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Cover Page Footnote
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Antibiotic Residues in Milk

COLOUR TEST FOR ANTIBIOTICS IN MILK

Readily detectable colour variations result from acid production by *Streptococcus thermophilus* when this organism is added to milk containing the dye brom cresol purple. Where antibiotics are present little growth occurs, acid production is limited accordingly, and the colour remains blue. The change in colour gives a measure of the amount of antibiotic present.
ANTIBIOTIC RESIDUES IN MILK
—The Farmer’s Responsibility

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THE LEVEL of antibiotic residues in commercial milk supplies has been steadily increasing over the past few years. These residues find their way into the bulk milk as a result of milk from cows under antibiotic treatment for mastitis or other infections being included in the bulk milk for sale.

As the use of antibiotics for treatment of diseases—particularly mastitis—in dairy herds has increased, so the level of residues in commercial milk has risen. It has now become a threat to the welfare of the dairy industry.

The responsibility for inclusion of these undesirable residues falls squarely on the farmer. It is well known that no milk from antibiotic-treated cows should be included for sale until three days after the last treatment.

Those affected by antibiotic residues in dairy products are:

- The consumer, because antibiotic residues in milk and milk products can cause allergies and sensitisation, or can affect people already sensitive to antibiotics as a result of previous medical treatment. Serious illness or death is possible in either case.
- The manufacturer, because residues affect his manufacturing processes. The results are higher costs, lower quality products, and dwindling markets.
- The farmer, because he and his family fall into the first group, and because his economic interests are threatened in the same way as those of the manufacturer.

PUBLIC HEALTH
The presence of antibiotic residues in milk and its products continues to cause concern to public health authorities throughout the world.

Consumers have begun to take an active interest, as many cases of allergy conditions and sensitisation through the consumption of contaminated milk or its products have been recorded.

It has been estimated (1) that in the United States of America, about five per cent. of all individuals are sensitive to some form of penicillin, and there is a mortality rate of 10 per cent. among the 3,000 to 4,000 cases of anaphylactoid shock (allergy reaction) reported each year.

This has created a greater recognition of the importance of penicillin and other antibiotic residues in milk utilised for human consumption.

Another factor which is causing concern is the appearance of antibiotic-resistant staphylococcal organisms in milk and dairy products. This introduces a risk of food poisoning.

MANUFACTURED PRODUCTS
Antibiotic residues in milk can easily reach the level where they kill or inhibit bacteria added to the milk for cheese manufacture. The immediate result is an extension of the manufacturing time because the bacteria take longer to develop and do their work. This in turn causes a rise in costs.

A lower quality product also results, because without the necessary production of acid by lactic acid bacteria the cheddar cheese characteristics do not develop.

Markets are no longer available for low quality products; in fact, newer markets are demanding that products be of high

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quality and contain no antibiotic, weedicide or pesticide residues, oestrogenic substances, organisms of the *E. coli* group, or staphylococcal organisms.

**TRENDS IN MASTITIS**

Antibiotic therapy has resulted in a shift from streptococci to staphylococci as the major cause of mastitis. Other organisms capable of causing mastitis are: *S. pneumoniae, E. coli, Ps. pyocyanea, P. cereus* and some fungal agents, but these are comparatively rare.

*Streptococcus agalactiae* still causes some mastitis, but this has been greatly reduced by antibiotic therapy. One American survey showed that the incidence of this form of mastitis dropped from 34 per cent. to 10.5 per cent. of the total of all forms during 1949-50. The incidence of penicillin-resistant *Staphylococcus aureus* in herd milk increased from 9.7 per cent. in 1954 to 37 per cent. in 1956.

A limited survey involving 1,300 cows in Western Australia indicated that 16.8 per cent. had mastitis of one form or another. Of the 1,300 cows 1.4 per cent. had streptococcal mastitis and 15.4 per cent. had staphylococcal mastitis; 20.6 per cent. of all mastitis cases failed to respond to penicillin.

The emergence of antibiotic resistant staphylococci (3) gives rise to increasing numbers of this type of organism being shed in the milk. This predisposes to food poisoning outbreaks if the milk is improperly handled.

It is believed (2) that recent American cases of food poisoning involving dried milk and cheddar cheese could have resulted from such contamination. Similar forms of *Staphylococcus aureus* were isolated from the cheese, and from the milk from two out of eight herds which supplied the factory where the toxic cheese was made.

**TREATMENT OF MASTITIS**

In Western Australia, antibiotics for animal treatment appear to be readily available to the farmer from most pharmacists, and sometimes from other retail outlets. The dosage rate of many products for intra-mammary infusion is 100,000 units of penicillin, but recent dosages of up to 500,000 units have been noted.

For other infections, such as foot rot, intra-muscular injection rates of 4,500,000 units of penicillin are common.

Antibiotic residues are found in the milk from cows treated either by intra-mammary infusion or intra-muscular injection, and in both cases the levels take some time to fall to zero, as shown by the graphs in Figs. 1 and 2.

![Graph 1](301)

**Fig. 1.**—Penicillin levels in milk following intra-mammary infusion. Dose: 100,000 units aqueous solution

![Graph 2](301)

**Fig. 2.**—Penicillin levels in milk following intra-muscular injection. Dose: 4,500,000 units aqueous solution

When penicillin is injected (one dose of 4,500,000 units) it takes 48 hours before the level of antibiotic in the milk falls to zero. Where dosage is by intra-mammary infusion (one dose of 100,000 units) this takes between 48 and 72 hours.
All milk from a cow treated by intra-mammary infusion must be withheld for 72 hours. This applies also when only one quarter is treated, as the other quarters receive the antibiotic through the bloodstream.

The graphs in Figs. 1 and 2 relate to penicillin in aqueous solutions. When oil base suspensions are used, the antibiotic levels in milk may be detectable for longer periods.

METHODS OF DETECTION
Detection of milk containing antibiotics by a test which would yield almost immediate results would be extremely desirable wherever milk is received. Such a test would probably be chemical rather than biological, but as yet this type of test has not been devised.

However, we do have two tests which when used either singly or together, detect low levels of antibiotics in milk, although the results are only available some time after the milk has been received and processed.

1.—Colour Test
To 10 ml. of a sample which has been heated for five minutes, is added a fast growing acid producing sensitive culture of *Streptococcus thermophilus*, and brom cresol purple dye. The test tubes are incubated for 2½ hours, together with a series of known concentrations and blanks, the colour change being matched with the known concentrations. This is a quick and satisfactory test. It was first suggested by Berridge (4) and has since been modified by Keogh (6). With this test, levels down to 0.01 units of penicillin or its equivalent are readily detectable, as shown by the colour plate.

2.—Disc Assay Test
A culture of the sensitive organism *S. lutea* is plated in dishes with agar based media. Small filter paper discs of a diameter less than 0.5 in. are saturated in the unknown milks and pressed lightly onto the surface of the media. The antibiotic, if present, diffuses from the disc.
and a zone of inhibition is created which prevents the culture from growing. This method detects levels of 0.005 units or lower of penicillin per ml. but it requires an overnight incubation period. The method is described by Naylor (5).

Both methods are made specific for penicillin by adding the enzyme penicillinase to the second sample of the unknown. Penicillinase destroys any penicillin present in this sample, so a positive reaction from the first sample and a negative reaction from the second sample indicates the presence of penicillin in the milk under test.

These methods have been in use at the Department of Agriculture's Dairy Laboratory for some time, and have been found to be very satisfactory.

DYE PREPARATIONS
The inclusion of a dye preparation with the antibiotic for intra-mammary infusion has had a good deal of success experimentally. With further improvement, this could be a means of detecting the presence of antibiotics, as both are secreted at the same rate.

LEVELS OF ANTIBIOTICS IN WEST AUSTRALIAN MILKS
Over 700 samples taken at receiving platforms in Western Australia since June of this year have revealed that 10 per cent. are positive reactors. Of these, 40 per cent. are above the arbitrary level of 0.05 units per ml. which is considered the danger level for cheese milk.

From this, it is apparent that very little attempt is being made to withhold milk from treated cows.

IMPOSITION OF CONTROLS
The question of imposing controls for the contamination of milk with antibiotics has been considered, and the introduction of regulations is a very real possibility so long as the problem continues.

Farmer education campaigns in the United States largely failed to arrest the trend, and Federal Health legislation was introduced to cover the situation for the nation. Milk found to contain residues of antibiotic is now destroyed and the responsible person identified and penalised. In one celebrated instance, the farmer had his licence revoked and finally his herd was sold after continued breaches.

This stage has not been reached in Western Australia or Australia, and those in the industry have tended to iron out their own problems. In recent times, South Australia has limited intra-mammary injection to 100,000 units, under its Veterinary Medicines Act, and this has proved helpful.

The question is being continually discussed at high levels in the industry and there appears to be a commendable reluctance to take recourse in legislation. It is possible that this step may not have to be taken.

The matter rests entirely in the hands of the farmer. It is up to the farmer to prevent antibiotic loading of his product.

ACKNOWLEDGMENTS
The help of Mr. J. Craig and Mr. G. Robinson for material supplied and to Mr. K. Needham for encouragement and helpful suggestions is gratefully acknowledged.

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