

The Disappearance of Residual Penicillin in Milk

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Results are given of experiments to show the rate of disappearance of penicillin at four different temperatures and to demonstrate that penicillin does not disappear in milk if stored in a deep freeze unit. Differences in sample storage conditions could lead to the presentation of conflicting analytical results in court proceedings and the implications are discussed.

Antibiotic therapy has been used by veterinary practitioners in the treatment of bovine mastitis for more than two decades, and the most common substance employed has been penicillin. The penicillin is infused into the infected quarter or quarters of the udder and, inevitably, some of the unmetabolised antibiotic diffuses into the milk.

The Report of the Milk Hygiene Sub-Committee of the Milk and Milk Products Technical Advisory Committee¹ pointed to the undesirability of antibiotic residues in milk, particularly since some individuals could become sensitised and others allergic to these antibiotics.

Factors which influence the quantity of penicillin in milk from treated cows are the concentration of the preparation, the vehicle employed in the preparation, the amount of milk drawn and the time interval between treatment and milking². Farmers are instructed to withhold such milk from sale until either forty-eight or seventy-two hours after administration of the penicillin, depending on the particular preparation used. The Milk Marketing Board makes regular checks on milk for penicillin residues and applies a financial levy system against milk containing more than 0.05 International Units (I.U.) of penicillin per millilitre of milk. This unofficial limit has been adopted by many Public Analysts, and farmers who sell milk in excess of this limit have been prosecuted under Section 2 of the Food and Drugs Act, 1955.

The majority of milk samples which contain penicillin residues are untreated milks which have been taken directly from a churn, the content of which would represent the combined output of three or four cows. When milk is bulked, as is commonly done for its conveyance to the dairy by tanker, the dilution effect of a preponderance of penicillin-free milk together with some penicillin-containing milk means that the discovery of a residue in such a sample is a rarity.

Repetitive analysis, in this laboratory, of some samples of milks refrigerated for several days showed a decrease in penicillin concentration. This prompted an investigation into the possible disappearance of penicillin in milk on storage.

Experimental

Samples of fat-free milk were spiked with penicillin to give a concentration of 0.2 I.U. per ml and were held at temperatures of 37°C, 18°C, 4°C and minus

10°C. These samples were analysed at intervals ranging from 25 hours to 200 hours after spiking. The method used was that of Liska and Calbert³, as modified by Wright and Tramer⁴. The microbiological method is based on the 2,3,5-triphenyl-tetrazolium chloride (T.T.C.) test.

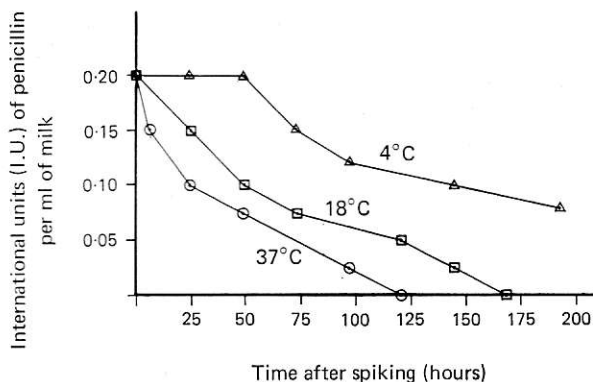


FIG. 1. The disappearance of Penicillin added to milk at 4°C, 18°C and 37°C.

Results

Fig. 1 shows the decrease in penicillin concentration with time. The results are an average of nine separate experiments. All penicillin had disappeared in five days at 37°C, in seven days at 18°C and in one hundred days at 4°C. There was no decrease in penicillin concentration at 10°C below zero after eighteen months' storage. A sample of fat-free milk was spiked with penicillin to give a concentration of 1.5 I.U. per ml and the rate of disappearance of penicillin was followed on storage at 4°C. This rate was similar to that of the sample containing 0.2 I.U. of penicillin per millilitre of milk but it took longer for the antibiotic to disappear completely.

Further experiments were carried out using samples of fat-free milk spiked with penicillin at the two concentrations already mentioned, keeping some in daylight and the others in darkness, all samples being at a temperature of 18°C. The rate of disappearance was the same in darkness and light.

Conclusions

In order to retain the whole of the penicillin in milk after sampling, it should be kept in a deep freeze unit at 10°C below zero. Samples can be retained in a refrigerator at 4°C for up to two or three days without appreciable loss of penicillin. Keeping samples at room temperature or higher for any length of time is obviously undesirable, although Vigue⁵ has found that flash pasteurisation or even boiling the milk for 15 min. does not destroy the penicillin. This suggests that time is probably a more important factor than temperature on the disappearance of penicillin in milk. Normally, milk samples should be analysed for penicillin residues as soon as possible after milking and

provided the samples are refrigerated for no more than two days, true results should be obtained. Where the laboratory is situated some distance from the sampling point, refrigerated conditions ought to be used in transportation. A problem arises concerning the storage of the third portion of a formal sample. If such a portion is refrigerated, it will obviously contain a diminished amount of penicillin when analysed some weeks later, and examination of it by the Laboratory of the Government Chemist would be pointless. The foregoing results show that it is imperative to keep this third portion in a deep freeze unit at 10°C below zero if the analytical result is to have any meaning in any subsequent dispute.

There is also the problem of the conditions under which the vendor keeps his portion of the sample prior to analysis. If he delays transportation of the sample to his analyst and stores it at room temperature in the meantime, all the penicillin could have disappeared.

A "formal" sample of milk examined in the Bristol laboratory contained penicillin at a concentration of more than 1.5 I.U. per ml. The farmer had his portion analysed one week later by an independent analyst who found no penicillin present in the sample, which, by that time, was sour. The appropriate proceedings were taken against the farmer who pleaded "Guilty" and he was fined by the Court. The conflicting analytical evidence was not used in the proceedings, but had Defending Counsel called upon this (having previously instructed his client to plead "Not Guilty"), it is probable that the Defendant would have been cleared. The third portion of the milk had been refrigerated by the Weights and Measures Inspectorate and if this had been analysed by the Laboratory of the Government Chemist it is probable that the concentration of penicillin found would have been in between the figures given by the other two analysts.

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