the field, could not be detrimental to a pregnant animal. Even if the test would terminate pregnancy in a tuberculous or sensitized animal and the animal was classified as a reactor and sent to slaughter, the fact that an abortion occurred would make the test esthetically undesirable because of the stigma attached to abortion. There was no evidence that abortion occurred in the field cattle.

The next problem that was encountered and worked out was the matter of dosage. Various levels of tuberculin were used in the four cattle sensitized with Myco. bovis and it was found that the response was independent of the dose of tuberculin. In an unreported case, one steer with generalized tuberculosis was killed when 3 ml. of tuberculin was injected. It was also found in the field studies that either 3/4 ml. or 1-1/2 ml. would detect tuberculosis; therefore, it was decided that the highest dose of tuberculin, 1-1/2 ml., which had given no problems would be used throughout the remainder of the experiment.

The animals infected with Myco. paratuberculosis, while they reacted to johnin, did not react as dramatically to tuberculin. This was fortunate, because it meant that a herd could be tested and animals with Johne's disease would not cause false positive reactions. This indicates that Myco.

paratuberculosis has antigens which are dissimilar to those of Myco. bovis, and therefore cross reaction presents no significant problem.

The possibility that infection of cattle with Group III atypical Mycobacterium might interfere with the use of IV tuberculin had to be considered. Before cattle were selected for use on this project, they were intravenously tested to insure that only negative animals were used. The first groups tested showed a positive response to the intravenous test. This testing continued until enough negative animals were found so that a group of 3 and a group of 4 were obtained for a study of thermal response of cattle infected with the Group III organisms. These 2 groups were infected and tested and the results were almost identical to those seen in the other cattle in the field testing previous to purchase. These animals showed a varied thermal response sometimes reaching as high as 104° or a little higher, but even more important was the fact that the temperature always returned to normal before 8 hours. This response was also obtained when tuberculin was used on cattle with Johne's disease.

The 76 animals reported on by Larsen and Kopecky (1965), however, did not show any indication of the Group III sensitization. Every animal tested and found positive to either the skin test or the IV test also had lesions at slaughter with an exception of one animal that was in a herd where tuberculosis was a chronic problem. This is explained on the basis that

this one animal either had lesions but not visible granulomata, was highly sensitized to Myco. bovis, or the lesion was overlooked.

At this point in the study, the hypothesis was made that there is a definite difference between the type of thermal response seen in animals infected with the Johne's bacillus or Group III organism and tested with ARS tuberculin and animals infected with Myco. bovis and tested with ARS tuberculin. It was concluded that the use of tuberculin in some field animals supposedly uninfected with Group III organisms and animals with Johne's disease produces, in some cases, a low grade temperature increase which always returned to normal by 6 to 8 hours. In animals with tuberculosis, the temperature elevation was generally greater, the peak temperature occurred later and the temperature did not return to normal during the 8-hour test period.

In an unpublished study in which this author was associated, a portion of a dairy herd which had many NGL tuberculin skin reactors was tested with the intravenous tuberculin test, 12 of these animals showed a reaction that peaked between 3-5 hours, returned to normal within the 8 hours and the temperature rise did not go above 104.4°. Many of the reactors had palpable lymphangitis in both front and rear limbs but no Myco. bovis was ever isolated from the draining lymph nodes. The pattern of response seen in these animals was similar to those experimental animals which were infected

with Group III atypical Mycobacterium.

The results obtained in the sixth experiment tend to bear out this hypothesis, especially when compared with the field experiment and with animals infected with Myco. paratuberculosis and with Group III organisms. The best reason in this authors opinion, is that a good source of antigen such as a granuloma either natural or artificial is required to produce a high level of fixed antibody homologous to the sensitizing agent needed to give a prolonged systemic response. This cannot occur in Group III infection because a granulomatous lesion is not produced and secondly, the tuberculin is heterologous to the antibody produced. In Johne's disease, there is a good focus of infection but again the antibody is of a heterologous nature to tuberculin. Since a low-grade response is seen in some animals supposedly not infected, the supposition is made that these animals are infected with acid-fast organisms, other than Group III or the Johne's bacillus.

The average ΔT and peak temperature in animals tested with ARS tuberculin and infected with Myco. paratuberculosis was 1.0° F. and 102.75° F., Group III Mycobacterium infection, 2.7° F. and 103.65° F., and with tuberculosis, 3.42° F. and 104.9° F. Further proof can be found when the data from cattle infected with Myco. paratuberculosis and tested with johnin are compared. The average ΔT was 3.15° F. and the average peak temperature was 104.9° F. This is substantial proof that the change in temperature and peak temperature is greater when the

infecting agent and test antigen are homologous. Therefore, if the IV test is to be used in the field, the criteria for designating a positive reaction should be a ΔT exceeding 3° F. and a peak temperature exceeding probably 104° F. This differs from the first report by Larsen and Kopecky (1965) in which any peak temperature exceeding 103.2° F. indicated a positive reaction. Data in this thesis does not support the 103.2° F. peak temperature criterion.

Another aspect of the sixth experiment was the use of various preparations of ARS tuberculin and of tuberculin PPD. The only drastic manipulation of the ARS tuberculin was the dialysis against water. The active principal was not lost. Therefore, its molecular size must be large enough not to be dialyzable, or if it is of a smaller size it must be bound in such a way that it is not dialyzable. The ARS tuberculin can be injected either undiluted or diluted with saline. It is probably preferable in the field to use the tuberculin undiluted, so that pyrogen is not introduced.

In a comparison of the data with the report of Rivera (1937), there was some similarity. In both studies, the temperature began to rise within four hours, but peaked after five to nine hours. Unfortunately, Rivera's complete report was not available, so this was the only comparison that can be drawn.

The work reported by Ukrainezyk-Laborie and associates (1952) seems in contrast to the work reported in this thesis.

The statements by these investigators tend to indicate that they were dealing with a nonspecific infection but when their published data was examined in detail and interpreted along the same lines as the data presented in this thesis, it could be seen that no contrast existed. The peak temperatures were reached at the fifth to seventh hour, the change in temperature varied from 4.0° F. to 5.6° F. and the temperature did not return to normal within 8 hours.

It is very unfortunate that Kovats (1956) did not complete his study of the intravenous tuberculin test by doing post-mortem examinations of his animals. His findings support the data reported in this project. Kovats varied the dose of tuberculin and found, as this research has tried to show, that dose and reaction within the limits studied are independent. However, he did find that if too high a concentration of tuberculin was used a severe reaction can occur.

Kutleša and Marič in 1961 claimed that they could distinguish between sensitization to Myco. bovis and sensitization to other Mycobacterium species by comparing the number of reactors in a herd, and the ΔT in the reactors, the reaction to the skin test and epizootiological data. The results in this thesis tend to indicate that nonspecific and specific sensitization can be distinguished by the time which the maximum temperature was reached, the ΔT and whether or not the temperature elevation returns to normal by 8 hours.

Kutleša and Marič also used PPD, but these workers used

bovine (Myco. bovis) PPD. Since one of the objects of this present research was to develop a test that could be readily adapted to field use, only commercially available PPD was used and this was prepared from Myco. tuberculosis. The results were quite contrasting. Human PPD gave results which indicated it was not a satisfactory antigen for use in the intravenous tuberculin test while the bovine PPD used by Kutlesa and Maric gave results similar to the results in this project obtained by using ARS tuberculin.

There appears to be two main uses for the intravenous tuberculin test. The first use which was reported by Larsen and Kopecky in 1965 and developed to a greater extent in this thesis is the elimination of hidden spreaders in herds of dairy cattle where frequent testing fails to remove the source of infection. This was tried and proved to be satisfactory since animals which continuously failed to react to the skin test were positive to the IV test and found to have widespread open tuberculosis.

The second use could be in the herd where a large number of newly disclosed reactors were found upon routine skin testing. Instead of sending all the animals to slaughter, an IV test can be run and the IV reactors sent to slaughter to determine whether or not the herd has tuberculosis. The pattern of reaction can also give some information. This second use has not been tried in newly-found diseased herds and an investigation of this nature could prove useful and interesting.

CONCLUSIONS

1. The intravenous tuberculin test, in conjunction with the tuberculin skin test, did detect all cases of tuberculosis in field test.

2. It did not produce thermal reactions in a herd known to be free of Myco. tuberculosis or Myco. paratuberculosis.

3. The following criteria were established for a positive reaction in tuberculosis.

   a. The peak temperature is reached between four to six hours following injection and should exceed 104° F.

   b. The ΔT should exceed 3° F. when tested with ARS tuberculin.

4. The ΔT and peak temperature are lower in cattle infected with a Mycobacterium other than Myco. bovis and the temperature returns to normal range by 8 hours.

5. The test antigen must be homologous to the infecting agent. It must satisfy the above mentioned criteria (Part 3) in animals known to be infected with the homologous agent.

6. Tuberculin PPD which is commercially available, in the United States is not a satisfactory antigen for use in the intravenous tuberculin test.

7. The test is not detrimental to animals and will not produce abortion in cattle if they are not infected with Myco. tuberculosis.

8. If the temperature of the animal rises early in the test period, but rapidly returns to normal within five to seven hours following the beginning of the test, a nonspecific infection can be suspected but not always proved.

SUMMARY

An intravenous tuberculin test which had been previously described was extensively studied to more fully evaluate the use of this test in the detection of bovine tuberculosis. The test was applied to a group of non-infected cattle and four groups of experimentally infected animals. The data was extracted from a group of cattle with naturally occurring tuberculosis and compared with the data of the experimentally infected cattle.

The first group was free from infection with either Mycobacterium bovis or Mycobacterium paratuberculosis. The test did not cause any thermal reaction in this group nor did the IV test sensitize these cattle to subsequent IV tests.

The second group, which were pregnant cows, was sensitized with killed Myco. bovis. The test did not effect the state of pregnancy in any cow. Four different doses of tuberculin were used and the response appeared to be independent of the dose.

Cattle infected with Myco. paratuberculosis and cattle infected with an atypical Group III Mycobacterium made up the third and fourth groups, respectively. These groups responded differently than the group with bovine tuberculosis in that the temperature rise, if any, occurred early and the temperature of the animals returned to normal by the end of the test period.

Naturally infected and artificially infected tuberculous

cattle reacted similarly. The temperature peak was reached from 3-9 hours, usually from 5-7, the temperature did not return to normal at the end of the test period. The peak temperature ranged from 103.0° F. to 107.4° F.

There was no significant difference in the use of ARS tuberculin either undiluted, diluted with saline, or dialyzed against water for the detection of tuberculous animals. Commercial PPD tuberculin was not satisfactory for this purpose. ARS tuberculin did not cause a significant thermal response in cattle infected with Myco. paratuberculosis or in cattle infected with atypical Group III Mycobacterium. It is therefore concluded that the antigen must be homologous to the infecting agent.

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APPENDIX

Complete Set of Data on Animals to which the Intravenous Tuberculin Test was Applied

Experiment I (normal cattle)

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/4	5-1/2	6-3/4	8	Δt	Stage of preg.
			Barn 129 - 1/3/63							
4958	2-1/2	F	102.4	102.3	101.8	101.4	101.6	100.4	-2	2 mo.
4815	3-1/2	F	101.8	102.0	102.0	102.4	102.0	101.6	-0.2	5 mo.
4961	2-1/2	F	102.5	102.4	101.8	101.4	101.4	101.4	-1.4	1 mo.
3907	9	F	102.0	101.6	101.2	100.8	101.0	100.8	-1.2	5 mo.
3571	11	F	102.0	102.2	101.8	101.6	101.4	100.6	-1.4	5 mo.
4437	6	F	102.2	102.4	101.8	101.4	101.4	101.2	-1.0	4 mo.
3615	11	F	102.0	101.6	101.4	101.2	101.4	101.2	-0.8	6 mo.
3892	9	F	101.8	101.6	101.4	101.2	101.4	101.2	-0.6	5 mo.
3739	10	F	102.0	101.4	101.2	101.0	100.8	100.6	-1.4	Not bred
4810	3	M	101.8	101.8	101.6	101.2	100.2	101.2	-0.6	
4923	2-1/2	F	102.2	101.6	101.4	101.2	101.4	100.6	-1.6	2 mo.
4940	2-1/2	F	102.8	101.8	101.8	101.2	101.4	101.0	-1.8	2 mo.
			Barn 136 - 1/8/63							
5081	1-1/2	F	103.2	103.2	101.4	101.0	101.2	101.6	-2.6	Not bred
5106	1-1/2	M	102.4	101.2	100.6	101.6	100.6	101.2	-1.4	Not bred
5102	1-1/2	M	102.8	101.4	101.2	101.4	101.2	100.8	-2.0	Not bred
5083	1-1/2	F	102.4	101.8	102.0	101.4	101.6	101.4	-1.0	Not bred
5089	1-1/2	F	102.4	102.2	101.8	102.2	101.8	101.4	-1.0	Not bred
5088	1-1/2	F	102.8	101.2	101.4	101.8	101.6	101.6	-1.2	Not bred
5084	1-1/2	F	102.6	102.2	101.2	101.4	101.6	101.6	-1.0	Not bred
5086	1-1/2	F	103.2	102.6	102.0	102.4	101.8	102.2	-1.0	Not bred
5091	1-1/2	F	102.8	102.2	101.6	102.0	101.8	101.4	-1.4	Not bred
5087	1-1/2	M	103.0	102.6	102.2	102.4	102.2	102.0	-1.0	Not bred
5092	1-1/2	F	102.4	102.2	102.0	102.4	102.2	102.0	-0.04	Not bred
5076	1-1/2	F	102.6	102.2	101.6	101.8	101.6	101.6	-1.0	Not bred
5080	1-1/2	F	102.4	102.6	101.2	101.8	101.2	101.6	-0.8	Not bred

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/4	5-1/2	6-3/4	8	Δt	Stage of preg.
5078	1-1/2	M	103.2	102.4	102.8	101.4	101.4	101.4	-1.8	Not bred
5090	1-1/2	M	102.8	102.6	102.4	100.2	101.4	101.4	-1.4	Not bred
5077	1-1/2	F	103.0	102.6	101.8	101.8	101.4	101.6	-1.4	Not bred
5094	1-1/2	F	103.2	103.0	102.6	100.0	101.2	101.4	-1.8	Not bred
5085	1-1/2	F	103.0	102.6	100.8	100.8	101.4	101.4	-1.6	Not bred
5079	1-1/2	F	102.4	102.4	101.8	101.8	101.8	101.6	-0.8	1 mo.
			Barn 128 - 1/15/63							
4739	4	F	100.8	100.6	101.2	101.2	101.2	100.8	0.0	2 mo.
4697	4	F	101.4	100.6	100.6	100.6	100.4	100.8	-0.6	1 mo.
4696	4	F	102.2	101.2	101.4	101.2	101.2	101.0	-1.2	2-1/2 mo.
4699	4	F	102.2	100.6	101.4	101.2	100.8	101.0	-1.2	1 mo.
4698	4	F	102.6	101.8	101.6	101.4	101.4	101.2	-1.0	1 mo.
4675	4	F	102.0	101.8	101.4	101.4	101.2	100.8	-1.2	2 mo.
4637	4	M	100.8	100.8	101.0	100.6	100.8	101.0	+0.2	male
3972	8	F	102.2	101.8	101.2	100(?)	101.0	101.4	-1.2	1 mo.
3893	8	F	102.0	101.6	101.2	101.4	100.8	101.4	-1.2	2 mo.
4291	6-1/2	F	102.0	101.6	101.0	101.2	101.4	101.0	-1.0	2 mo.
3767	9	F	101.8	101.2	100.8	101.4	101.0	100.6	-1.2	1 mo.
3796	9	F	101.8	101.2	100.8	100.8	101.4	101.4	-1.4	2 mo.
			Barn 127 - 1/31/63							
3769	9	F	102.2	101.4	101.0	101.2	101.0	101.2	-1.2	1 mo.
3743	9	F	101.0	101.4	101.2	101.0	100.2	100.6	-0.8	2 mo.
3953	8	F	102.6	102.2	102.0	102.0	101.2	101.2	-1.4	1 mo.
3942	8	F	102.2	101.2	101.4	100.8	100.6	100.4	-1.8	2-1/2 mo.
3931	8	F	102.2	101.6	102.0	101.2	101.0	100.2	-2.0	3 mo.
3771	9	F	100.8	101.2	100.8	101.0	101.0	100.4	-0.4	4 mo.
4013	8	F	101.8	102.0	101.4	101.4	101.0	101.0	-0.8	3-1/2 mo.
3859	8-1/2	F	101.6	101.6	101.6	101.8	101.0	100.2	-1.4	3-1/2 mo.
3903	8	F	101.4	101.0	100.8	101.0	100.2	101.2	-1.2	3 mo.
4060	7-1/2	F	102.2	101.6	101.2	101.2	101.2	101.0	-1.2	3 mo.

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/4	5-1/2	6-3/4	8	Δt	Stage of preg.
4053	7-1/2	F	102.0	102.2	101.6	101.8	100.8	101.0	-1.2	1/2 mo.
			Barn 135 - 1/17/63							
4836		M	100.2	101.2	100.4	100.6	100.8	100.8	+0.6	
4843		M	100.8	100.8	100.8	101.2	101.0	101.2	+0.4	
			Barn 125 - Mastitis - 1/29/63							
4812		F	102.4	103.0	102.0	101.6	102.0	102.2	-0.2	
4816		F	101.4	101.4	101.4	101.4	101.4	101.4	0.0	
			Barn 132 - 2/5/63							
5116	1	F	102.2	102.0	101.4	101.6	101.4	101.0	-1.2	Not bred
5112	1	F	102	101.8	101.8	101.8	101.4	101.0	-1.0	Not bred
5104	1	F	102.4	101.4	101.6	101.8	101.4	101.2	-1.2	Not bred
5110	1	F	102.4	101.4	101.6	101.6	101.2	101.4	-1.2	Not bred
5119	1	F	102.2	101.0	101.2	101.2	100.4	101.2	-1.8	Not bred
5120	1	F	102.4	102	102.4	102.2	101.8	101.4	-1.0	Not bred
5109	1	F	102.6	102.0	102.2	101.8	101.8	101.8	-0.8	Not bred
5111	1	F	102.0	101.8	101.8	101.0	101.0	101.8	-1.0	Not bred
5103	1	F	102.6	101.8	102.4	101.2	101.2	102.0	-1.4	Not bred
5105	1	F	102.2	102.8	103.0	102.6	102.0	101.6	+0.8	Not bred
5117	1	F	102.2	102.0	102.0	102.0	101.6	102.0	-0.6	Not bred
5125	1	F	102.4	102.0	101.8	102.0	101.8	102.0	-0.6	Not bred
5141	6 mo.	F	101.8	102.2	102.2	102.2	101.8	102.2	+0.4	Not bred
5147	5-1/2 mo.	F	102.4	102.4	102.8	102.6	102.4	102.6	+0.2	Not bred
5130	6 mo.	F	102.6	103.0	101.8	102.4	102.6	101.8	+0.4	Not bred
5143	6 mo.	F	102.6	102.8	103.0	102.8	102.4	102.6	+0.4	Not bred
5138	6 mo.	F	102.0	102.4	102.2	102.6	101.2	101.8	+0.6	Not bred
5146	5-1/2 mo.	F	102.4	102.8	102.4	102.6	102.0	102.0	+0.4	Not bred
5154	5 mo.	F	102.6	102.4	103.2	102.2	102.0	101.6	+0.6	Not bred

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/4	5-1/2	6-3/4	8	Δt	Stage of preg.
					Barn 134 - 2/7/63					
5017	2	F	102.4	101.8	102.0	101.6	101.4	101.2	-1.2	
5033	2	F	102.8	101.2	101.4	101.2	102.0	100.8	-2.0	3-1/2 mo.
5045	2	F	102.6	101.4	101.4	101.4	101.6	100.8	-1.8	
5008	2	F	102.6	102.0	101.6	101.4	101.8	102.0	-1.2	
4965	2-1/2	F	102.2	101.4	102.4	102.0	101.6	102.0	+0.2	
5024	2	F	102.8	102.0	101.6	102.0	102.0	102.0	-1.2	
5048	2	F	103.6	102.6	102.8	102.4	102.2	101.6	-2.0	
5037	2	F	102.2	102.0	101.6	101.6	101.4	101.4	-0.8	
5023	2	F	102.6	102.0	101.8	101.8	101.6	101.6	-1.0	
4948	2-1/2	M	101.6	101.6	101.6	101.8	101.4	101.6	+0.2	
					Barn 133 - 2/12/63					
5038			102.8	101.0	101.0	101.0	101.8	101.2	-1.6	
5035			102.6	102.2	101.8	101.6	101.6	100.6	-2.0	
5045			101.8	101.6	101.8	101.0	101.2	101.6	-0.8	
5025			102.8	102.0	101.6	101.4	102.0	101.0	-1.0	
5036			101.2	101.6	101.8	101.6	101.8	101.6	+0.6	
5020			101.0	100.8	101.6	101.6	101.6	101.4	+0.6	
4951			101.2	101.4	101.4	101.6	101.8	101.0	+0.4	
5006			102.6	102.4	102.4	102.2	102.2	101.6	-1.0	
4990			102.4	102.2	101.8	101.6	102.0	101.2	-1.2	
5016			102.6	102.0	102.8	101.8	101.6	101.6	-1.0	
5031			102.2	102.0	101.8	102.0	102.0	101.4	-0.8	
5032			102.8	102.6	102.4	101.8	101.8	101.4	-1.4	
					Barn 130 - 2/14/63					
3925		F	101.6	101.6	101.2	101.0	101.0	100.6	-1.0	6-1/2 mo.
3975			101.8	101.2	101.4	100.8	101.2	100.6	-1.2	7 mo.
3908			101.6	101.4	101.0	101.0	100.8	100.4	-1.2	8 mo.
3930			101.8	101.8	101.2	101.0	100.8	101.0	-1.0	7 mo.

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/4	5-1/2	6-3/4	8	Δt	Stage of preg.
4078			101.2	101.0	100.4	100.4	100.2	100.8	-1.0	7-1/2 mo.
3775			101.6	101.6	101.2	101.2	101.0	100.8	-0.8	7 mo.
3914			101.8	102.4	101.4	101.4	101.4	101.6	+0.6	7-1/2 mo.
3740			102.4	101.8	101.2	101.2	101.2	101.4	-1.2	7-1/2 mo.
3900		F	101.2	101.6	101.2	101.8	101.2	101.2	0.6	7-1/2 mo.
3929		F	101.8	101.6	101.8	101.0	101.4	101.2	-0.8	7-1/2 mo.
3650		F	102.0	102.0	101.8	101.0	101.2	101.2	-1.0	7-1/2 mo.
5027		M	100.8	101.4	101.4	101.0	101.0	101.4	+0.6	
					Barn 129 - 2/14/63					
4958	2-1/2	F	101.0	101.4	101.4	101.2	101.2	100.6	0.4	3 mo.
4815	3-1/2		101.0	101.2	101.2	100.8	101.4	100.8	0.4	7 mo.
3571	11		101.4	101.2	101.2	101.4	101.0	101.0	-0.4	7-1/2 mo.
3907	9		100.8	101.2	101.2	101.0	101.2	100.8	+0.4	7-1/2 mo.
4961	3-1/2		101.8	101.4	101.4	101.2	100.0	100.4	-0.8	3 mo.
					3/16/63					
4961			101.8	101.4	100.2	101.0	100.8	100.8	-1.6	
4958			102.2	102.0	100.6	101.4	101.6	101.2	-1.6	
4815			102.6	101.6	101.6	101.6	102.0	102.0	-1.0	
3907			101.8	101.0	100.6	100.6	100.6	100.8	-1.2	
4437			102.4	102.0	101.8	101.6	101.6	101.6	-0.8	
3571			102.2	102.2	101.4	101.4	101.6	101.4	-0.6	
3615			102.0	101.6	101.8	102.0	101.6	102.0	-0.4	
3892			101.8	101.2	101.6	101.6	101.4	101.8	-0.2	
4923			102.4	101.6	101.0	100.8	101.6	101.6	-1.4	
3739			101.8	101.2	100.4	100.8	101.0	101.0	-1.0	
					4/18/63					
4958			101.8	101.6	101.0	101.0	101.0	101.2	-0.8	
4961			102.0	101.0	101.0	101.6	101.6	101.2	-1.0	

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/2	5-1/2	6-3/4	8	t	Stage of preg.
										calved
4815			102.6	102.2	101.0	102.2	101.4	101.8	-1.6	4/4/63
3907			101.2	101.2	100.6	100.6	100.8	101.0	-0.6	4/12/63
3571			103.4	102.6	102.2	102.8	102.6	101.8	-1.8	4/18/63
3892			101.4	101.4	100.6	101.4	101.8	101.4	-0.8	4/17/63
3739			101.2	100.8	100.8	100.4	101.0	101.0	-0.6	Not preg.
4940			101.4	101.8	101.0	101.8	101.2	101.4	+0.4	
4923			101.6	101.8	101.0	101.8	101.0	101.2	+0.2	
3615			101.4	101.2	100.4	101.0	100.8	101.0	-0.6	3/29/63
4437			102.0	102.0	101.6	102.0	101.2	102.2	+0.2	
					Barn 128 - 4/18/63					
3796			101.6	100.8	101.2	100.6	100.8		-1.0	
4696			101.8	100.4	100.8	101.6	101.2		-1.0	
4697			101.6	101.6	101.6	100.6	101.4		-1.0	
3767			101.6	101.6	101.6	101.2	101.4		-0.4	
					Barn 129 - 5/9/63					
4958			102.0	101.6	101.2	101.4	101.0		-0.8	
4961			102.2	101.6	101.4	101.4	101.6		-0.8	
4815			102.4	102.0	101.4	102.0	102.4		-1.0	
3907			101.6	100.6	100.6	101.0	101.4		-1.0	
3571			101.4	101.4	101.4	101.4	101.6		+0.2	
4437			104.0	103.2	103.0	103.2	103.0		-1.0	
3892			101.2	101.4	101.4	101.0	101.4		+0.2	
3615			101.4	101.2	101.0	100.4	100.8		-1.0	
4923			100.8	101.4	101.2	101.4	101.6		+0.8	
3739			101.4	100.8	100.6	100.8	101.0		-0.8	
4940			102.0	101.4	101.4	101.6	101.6		-0.6	
					Barn 128 - 5/16/63					
3796			100.6	100.8	100.8	101.2	101.4	101.6	+1.0	
4696			101.6	101.4	100.2	101.0	100.2	101.0	-1.4	

Animal no.	Age	Sex	Temperature (in hours) 0	3	4-1/4	5-1/2	6-3/4	8	Δt	Stage of preg.
4697			101.8	101.4	100.8	101.2	101.2	101.4	-1.0	
3767			101.6	100.8	101.4	100.6	101.0	100.8	-1.0	
			Barn 129 - 5/16/63							
4958			101.8	101.6	100.6	100.6	101.4	101.4	-1.2	
4715			102.0	101.4	100.4	100.0	100.0	100.0	-2.0	
3571			101.4	100.8	101.0	100.6	99.4	99.8	-2.0	
3892			100.8	101.2	101.0	100.4	100.4	100.8	+0.4	
3739			101.4	101.2	101.0	101.2	101.0	101.0	+0.4	
4940			102.0	101.6	101.2	101.6	102.0	102.0	-0.4	
4923			101.8	101.0	101.0	101.2	101.0	101.2	-0.8	
3615			101.4	101.2	101.0	101.2	101.0	101.2	-0.4	
4437			101.8	101.4	101.4	101.2	101.4	101.4	0.4	
3907			101.6	100.6	100.2	101.0	100.4	100.6	-1.4	
4961			102.2	101.4	101.4	101.2	101.0	101.0	-1.2	

Experiment II (cattle infected with killed Myco. bovis)

Animal no.	Dosage tuberculin in ml.	0	1-1/2	3	4-1/2	6	7-1/2
4998	3/4	100.6	101.2	102.0	102.2	103.8	106.0
	6	101.0	101.8	102.8	104.0	104.8	104.0
5004	1-1/2	102.2	102.6	103.6	104.2	104.8	104.0
	3	101.4	102.2	103.0	103.6	103.4	102.2
4978	3	101.6	101.8	102.8	104.4	106.2	107.8
	1-1/2	103.2	103.8	103.8	103.4	103.4	102.8
4987	6	101.4	102.6	104.8	105.4	106.4	105.6
	3/4	101.6	102.0	102.8	102.8	103.6	103.4

Experiment III (cattle infected with Myco. paratuberculosis)

Animal no.	Date	Antigen	Dose	0	2	3	4	6	8
5114	6/21/62	J	3	101.8	103.4	104.4	105.4	104.4	102.4
	11/20/62	TBC	3	102.0	102.6	102.6	102.2	101.4	101.4
	11/29/62	J	3	102.0	103.6	102.6	102.8	105.6	106.2
5122	6/21/62	J	3	101.6	103.9	104.0	106.0	106.6	105.9
	11/20/62	TBC	3	102.2	102.8	103.0	102.2	102.2	102.2
	11/29/62	J	3	101.2	102.0	102.0	103.4	104.8	106.2
5123	6/21/62	J	3	101.6	103.2	102.4	102.2	102.2	102.2
	11/20/62	TBC	3	101.0	101.2	102.6	101.6	102.2	101.6
	11/29/62	J	3	101.3	102.2	102.2	101.8	101.0	103.8
5127	6/21/62	J	3	101.5	102.8	102.8	103.6	103.8	103.6
	11/20/62	TBC	3	102.0	102.4	103.0	102.2	102.8	102.0
	11/29/62	J	3	102.0	102.8	102.6	102.5	101.8	103.4
				0	3	4-1/4	5-1/2	6-3/4	8
5127	5/22/63	TBC	1-1/2	102.0	103.2	103.0	103.0	103.0	102.0
	5/28/63	J	5	102.0	104.2	105.4	106.6	105.0	104.0
	7/3/63	TBC	3/4	101.8	101.8	101.6	102.5	102.0	103.0
	8/30/63	TBC	1-1/2	101.2	101.8	102.2	101.8	102.4	102.6
	9/3/63	J	5	101.8	102.4	103.6	105.0	105.2	104.0
5170	5/22/63	J	5	101.8	102.8	103.4	104.8	105.0	104.6
	7/3/63	TBC	3/4	101.6	102.2	101.8	101.6	101.4	102.0
	8/30/63	TBC	1-1/2	101.8	101.6	101.6	102.4	102.6	101.6
	9/3/63	J	5	101.6	100.0	101.0	101.8	102.2	103.2
	9/4/63	J	5	102.2	102.2	102.2	102.4	102.2	102.6

Experiment IV (cattle infected with Runyon Group III atypical Mycobacterium)

Animal no.	Date	Antigen	Dose	Group 1[a] 0	2	3	4	6	8
5278	5/7/64	TBC	1-1/2	101.4	101.8	102.8	102.0	101.4	101.4
	6/24/64	TBC	1-1/2	101.4	100.6	100.6	100.8	101.0	101.2
	7/2/64	J	4	100.0	101.2	101.8	102.8	103.0	103.2
5279	5/7/64	TBC	1-1/2	101.0	103.6	103.0	102.2	101.4	101.4
	6/24/64	TBC	1-1/2	100.8	102.0	103.0	103.4	102.0	101.0
	7/2/64	J	4	100.0	101.4	101.4	103.8	103.8	103.0
5280	5/7/64	TBC	1-1/2	101.2	102.6	103.2	103.0	101.4	101.2
	6/24/64	TBC	1-1/2	100.4	102.6	102.8	102.0	101.2	101.2
	7/2/64	J	4	101.2	100.8	101.2	103.2	104.2	103.8

				Group 2[b] 0	2	3-1/2	5	6-1/2	8
5337	10/30/64	TBC	1-1/2	101.2	101.4	104.2	103.0	102.0	101.2
5338	10/30/64	TBC	1-1/2	101.4	103.0	102.8	103.4	101.6	101.8
5334	10/30/64	TBC	1-1/2	100.4	102.0	104.2	104.8	101.2	101.6
5340	10/30/64	TBC	1-1/2	100.4	102.6	104.0	104.6	101.6	100.6

Animal no.	Date	Antigen	Dose	0	1	2	3	4	5	6	7	8
5337	12/10/64	J	4 ml.	101.0	102.0	102.0	101.4	101.8	102.0	102.0	102.0	100.8
5338	12/10/64	J	4 ml.	101.6	102.0	101.8	102.4	102.0	102.0	102.0	102.0	101.6

[a]Infected on April 8, 1964.

[b]Infected on September 4, 1964.

Animal no.	Date	Antigen	Dose	0	1	2	3	4	5	6	7	8
5334	12/10/64	J	4 ml.	100.2	101.6	102.6	101.6	102.2	102.2	102.4	101.0	101.8
5340	12/10/64	J	4 ml.	100.4	101.6	102.4	101.6	102.2	102.4	101.4	102.0	.....

Experiment V (naturally infected cattle)

Animal no.	ARS	Dosage tuberculin in ml.	0	2	3-1/2	5	6-1/2	8	
5108		1-1/2	102.4	103.4	104.4	105.6	104.6	103.6	
5172			101.4	103.0	103.6	103.8	103.2	103.0	
5215			100.4	102.8	103.2	106.8	105.4	103.6	
5216			101.2	103.0	104.8	104.4	104.2	103.6	
S-6777		3/4	100.4	101.4	101.0	102.4	104.4	107.0	
6783		3/8	100.3	102.0	101.8	103.8	105.8	106.6	
6780		3/4	101.9	102.3	103.2	104.0	104.5	105.0	
			0	3	4-1/4	5-1/2	6-3/4	8	9
R-92698		3/4	99.8	101.4	100.8	100.8	101.8	103.2	104.6
13300		3/4	101.0	101.0	101.2	101.8	103.4	103.2	103.8
13280		3/4	102.0	101.8	101.2	102.2	102.0	103.0	103.2
			0	3	4-1/4	5-3/4	7-1/2		
6512		3/4	100.2	103.4	103.6	102.4	102.2		
6511		1-1/2	101.2	102.0	102.8	105.6	107.4		
6510		3/4	101.4	103.8	106.0	106.7	104.5		
6502		1-1/2	101.5	103.6	104.2	104.6	105.4		

Animal no.	ARS	Dosage tuberculin in ml.	0	3-1/2	5	6-1/2	8
4585		1-1/2	101.6	104.2	104.0	102.4	102.2
0985		1-1/2	101.4	105.0	104.8	103.0	102.8
4584		1-1/2	101.8	104.0	105.0	103.0	101.4
6602		1-1/2	101.4	104.4	105.2	104.0	102.8
6278			101.4	105.2	104.4	102.6	101.6
6277			102.0	103.4	102.0	101.4	101.8
4583			101.4	105.2	104.6	102.2	101.6

Experiment VI (cattle artificially infected with Myco. bovis)

Key to tables for Experiment VI

Animals	Antigen	Sequence dates: Temperature #1 (before infection)	Sequence dates: Temperature #2 (after infection)
1 - 5306	1 - No treatment	1 - 1/19/65	8/24/65
2 - 5302	2 - 10 ml. saline	2 - 2/2/65	9/7/65
3 - 5286	3 - 10 ml. dil. tuberculin	3 - 2/23/65	9/21/65
4 - 5304	4 - 1-1/2 ml. dialyzed TBC	4 - 3/2/65	10/5/65
5 - 5287	5 - 1-1/2 ml. reg. ARS tuberculin	5 - 3/16/65	10/19/65
6 - 5294	6 - 75,000 T.U. PPD Parke-Davis	6 - 3/30/65	11/1/65
7 - 5282			
8 - 5276			

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
1	1	1	0	101.4	101.0
1	1	1	1	100.8	101.0
1	1	1	2	101.8	101.2
1	1	1	3	101.0	101.2
1	1	1	4	101.4	101.6
1	1	1	5	102.0	100.4
1	1	1	6	101.8	99.4
1	1	1	7	102.0	99.4
1	1	1	8	101.0	101.2
1	1	1	12		
1	2	2	0	101.0	101.4
1	2	2	1	101.8	101.0
1	2	2	2	101.8	101.2
1	2	2	3	102.0	101.4
1	2	2	4	101.2	101.4
1	2	2	5	101.6	101.8
1	2	2	6	102.2	100.2
1	2	2	7	102.0	100.6
1	2	2	8	101.6	100.6
1	2	2	12		100.8
1	3	3	0	101.4	100.6
1	3	3	1	102.2	100.8
1	3	3	2	102.4	101.6
1	3	3	3	102.4	102.8
1	3	3	4	103.0	104.4
1	3	3	5	104.0	105.4
1	3	3	6	104.6	106.8
1	3	3	7	103.2	105.4
1	3	3	8	102.6	104.0
1	3	3	12		102.6

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
1	4	4	0	101.2	102.4
1	4	4	1	101.6	100.8
1	4	4	2	100.8	102.4
1	4	4	3	102.0	101.8
1	4	4	4	101.0	101.2
1	4	4	5	102.0	101.6
1	4	4	6	102.2	103.2
1	4	4	7	101.6	105.4
1	4	4	8	101.4	105.4
1	4	4	12		103.4
1	5	5	0	101.4	101.4
1	5	5	1	102.0	101.8
1	5	5	2	101.6	101.4
1	5	5	3	102.4	101.6
1	5	5	4	102.8	105.2
1	5	5	5	103.2	106.0
1	5	5	6	103.0	105.6
1	5	5	7	102.6	104.6
1	5	5	8	102.0	104.6
1	5	5	12		101.6
1	6	6	0	101.4	101.2
1	6	6	1	101.4	100.6
1	6	6	2	101.2	101.6
1	6	6	3	102.0	102.2
1	6	6	4	102.0	101.6
1	6	6	5	102.3	101.6
1	6	6	6	102.0	100.2
1	6	6	7	101.8	101.8
1	6	6	8	101.6	101.4
1	6	6	12		

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
2	2	1	0	101.4	101.4
2	2	1	1	102.0	101.8
2	2	1	2	102.8	100.0
2	2	1	3	101.6	101.4
2	2	1	4	101.6	101.8
2	2	1	5	101.6	101.0
2	2	1	6	102.4	102.4
2	2	1	7	102.0	100.8
2	2	1	8	102.4	101.6
2	2	1	12		
2	3	2	0	101.0	102.2
2	3	2	1	102.0	102.2
2	3	2	2	102.8	102.6
2	3	2	3	102.4	102.6
2	3	2	4	102.2	104.0
2	3	2	5	102.8	104.2
2	3	2	6	103.4	103.8
2	3	2	7	102.6	102.4
2	3	2	8	102.6	102.4
2	3	2	12		101.6
2	4	3	0	101.6	101.2
2	4	3	1	102.6	101.8
2	4	3	2	101.6	101.0
2	4	3	3	102.2	100.8
2	4	3	4	101.4	100.6
2	4	3	5	101.6	101.6
2	4	3	6	102.4	103.0
2	4	3	7	102.4	105.6
2	4	3	8	101.8	105.8
2	4	3	12		102.0

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
2	5	4	0	100.6	102.2
2	5	4	1	101.2	102.2
2	5	4	2	101.6	103.4
2	5	4	3	102.4	102.2
2	5	4	4	101.8	102.6
2	5	4	5	101.6	103.6
2	5	4	6	102.2	104.8
2	5	4	7	101.6	103.4
2	5	4	8	101.4	102.4
2	5	4	12		100.6
2	6	4	0	101.8	101.0
2	6	4	1	102.2	102.0
2	6	4	2	101.4	101.4
2	6	4	3	102.2	100.0
2	6	4	4	101.8	102.4
2	6	4	5	102.0	101.4
2	6	4	6	102.6	101.6
2	6	4	7	101.4	101.6
2	6	4	8	101.8	102.4
2	6	4	12		101.4
2	1	4	0	101.2	101.2
2	1	4	1	101.6	99.4
2	1	4	2	101.0	101.6
2	1	4	3	102.6	102.2
2	1	4	4	102.2	100.8
2	1	4	5	101.2	98.6
2	1	4	6	101.6	101.4
2	1	4	7	101.8	100.6
2	1	4	8	101.6	101.4
2	1	4	12		

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
3	3	1	0	100.8	101.8
3	3	1	1	102.8	101.2
3	3	1	2	102.8	102.4
3	3	1	3	102.4	102.4
3	3	1	4	102.4	102.6
3	3	1	5	103.2	103.4
3	3	1	6	102.4	102.8
3	3	1	7	102.0	103.2
3	3	1	8	101.8	102.6
3	3	1	12		
3	4	2	0	101.0	101.6
3	4	2	1	102.0	103.0
3	4	2	2	102.8	102.4
3	4	2	3	102.4	102.0
3	4	2	4	102.4	101.2
3	4	2	5	101.6	102.2
3	4	2	6	103.6	102.0
3	4	2	7	102.4	102.2
3	4	2	8	101.4	102.0
3	4	2	12		101.6
3	5	3	0	100.4	101.2
3	5	3	1	102.4	102.6
3	5	3	2	102.0	103.0
3	5	3	3	102.6	102.2
3	5	3	4	102.4	101.8
3	5	3	5	102.4	104.0
3	5	3	6	102.0	103.0
3	5	3	7	101.2	102.8
3	5	3	8	102.4	101.2
3	5	3	12		101.0
3	6	4	0	100.6	102.2
3	6	4	1	101.6	102.2
3	6	4	2	102.2	101.8
3	6	4	3	103.0	100.4
3	6	4	4	102.6	100.0
3	6	4	5	103.4	100.0
3	6	4	6	103.4	102.0
3	6	4	7	103.2	100.2
3	6	4	8	102.6	101.6
3	6	4	12		102.2
3	1	5	0	101.0	101.6
3	1	5	1	102.2	102.6
3	1	5	2	102.0	102.6
3	1	5	3	102.2	102.6
3	1	5	4	101.8	100.2
3	1	5	5	102.6	100.8
3	1	5	6	103.6	102.4
3	1	5	7	102.4	102.4
3	1	5	8	102.2	100.8
3	1	5	12		102.0
3	2	6	0	101.4	99.6
3	2	6	1	102.0	101.2
3	2	6	2	101.8	102.0
3	2	6	3	101.8	101.0
3	2	6	4	102.4	101.2
3	2	6	5	102.8	101.2
3	2	6	6	102.6	101.8
3	2	6	7	103.6	101.6
3	2	6	8	103.0	101.6
3	2	6	12		

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
4	4	1	0	103.0	101.6
4	4	1	1	102.6	101.8
4	4	1	2	102.6	102.6
4	4	1	3	102.0	102.0
4	4	1	4	102.0	103.4
4	4	1	5	102.0	106.2
4	4	1	6	101.8	106.4
4	4	1	7	101.8	106.0
4	4	1	8	102.0	105.0
4	4	1	12		
4	5	2	0	101.6	102.4
4	5	2	1	103.0	102.0
4	5	2	2	103.2	102.2
4	5	2	3	102.2	103.0
4	5	2	4	102.2	103.2
4	5	2	5	102.2	105.6
4	5	2	6	103.0	106.6
4	5	2	7	102.8	105.6
4	5	2	8	102.0	104.0
4	5	2	12		102.2
4	6	3	0	101.6	101.6
4	6	3	1	103.4	102.0
4	6	3	2	102.6	101.4
4	6	3	3	102.0	100.0
4	6	3	4	102.4	100.6
4	6	3	5	103.0	101.0
4	6	3	6	103.0	101.6
4	6	3	7	102.2	101.8
4	6	3	8	102.2	101.6
4	6	3	12		100.2

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
4	1	4	0	101.0	100.6
4	1	4	1	101.4	100.6
4	1	4	2	101.4	100.4
4	1	4	3	101.8	101.4
4	1	4	4	101.4	100.2
4	1	4	5	102.0	100.4
4	1	4	6	102.6	101.6
4	1	4	7	101.8	100.4
4	1	4	8	101.4	101.6
4	1	4	12		100.8
4	2	5	0	101.8	102.6
4	2	5	1	102.0	102.0
4	2	5	2	101.6	100.8
4	2	5	3	101.8	102.0
4	2	5	4	101.6	101.6
4	2	5	5	101.8	101.4
4	2	5	6	103.4	102.0
4	2	5	7	102.2	102.6
4	2	5	8	102.2	101.2
4	2	5	12		101.0
4	3	6	0	100.8	101.6
4	3	6	1	102.0	101.4
4	3	6	2	101.6	102.0
4	3	6	3	101.8	102.6
4	3	6	4	101.6	101.0
4	3	6	5	102.0	102.0
4	3	6	6	101.6	101.4
4	3	6	7	102.4	103.0
4	3	6	8	102.0	102.4
4	3	6	12		

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
5	5	1	0	101.6	100.8
5	5	1	1	102.0	101.4
5	5	1	2	102.0	101.4
5	5	1	3	101.8	102.6
5	5	1	4	101.6	104.0
5	5	1	5	101.6	105.0
5	5	1	6	102.4	104.6
5	5	1	7	101.6	104.2
5	5	1	8	101.8	103.4
5	5	1	12		
5	6	2	0	101.6	100.6
5	6	2	1	102.4	101.6
5	6	2	2	102.8	101.0
5	6	2	3	102.4	102.4
5	6	2	4	101.8	102.2
5	6	2	5	102.2	102.0
5	6	2	6	102.4	102.4
5	6	2	7	102.2	103.0
5	6	2	8	102.0	102.8
5	6	2	12		102.4
5	1	3	0	101.2	100.6
5	1	3	1	101.6	101.2
5	1	3	2	102.0	101.0
5	1	3	3	101.6	100.6
5	1	3	4	101.4	101
5	1	3	5	101.4	101
5	1	3	6	102.6	101.4
5	1	3	7	101.4	100.6
5	1	3	8	102.0	100.8
5	1	3	12		101.6

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
5	2	4	0	101.0	101.0
5	2	4	1	101.4	100.8
5	2	4	2	102.0	101.2
5	2	4	3	102.8	101.6
5	2	4	4	101.2	99.4
5	2	4	5	101.0	99.8
5	2	4	6	101.4	101.2
5	2	4	7	101.4	101.4
5	2	4	8	101.0	101.6
5	2	4	12		102.0
5	3	5	0	101.6	101.2
5	3	5	1	102.4	102.0
5	3	5	2	102.4	101.8
5	3	5	3	102.0	101.6
5	3	5	4	102.4	102.2
5	3	5	5	102.6	103.4
5	3	5	6	103.4	104.4
5	3	5	7	102.2	105.2
5	3	5	8	101.8	105.0
5	3	5	12		103.0
5	4	6	0	101.4	101.2
5	4	6	1	102.0	100.6
5	4	6	2	101.6	101.4
5	4	6	3	102.0	101.0
5	4	6	4	102.0	101.4
5	4	6	5	102.0	102.2
5	4	6	6	102.6	102.6
5	4	6	7	103.0	102.6
5	4	6	8	101.6	102.6
5	4	6	12		

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
6	6	1	0	102.0	101.0
6	6	1	1	100.6	102.0
6	6	1	2	101.2	101.6
6	6	1	3	101.6	102.0
6	6	1	4	101.4	101.0
6	6	1	5	102.2	102.4
6	6	1	6	102.6	102.8
6	6	1	7	101.4	102.6
6	6	1	8	101.8	103.0
6	6	1	12		
6	1	2	0	101.8	101.4
6	1	2	1	101.4	101.2
6	1	2	2	102.2	99.0
6	1	2	3	101.6	99.2
6	1	2	4	102.0	98.6
6	1	2	5	101.4	101.6
6	1	2	6	101.8	100.6
6	1	2	7	102.0	101.6
6	1	2	8	101.8	102.0
6	1	2	12		101.0
6	2	3	0	101.0	101.6
6	2	3	1	101.8	102.1
6	2	3	2	101.8	102.0
6	2	3	3	101.6	102.2
6	2	3	4	101.6	102.2
6	2	3	5	102.0	103.0
6	2	3	6	102.2	101.2
6	2	3	7	101.8	100.6
6	2	3	8	102.0	101.0
6	2	3	12		101.0
6	3	4	0	100.6	101.4
6	3	4	1	100.4	102.2
6	3	4	2	101.6	101.6
6	3	4	3	101.4	104.8
6	3	4	4	101.4	106.0
6	3	4	5	102.6	105.8
6	3	4	6	102.4	104.6
6	3	4	7	102.4	103.4
6	3	4	8	102.0	102.6
6	3	4	12		102.4
6	4	5	0	101.0	102.0
6	4	5	1	102.0	102.0
6	4	5	2	101.4	102.8
6	4	5	3	101.6	102.6
6	4	5	4	101.8	102.4
6	4	5	5	102.0	103.4
6	4	5	6	102.4	103.0
6	4	5	7	102.0	103.0
6	4	5	8	102.0	103.4
6	4	5	12		103.0
6	5	6	0	100.6	101.0
6	5	6	1	101.4	101.0
6	5	6	2	101.4	101.0
6	5	6	3	102.0	101.6
6	5	6	4	102.2	101.4
6	5	6	5	103.0	103.4
6	5	6	6	102.8	103.0
6	5	6	7	103.0	103.2
6	5	6	8	102.4	103.4
6	5	6	12		

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.	Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
7	1	1	0	101.8	101.2	7	4	4	0	101.2	101.2
7	1	1	1	101.8	100.8	7	4	4	1	101.6	100.8
7	1	1	2	101.6	100.4	7	4	4	2	102.0	100.8
7	1	1	3	102.0	101.2	7	4	4	3	102.0	101.6
7	1	1	4	101.2	100.8	7	4	4	4	102.2	101.8
7	1	1	5	102.4	101.2	7	4	4	5	101.6	100.8
7	1	1	6	102.6	101.8	7	4	4	6	101.0	100.8
7	1	1	7	102.0	102.0	7	4	4	7	101.8	101.8
7	1	1	8	101.8	102.0	7	4	4	8	102.4	102.0
7	2	2	0	100.8	101.4	7	5	5	0	101.8	101.4
7	2	2	1	100.0	100.4	7	5	5	1	101.6	101.4
7	2	2	2	101.2	100.8	7	5	5	2	102.6	102.0
7	2	2	3	101.6	101.8	7	5	5	3	102.8	101.0
7	2	2	4	100.0	101.4	7	5	5	4	102.4	101.2
7	2	2	5	101.0	100.8	7	5	5	5	103.0	101.0
7	2	2	6	101.6	101.0	7	5	5	6	102.6	102.0
7	2	2	7	100.8	101.2	7	5	5	7	102.0	101.6
7	2	2	8	101.8	101.2	7	5	5	8	100.8	102.0
7	3	3	0	101.6	100.4	7	6	6	0	100.0	100.8
7	3	3	1	101.6	100.4	7	6	6	1	102.4	100.8
7	3	3	2	101.6	102.4	7	6	6	2	101.8	100.4
7	3	3	3	101.8	102.6	7	6	6	3	101.8	101.4
7	3	3	4	101.4	102.0	7	6	6	4	101.6	101.2
7	3	3	5	101.0	101.8	7	6	6	5	101.6	100.6
7	3	3	6	101.4	101.4	7	6	6	6	102.0	101.2
7	3	3	7	101.0	102.6	7	6	6	7	101.8	101.2
7	3	3	8	101.8	101.4	7	6	6	8	102.0	100.6
8	4	1	0	101.6	101.6	8	1	4	0	100.2	101.4
8	4	1	1	101.4	101.6	8	1	4	1	101.0	101.0
8	4	1	2	101.8	101.2	8	1	4	2	101.8	101.6
8	4	1	3	102.0	101.4	8	1	4	3	101.8	101.6

Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.	Animal no.	Antigen no.	Sequence no.	Hour	#1 Temp.	#2 Temp.
8	4	1	4	101.4	101.0	8	1	4	4	101.8	101.4
8	4	1	5	102.2	101.2	8	1	4	5	101.8	100.2
8	4	1	6	102.2	101.4	8	1	4	6	101.2	102.0
8	4	1	7	101.4	101.6	8	1	4	7	102.4	102.0
8	4	1	8	101.8	101.6	8	1	4	8	102.0	101.4
8	5	2	0	100.4	101.2	8	2	5	0	101.4	101.0
8	5	2	0	101.2	98.6	8	2	5	1	100.4	100.4
8	5	2	2	101.4	102.0	8	2	5	2	101.6	101.0
8	5	2	3	102.6	100.8	8	2	5	3	101.8	101.2
8	5	2	4	101.8	102.0	8	2	5	4	101.6	101.0
8	5	2	5	101.6	101.6	8	2	5	5	102.8	100.0
8	5	2	6	102.0	101.2	8	2	5	6	102.4	99.8
8	5	2	7	102.6	102.2	8	2	5	7	101.0	101.0
8	5	2	8	102.0	101.2	8	2	5	8	101.2	102.0
8	6	3	0	100.4	101.8	8	3	6	0	101.4	100.8
8	6	3	1	101.8	101.4	8	3	6	1	102.0	100.0
8	6	3	2	101.8	101.6	8	3	6	2	102.4	101.8
8	6	3	3	102.4	101.6	8	3	6	3	101.6	100.6
8	6	3	4	101.4	101.2	8	3	6	4	101.8	100.4
8	6	3	5	101.2	101.8	8	3	6	5	101.8	101.4
8	6	3	6	101.4	101.6	8	3	6	6	101.2	102.0
8	6	3	7	101.4	102.2	8	3	6	7	100.8	101.4
8	6	3	8	101.4	102.4	8	3	6	8	102.4	101.6