# AN INVESTIGATION INTO THE EFFECT OF PASTEURISATION ON THE BOVINE TUBERCLE BACILLUS IN NATURALLY INFECTED TUBERCULOUS MILK.

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(With 1 Text-figure.)

The following investigation into the effect of pasteurisation on the tubercle bacillus was commenced in 1923, in order to ascertain whether the temperatures used in commercial pasteurisation are at all times sufficient to destroy the virulence of the tubercle bacillus in naturally infected tuberculous milk, *i.e.* milk as secreted by a cow suffering from tuberculosis of the udder.

Before this investigation was begun the results obtained by various workers on this problem showed wide differences both in time and temperature. Lists of these investigations which have been published from time to time show variations from 60° C. (140° F.) for 20 minutes reported by Rosenau in 1907, to 70° C. (158° F.) for 45 minutes reported by Woodhead in 1895 as necessary for the effective destruction of the tubercle bacillus in milk.

Since 1923 papers have been published recording the following results:

(1) Campbell-Brown (1923, p. 317) found that by employing cultures of human and bovine tubercle bacilli 60° C. (140° F.) for 20 minutes was sufficient to render the contaminated milk innocuous. In a criticism of Delépine's experiments, Campbell-Brown suggests that Delépine's statement (1914, p. 41) that tubercle bacilli are not killed in 18 minutes at a temperature rising steadily from 60° C. to 94° C. (201·2° F.) cannot be relied upon to disprove Rosenau's belief that tubercle bacilli are killed in 20 minutes at 60° C. As the difference in time is only two minutes and the temperature in Delépine's experiments rose steadily from 60° C. to 94° C., the different results obtained by the two workers may be explained by the circumstance that Delépine used naturally infected milk whilst Rosenau used cultures.

(2) Cameron-Macaulay (1925, p. 297) used tuberculous sputum and ground-up tuberculous glands from condemned carcasses and found that both the human and bovine varieties of tubercle bacilli in milk are destroyed by pasteurisation for 30 minutes at 62·7° C. to 65·5° C. (145 to 150° F.).

(3) Hermima Jenkins (1926, p. 273) carried out two experiments only, at 60° C. and 62·8° C. (145° F.) respectively for 30 minutes, using naturally infected milk, and failed to produce tuberculosis within six weeks in inoculated guinea-pigs.

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(4) R. G. White (1926, p. 222) inoculated 30 guinea-pigs with naturally infected tuberculous milk heated at 62·5° C. (144·5° F.) for 30 minutes and reports one positive result which he explains in the following words: "It is possible that an accident may have occurred. The result, which proved to be the one exception in the tests, I am, therefore, inclined to interpret in this light." In view of the experiments to be presently described I disagree with this statement. With regard to Dr White's experiments it is well to remember that a period of eight minutes was taken to raise the milk to the required temperature—the time taken for cooling the milk is not stated.

(5) C. W. Andersen (1924, p. 177) with an apparatus similar in principle to that used in the present investigation, found that by heating apparently normal milk from cows suffering from tuberculosis of the udder, one guinea-pig out of six injected with the centrifugalised deposit and cream of 20 c.c. of this milk, which had been heated at 63° C. (145·4° F.) for 30 minutes, developed generalised tuberculosis after three and a half months.

(6) In a publication on pasteurisation by American authors (*Public Health Bull.* No. 147, p. 121) tubercle bacilli were found to be destroyed at 59° C. (138° F.) for 30 minutes, 61·1° C. (142° F.) for 30 minutes and 64·4° C. (148° F.) for 30 minutes; the three latter conclusions were based on the results of single experiments made on milk inoculated with cultures of tubercle bacilli.

The foregoing epitome of recent work on this subject shows that of the three workers who used naturally infected milk in their experiments, two report positive results after heating at 62·5° C. to 63° C. (144·5 to 145·4° F.) for 30 minutes; the workers using material other than naturally infected milk report that the tubercle bacillus is killed at temperatures of 62·8° C. (145° F.) for 30 minutes and 60° C. (140° F.) for 20 minutes respectively.

In the present series of experiments particular attention has been paid to the possibilities of "latent infection" described by Delépine (1916, p. 600) who states:

> From these results it would appear that the heated bacilli were at first barely capable of overcoming the resistance offered to them by the phagocytes in the region first infected, but that some bacilli had escaped and had been carried away probably by some of the migratory cells. These bacilli had been able to multiply and to regain their pathogenic power.
>
> The facts observed seem also to indicate that the recuperation of this pathogenic power became more marked as the distance from the seat of inoculation increased, for, while the local lesions remained insignificant, those of distant organs were practically as extensive as those found in the animals inoculated with untreated milk.

With this possibility of latent infection in mind the majority of the guinea-pigs inoculated with heated milk in the present investigation were kept until at least 100 days after inoculation and in some cases the animals were kept 600 days.

The experiments described in the following pages record an attempt to reproduce in the laboratory the conditions under which milk is pasteurised commercially in the pocket type of pasteuriser, great care being taken to ensure that every particle of milk received the same amount of heat.

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### *Apparatus used.*

The milk container or "holder" (Fig. 1) consisted of a cylindrical tinned copper vessel narrowing towards the neck and fitted with a large india-rubber bung. Through the rubber bung were inserted two long thermometers and a long thin piece of metal tubing enclosing the stem of the milk stirrer. The stirrer consisted of a solid bar of tinned copper hollowed out at the base to fit on to a projecting pin on the bottom of the container; soldered to the base of this stirrer rod was a piece of sheet metal shaped like a propeller. The thermometers and the metal tube enclosing the stirrer rod were all embedded tightly in the rubber bung so that when the apparatus was in use the whole could be totally immersed in water without fear of leakage; the only part remaining out of the water was the top of the metal tube which projected several inches above the top of the rubber bung. The only access to the atmosphere was through the small space between the stirrer rod and its enclosing metal tube. The heating and the holding processes were carried out in tall cylindrical vessels containing water heated by means of gas rings. The holding vessel was enclosed in a round tin to prevent draughts and to protect the small flame used.

### *Technique of pasteurisation.*

In most of the experiments the quantity of milk used was 1000–1200 c.c. of the infected milk taken from a single milking of the experimental cow; when the source of this milk was varied reference has been made in the tables to the variation.

The milk was carried to the laboratory and the quantity required for pasteurisation was poured into the previously sterilised holder; at the same time a control sample of the raw milk was taken from the bulk. The stirrer rod was then placed in position and the rubber bung with the thermometers inserted firmly into the neck of the holder so that the two thermometer bulbs reached (*a*) to the lower part of the milk, and (*b*) to a point just under its surface respectively. The temperature of the milk was recorded and the holder immersed up to the milk level in a container of rapidly boiling water. During this pre-heating, which was always completed in less than one and a half minutes, the milk was kept vigorously stirred. When the milk thermometers recorded a temperature of 2° F. below that required for pasteurisation the holder was transferred to the water bath which was held at the pasteurising temperature. Preliminary experiments showed that by removing the holder from the pre-heater at this time the true pasteurising temperature was reached almost immediately the holder was placed in the water bath. The holder was then clamped by means of a retort stand so that the whole of the holder and the bung were suspended and entirely immersed in the water bath. The milk was kept gently stirred during the holding period and any slight variations in the temperature of the water bath were at once adjusted by the addition of hot or cold water; the temperatures of the milk and water bath were recorded every two minutes. At the completion of the holding period the milk was immediately passed over a sterilised milk cooler and the temperature reduced to below 32·2° C. (90° F.) in less than one minute. Samples of the pasteurised milk were immediately centrifugalised and the cream and deposit of 200 c.c. inoculated subcutaneously into the inner aspect of the hind legs of four guinea-pigs. Throughout this investigation the guinea-pigs inoculated with the raw milk before heating developed a tuberculous infection in less than six weeks.

### *Experimental animals.*

The milk of three cows suffering from tuberculosis of the udder was used in this investigation.

*Cow* No. 1. Red Shorthorn cow "Rose." This cow was reported by a Veterinary Inspector as showing typical signs of tuberculosis of the udder. Tubercle bacilli were demonstrated in all four quarters. The milk was abnormal in appearance, a flocculent mass separating out on standing.

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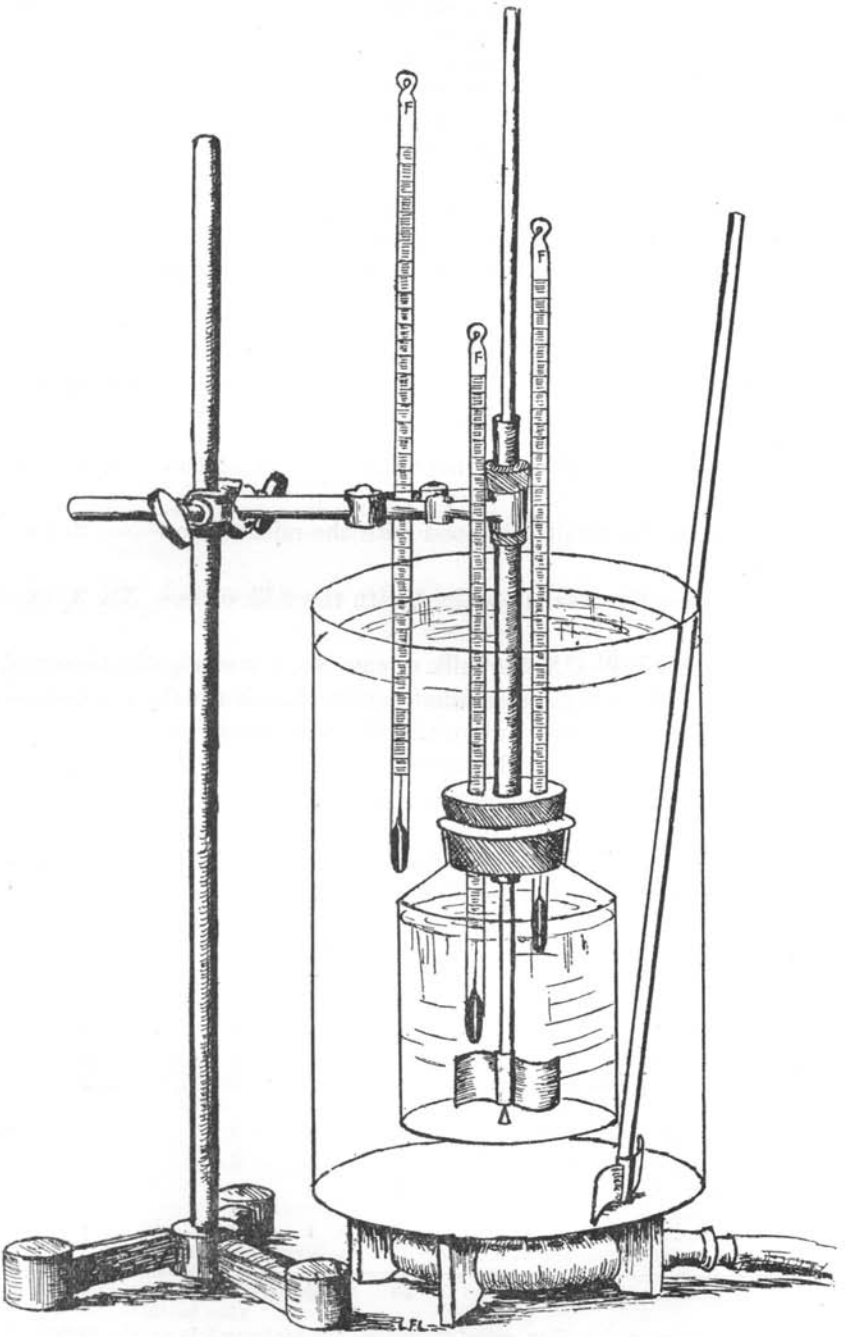


Fig. 1.



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*Cow* No. 2. British Friesian cow "Dorothy." The milk from this cow had previously been proved to contain living tubercle bacilli. The clinical signs of disease were very slight. The right supra mammary gland showed slight enlargement and the udder had a distinct hard swelling high up in the right hind quarter; all the other quarters were apparently normal. It was found by guinea-pig inoculation that both hind quarters were yielding tuberculous milk. The two fore quarters remained unaffected throughout the experiments. The milk remained normal in appearance during the whole of the time that this cow was used as an experimental animal.

*Cow* No. 3. Roan Shorthorn cow "Floss." During the whole of the time that this cow was used as an experimental animal her appearance was that of a typical milking cow and under ordinary circumstances she would without doubt have remained in the dairy herd.

## First series of experiments.

### *Pasteurisation of naturally infected tuberculous milk at* 62·8° C. (145° F.) *for* 30 *minutes.*

Table I gives the results obtained with the milk of cow No. 1, experiments A–F.

Table II gives the results obtained with the milk of cow No. 2, experiments G–V.

Table III gives the results obtained with the milk of cow No. 3, experiments B W–C M.

In experiments B W–C M the milk of cow No. 3 was slightly abnormal in appearance, the colour being somewhat darker than is usually the case—but in the opinion of a number of authorities this milk would have been included in the bulk milk of a herd. Throughout these experiments small flakes of coagulated material were found on the cooler after pasteurisation and cooling.

Table I. *Pasteurisation of milk from cow No.* 1 *at* 62·8° C. (145° F.) *for* 30 *minutes.*

| Experiment | Milk used | Guinea-pigs inoculated | – | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|
| A | Mixed milk | 4 | 4 | 0 | 0 |
| B | Mixed milk | 4 | 3 | 0 | 1 |
| C | Mixed milk | 4 | 4 | 0 | 0 |
| | Coagulated material from cooler in experiment C | 2 | 0 | 1* | 1 |
| D | Milk from left quarters | 4 | 4 | 0 | 0 |
| E | Milk from left quarters | 4 | 4 | 0 | 0 |
| F | Milk from right fore quarter | 4 | 3 | 0 | 1 |
| | Coagulated material from cooler in experiment F | 2 | 1 | 0 | 1 |
| | Total ... ... ... | 28 | 23 | 1 | 4 |

* After pasteurising and cooling, some coagulated material was left on the surface of the cooler. A microscopical examination of this material showed the presence of many acid fast bacilli; when the coagulum was ground up with saline and inoculated into two guinea-pigs, a positive result was obtained in one case. This result was confirmed by re-inoculation into fresh guinea-pigs.

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Table II. *Pasteurisation of milk of cow No.* 2 *at* 62·8° C. (145° F.) *for* 30 *minutes.*

| Experiment | Milk used | Guinea-pigs inoculated | − | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|
| G | Milk from right hind quarter | 4 | 3 | 0 | 1 |
| H | ,, ,, | 4 | 4 | 0 | 0 |
| I | Milk from both hind quarters | 4 | 3 | 0 | 1 |
| J | ,, ,, | 4 | 4 | 0 | 0 |
| K | ,, ,, | 4 | 4 | 0 | 0 |
| L | ,, ,, | 4 | 4 | 0 | 0 |
| M | ,, ,, | 4 | 2 | 1* | 1 |
| N | ,, ,, | 4 | 4 | 0 | 0 |
| O | ,, ,, | 4 | 4 | 0 | 0 |
| P | ,, ,, | 4 | 4 | 0 | 0 |
| Q | Milk from right hind quarter | 4 | 3 | 0 | 1 |
| R | ,, ,, | 4 | 3 | 0 | 1 |
| S | ,, ,, | 4 | 3 | 0 | 1 |
| T | ,, ,, | 4 | 3 | 0 | 1 |
| U | ,, ,, | 4 | 3 | 0 | 1 |
| V | ,, ,, | 4 | 4 | 0 | 0 |
| | Total ... ... ... | 64 | 55 | 1 | 8 |

* Guinea-pig M 3 died of tuberculosis. *Post mortem* appearance—liver, spleen and lungs—advanced tuberculosis. Mesenteric glands enlarged and caseated, left superficial inguinal gland slightly enlarged and caseated. Re-inoculation of *post mortem* material into two fresh guinea-pigs gave positive results.

Table III. *Pasteurisation of milk from cow No.* 3 *at* 62·8° C. (145° F.) *for* 30 *minutes.*

| Experiment | Milk used | Guinea-pigs inoculated | − | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|
| B W | Coagulated material on cooler | 2 | 1 | 0 | 1 |
| B X | ,, ,, | 2 | 2 | 0 | 0 |
| B Y | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| B Z | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C A | Mixed milk | 2 | 1 | 0 | 1 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C B | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C C | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C D | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C E | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C F | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C G | Mixed milk | 2 | 1 | 0 | 1 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C H | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C I | Mixed milk | 2 | 1 | 0 | 1 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C J | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 1 | 0 | 1 |
| C K | Mixed milk | 2 | 1 | 0 | 1 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C L | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| C M | Mixed milk | 2 | 2 | 0 | 0 |
| | Coagulated material | 2 | 2 | 0 | 0 |
| | Total ... ... ... | 64 | 58 | 0 | 6 |



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*Summary of first series of experiments.*

A series of 39 experiments was made on the milk of three cows suffering from tuberculosis of the udder. The milk was in every case heated at 62·8° C. (145° F.) for 30 minutes.

Out of 118 guinea-pigs inoculated with the centrifugalised deposit and cream of the heated milk, one developed generalised tuberculosis; the remaining 117 gave negative results or died of non-tuberculous infection. The positive case (Guinea-pig M 3) died 110 days after inoculation, the infection resembling that described by Delépine in his paper on "Latent Tuberculosis." The local lymphatic glands were only slightly involved but the liver, spleen and lungs were the sites of an extensive infection.

Out of 38 guinea-pigs inoculated with coagulated material found on the cooler after pasteurisation one gave a positive result.

## Second series of experiments.

*Pasteurisation of naturally infected tuberculous milk at 60° C. (140° F.) for 30 minutes.*

Table IV gives the results obtained with the milk of cow No. 2, experiments A A–A R, and cow No. 3, experiments A S–A Y.

Throughout this series of experiments the milk used was apparently normal.

Table IV. *Pasteurisation of milk from cows Nos. 2 and 3 at 60° C. (140° F.) for 30 minutes.*

| Experiment | Milk used | Guinea-pigs inoculated | − | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|
| | Cow No. 2 | | | | |
| A A | Mixed milk | 4 | 4 | 0 | 0 |
| A B | ,, | 4 | 4 | 0 | 0 |
| A C | Milk from hind quarters | 4 | 4 | 0 | 0 |
| A D | ,, ,, | 4 | 3 | 0 | 1 |
| A E | Mixed milk | 4 | 3 | 0 | 1 |
| A F | ,, | 4 | 4 | 0 | 0 |
| A G | Milk from hind quarters | 4 | 4 | 0 | 0 |
| A H | Mixed milk | 4 | 4 | 0 | 0 |
| A I | ,, | 4 | 4 | 0 | 0 |
| A J | Milk from hind quarters | 4 | 4 | 0 | 0 |
| A K | ,, ,, | 4 | 4 | 0 | 0 |
| A L | Mixed milk | 4 | 4 | 0 | 0 |
| A M | ,, | 4 | 4 | 0 | 0 |
| A N | ,, | 4 | 4 | 0 | 0 |
| A O | ,, | 4 | 4 | 0 | 0 |
| A P | ,, | 4 | 4 | 0 | 0 |
| A Q | ,, | 4 | 4 | 0 | 0 |
| A R | ,, | 4 | 4 | 0 | 0 |
| | Cow No. 3 | | | | |
| A S | Mixed milk | 4 | 4 | 0 | 0 |
| A T | ,, | 4 | 4 | 0 | 0 |
| A U | ,, | 4 | 4 | 0 | 0 |
| A V | ,, | 4 | 4 | 0 | 0 |
| A W | ,, | 4 | 3 | 0 | 1 |
| A X | Milk from left hind quarter | 4 | 4 | 0 | 0 |
| A Y | Mixed milk | 4 | 3 | 0 | 1 |
| | Total ... ... | ... 100 | 96 | 0 | 4 |

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*Summary of second series of experiments.*

Twenty-five experiments were made, heating apparently normal tuberculous milk at 60° C. (140° F.) for 30 minutes. One hundred guinea-pigs inoculated with the centrifugalised deposit and cream of the heated milk gave negative results or died of non-tuberculous infection.

## THIRD SERIES OF EXPERIMENTS.

*Pasteurisation of naturally infected tuberculous milk at 60° C. (140° F.) for 20 minutes.*

Table V gives the results obtained with the milk of cow No. 3.

Experiments B A to B S were made at 60° C. (140° F.) for 20 minutes on the milk of cow No. 3. During this period the milk was apparently normal, though at the end of the series of experiments the quantity of milk yielded by the cow had dropped to one half-pint a day. At this stage the milk secreted was slightly darker than ordinary milk—although in the opinion of the writer and also a number of competent judges this milk would have been included in the bulk milk of a dairy herd if the quantity had been sufficient. At this stage small flakes were again found on the cooler after pasteurisation.

Table V. *Pasteurisation of milk of cow No. 3 at 60° C. (140° F.) for 20 minutes.*

| Experiment | Milk used | Guinea-pigs inoculated | − | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|
| B A | Mixed milk | 4 | 4 | 0 | 0 |
| B B | ,, | 4 | 4 | 0 | 0 |
| B C | ,, | 4 | 3 | 0 | 1 |
| B D | ,, | 4 | 4 | 0 | 0 |
| B E | ,, | 4 | 2 | 0 | 2 |
| B F | ,, | 4 | 3 | 0 | 1 |
| B G | ,, | 4 | 3 | 0 | 1 |
| B H | ,, | 4 | 3 | 0 | 1 |
| B I | ,, | 4 | 2 | 0 | 2 |
| B J | ,, | 4 | 3 | 0 | 1 |
| B K | ,, | 4 | 2 | 0 | 2 |
| B L | ,, | 4 | 3 | 0 | 1 |
| B M | ,, | 4 | 3 | 0 | 1 |
| B N | ,, | 4 | 3 | 0 | 1 |
| B O | ,, | 4 | 1 | 0 | 3 |
| B P | ,, | 4 | 4 | 0 | 0 |
| B Q | ,, | 2 | 0 | 1 | 1 |
| | Coagulated material from cooler in experiment B Q | 2 | 0 | 0 | 2 |
| B R | Coagulated material from cooler in experiment B R | 2 | 2 | 0 | 0 |
| B S | Coagulated material from cooler in experiment B S | 2 | 1 | 1 | 0 |
| | Total ... ... ... | 72 | 50 | 2 | 20 |

In experiments B Q, B R, and B S, coagulated material was again noticed on the cooler after pasteurisation. In experiment B Q all of the inoculated guinea-pigs died, but guinea-pig B Q 2 showed signs of tuberculosis. Material was removed from the site of inoculation and local lymphatic glands, and after treatment was re-inoculated into two fresh guinea-pigs, both of which developed generalised tuberculosis.

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*Summary of third series of experiments.*

Seventeen experiments were made with naturally infected tuberculous milk heated at 60° C. (140° F.) for 20 minutes. Out of 66 guinea-pigs inoculated with the centrifugalised deposit and cream of the pasteurised milk one developed tuberculosis, the remaining 65 gave negative results or died of non-tuberculous infections.

Three experiments were made in which coagulated material was found to be present on the cooler after pasteurisation. Out of six guinea-pigs inoculated with this coagulated material, one developed tuberculosis, the remaining five gave negative results or died of non-tuberculous infection.

Both of the positive results recorded in this series of experiments occurred towards the end of the lactation period of cow No. 3 when the milk was slightly abnormal.

FOURTH SERIES OF EXPERIMENTS.

*Pasteurisation of naturally infected tuberculous milk at 59·3° C. (138·8° F.) for 20 minutes.*

Table VI gives the results obtained with the milk of cow No. 3.

In this series of experiments coagulated material was again found on the cooler after pasteurisation.

Table VI. *Pasteurisation of milk of cow No. 3 at 59·3° C. (138·8° F.) for 20 minutes.*

| Experiment | Milk used | Guinea-pigs inoculated | − | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|
| D A | Mixed milk | 4 | 1 | 2 | 1 |
| | Coagulated material from cooler in experiment D A | 2 | 1 | 0 | 1 |
| D B | Mixed milk | 4 | 0 | 4 | 0 |
| | Coagulated material from cooler in experiment D B | 2 | 1 | 0 | 1 |
| D C | Mixed milk | 4 | 0 | 4 | 0 |
| | Coagulated material from cooler in experiment D C | 2 | 1 | 0 | 1 |
| | Total ... ... ... | 18 | 4 | 10 | 4 |

*Summary of fourth series of experiments.*

Three experiments were made by heating naturally infected tuberculous milk at 59·3° C. (138·8° F.) for 20 minutes; out of twelve guinea-pigs inoculated with the centrifugalised deposit and cream of the heated milk ten developed tuberculosis.

Out of six guinea-pigs inoculated with coagulated material from the cooler after pasteurisation all six gave negative results or died of non-tuberculous infection.

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## GENERAL SUMMARY OF RESULTS.

Table VII gives the results obtained with guinea-pigs inoculated with naturally infected tuberculous milk after pasteurisation at various temperatures.

Table VII. *General summary of experiments.*

| Series | Treatment | Material inoculated | Guinea-pigs inoculated | – | + | Died of non-tuberculous infection in less than 100 days |
|---|---|---|---|---|---|---|
| I | 145° F. for 30 min. | Centrifugalised deposit and cream | 118 | 103 | 1 | 14 |
| I (*a*) | „ „ | Coagulated material from cooler | 38 | 33 | 1 | 4 |
| II | 140° F. for 30 min. | Centrifugalised deposit and cream | 100 | 96 | 0 | 4 |
| III | 140° F. for 20 min. | Centrifugalised deposit and cream | 66 | 47 | 1 | 18 |
| III (*a*) | „ „ | Coagulated material from cooler | 6 | 3 | 1 | 2 |
| IV | 138·8° F. for 20 min. | Centrifugalised deposit and cream | 12 | 0 | 10 | 2 |
| IV (*a*) | „ „ | Coagulated material from cooler | 6 | 3 | 0 | 3 |

## DISCUSSION.

Since the discovery of the tubercle bacillus by Koch there has been controversy regarding its thermal death point, particularly in milk.

Owing to some workers having tested the thermal death point of bacilli in naturally infected material, whilst others used cultures of the bacilli, and that there have been variations in technique it is necessary to exercise some caution when attempting to correlate the results.

Tubercle bacilli are not easily cultivated on artificial media and it is probable that artificial cultivation yields organisms which are less resistant to outside influences than those occurring naturally. Further, cultures of bacilli can have no such protective covering as is provided by cells and tissue in natural infection.

Therefore results obtained with artificial cultures ought not to be accepted as criteria of those which would be obtained with naturally infected milk.

In any biological experiments, numbers are of the greatest importance because natural resistance to infection varies, sometimes very widely, from animal to animal. Conclusions should therefore be based only on numerous experiments.

In attempting to translate the results of experiments into practice it must be remembered that it is by no means always possible to exercise that strict control which obtains in the laboratory. An instance of this is found in the recent work of American investigators who found that with pasteurisers in ordinary commercial use at the time of the experiments, tubercle bacilli survived heating at 62·8° C. (145° F.) for 30 minutes. With machines specially constructed this combination of time and temperature proved to be effective

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in destroying cultures of tubercle bacilli in milk. The failure of the original machines to kill the organisms is explained by leaky valves and faulty construction which allowed some of the milk to escape exposure to the recorded temperature for the full period of time.

Further, the accuracy of the thermometers used to record the temperatures maintained during heating in commercial pasteurisation is one of great importance when the results of the author's experiments are considered. It was found that whilst at 140° F. the bacilli were usually destroyed, at 138·8° F. they survived on no less than ten out of twelve occasions, the exposure lasting 20 minutes in both cases. It is, therefore, evident that it is necessary to use standardised thermometers in commercial pasteurisation.

## Conclusions.

1. A temperature of 62·8° C. (145° F.) for 30 minutes does not invariably kill the tubercle bacillus in naturally infected milk, although in most cases this temperature is effective.

2. At a temperature of 60° C. (140° F.) for 20 minutes tubercle bacilli are in many cases destroyed, but this combination of time and temperature leaves no margin of safety.

## Acknowledgments.

I am indebted to Prof. R. Stenhouse Williams, Mr A. T. R. Mattick and to Mr W. A. Hoy of the National Institute for Research in Dairying for their help and advice throughout this investigation.

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(*MS. received for publication*, 19. v. 1927.—Ed.)

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