THE PROBLEM OF ANTIBIOTIC DETECTION IN MILK

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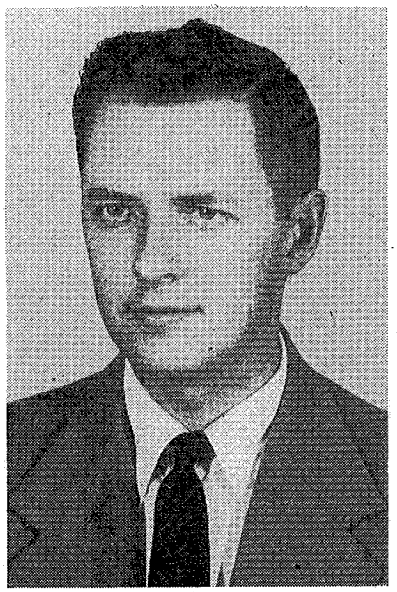
The potential importance of the presence of antibiotic compounds in raw milk has led to the development of several antibiotic detection methods. The majority of these procedures is based on the inhibition of the metabolic activities of dairy streptococci with antibiotics as evidenced by a decrease in acid production rate or in reductase activity. Considerations of the problems associated with the use of antibiotic-free control samples, the time required for the test and other factors are important in the application of these methods.

Several investigators have discussed the potential importance of the presence of microgram amounts of antibiotics in raw milk (1, 4, 5, 10, 14) where accepted chemotherapeutic procedures for the treatment of mastitis are primarily responsible for the introduction of these antibacterial compounds into milk (2). Elimination of antibiotic milk from dairy plant milk supplies would be facilitated by an antibiotic detection method which embodies the characteristics of speed and simplicity. Quite logically research on this problem has generally been based on the interference by antibiotics on the metabolic activities of dairy streptococci. It is the purpose of this review to present information on the existent methods and to discuss some of the applications and limitations of these procedures.

One might expect a high degree of sensitivity from detection methods involving only a small dilution of the unknown sample. To this end several procedures have evolved which consist essentially of inoculating with a known bacterial culture and estimating the rate of acid production or reductase activity. Following the findings of Krienke (14), Silverman and Kosikowsky (19) presented a detection method based on the retardation of lactate formation. With a commercial starter as the test organism, added low concentrations of penicillin, aureomycin and dihydrostreptomycin prevented normal acid formation as measured titrimetrically in a four hour test period. Earlier data on the retardation of lactic steptococci by penicillin were provided by Hunter (10) as well as by Berridge (3).

Other evidence for the interference of antibiotics with the metabolism and multiplication of bacteria in a milk medium has come from investigations in which nonspecific reductase activity is estimated by the use of oxidation-reduction indicators. Johns and Katznelson (12) demonstrated that penicillin inhibited the reductase activities of starter organisms as measured by methylene blue or resazurin reduction. Ruche (17) concluded that penicillin retarded only slightly the reduction of methylene blue in either normal milk to which antibiotic was added directly or in milk sampled after udder infusion. This investigation involved extended incubation as no inoculum was added.



Dr. L. A. Nutting received the B.S. degree from the University of New Hampshire in 1941 and later received the M.S. and Ph.D. degrees from Virginia Polytechnic Institute. His doctoral thesis research was completed under the auspices of the Oak Ridge Institute of Nuclear Studies. Since 1950 he has been connected with the National Dairy Research Laboratories, Inc. as a microbial physiologist.

Methylene blue reduction was also investigated by Schipper and Petersen (18) with a strain of *Bacillis cereus* as the test organism in the presence of added aureomycin. These investigators were able to detect concentrations well below 0.1 microgram per milliliter.

Resazurin reduction has been employed for the quantitative determination of nisin by Friedmann and Epstein (9). The principle of the reductive conversion of tetrazolium compounds to formazans in the presence of proliferating microorganisms has also been employed by Neal and Calbert (16) as a nonspecific test for inhibition. A single strain of *Streptococcus thermophilus* exhibited greater sensitivity to added penicillin, aureomycin or terramycin but not

streptomycin than a single commercial lactic starter. Total incubation time was two and one half hours and detectable antibiotic concentrations were similar to those generally regarded as deleterious to the manufacture of cultured dairy products.

Mattick *et al.* (15) have adapted the inhibition of nitrate reduction by *Micrococcus pyogenes* var. *aureus* for the assay of low concentrations of penicillin in milk. By using large inocula these workers were able to obtain sufficient rates of nitrate production to run a complete test in two hours.

In cases where one is willing to rely on more elaborate and time consuming procedures several basic methods which have been applied to the assay of antibiotics in nonmilk biological samples are available; these include dilution methods, diffusion methods and turbidimetric methods (7).

Any procedure applicable to milk which depends primarily upon some aspect of the inhibition of bacterial multiplication in the suspected sample is inherently subject to certain limitations. Of prime importance is a consideration of the type of medium employed as a control. One, of course, must be reasonably certain that the control medium contains inhibitors in a concentration below the level required to have an influence on the test culture. Silverman *et al* (19) have suggested the use of a reconstructed milk control, although no data with this medium were presented. Foster (8) has very adequately demonstrated both a beneficial and a deleterious effect on the growth of streptococci in heated milk which require consideration in any use of reconstituted milk controls. These heat effects might possibly be compensated for by heating suspected samples; however, such a procedure would entail the possible destruction of antibiotic (6, 10). A thorough investigation of the comparative behavior of the same organism in both reconstituted and raw milk would presumably provide background information which would contribute to a detection method utilizing a reconstituted milk control.

In addition the use of heated milk requires a consideration of the findings of Jenness (11) concerning the heat liberation of sulfhydryl groups and their influence on dye reduction.

Another important aspect concerns the minimum time required for completion of the test. The length of the incubation period is probably the principal factor although Neal *et al.* (16) have mentioned the importance of inoculum size. Presumably, increasing the inoculum decreases the required incubation time. However, in the case of large inocula one must consider the effect of dilution by the inoculum and its relationship to the sensitivity.

For measurements of reductase inhibition the choice of indicator affects the measurement and in this connection the blue diformazan obtained by the reduction of tetrazolium blue[1] might have desirable characteristics over the red formazans studied to date.

Most of the methods contained in the literature are nonspecific and serve only to detect general inhibition of the test organisms, a limitation which is probably not serious in most plant sample testing. Should a more precise knowledge of inhibitor type be required then one would be obliged to investigate further the positive test samples.

In general the detection procedures which are presently available are found wanting principally when one attempts to integrate these procedures with daily plant operations. The time factor is too great. Rapid milk rejection tests are an ever-present problem to individuals responsible for efficient plant practices as well as a demanding challenge to the investigator. One approach to rapid antibiotic detection method development which has been suggested (13) involves some type of labeling in the chemotherapeutic treatment materials prior to use. Presumably the incorporation of a dyestuff or a long half-life radio-active indicator might provide a means for the rapid detection of antibiotics in high dilution. However, the authors are not aware of any published findings on tracer experiments as applied to milk-antibiotic test systems.

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ITINERARY AND RATES TO SEATTLE CONTINUED FROM PAGE 161

	Station	*Railroad*	*Train*	*Time*	*Date*
No. 2-A					
LV Chicago	Union	Milw. R.R.	Olympian Hia.	3:00 PM	9/3
AR Seattle	Union	Milw. R.R.	Olympian Hia.	9:30 AM	9/5
Attend Convention Sept. 5-7, 1956					
LV Seattle	King St.	NP		11:45 PM	9/7
AR Portland	SP	NP		5:45 AM	9/8
LV Portland	SP	SP	Shasta Daylight	7:45 AM	9/8
AR San Francisco	Market St.	SP	Shasta Daylight	11:30 PM	9/8
LV San Francisco	Market St.	SP	C. of San Fran.	4:00 PM	9/9
AR Chicago	Union	Milw. R.R.	C. of San Fran.	11:15 AM	9/11
No. 3					
LV Chicago	Union	Milw. R.R.	Olympian Hia.	3:00 PM	9/3
AR Seattle	Union	Milw. R.R.	Olympian Hia.	9:30 AM	9/5
Attend Convention Sept. 5-7, 1956					
LV Seattle	CP Docks	CPSS	Princess SS	8:00 AM	9/8
AR Victoria, B.C.	CP Docks	CPSS	Princess SS	11:00 AM	9/8
LV Victoria, B.C.	CP Docks	CPSS	Princess SS	2:15 PM	9/8
AR Vancouver, B.C.	CP Docks	CPSS	Princess SS	5:00 PM	9/8
LV Vancouver, B.C.	CPR	CPR	Soo Dominion	7:30 PM	9/8
AR Field, B.C.	CPR	CPR	Soo Dominion	2:40 PM	9/9
One Day Tour from Field, BC to Lake Louise & Banff					
LV Banff, Alta.	CPR	CPR	Soo Dominion	6:05 PM	9/10
AR St. Paul, Minn	Union	SOO	Soo Dominion	8:00 AM	9/12
LV St. Paul, Minn	Union	Milw. R.R.	Am Hiawatha	8:25 AM	9/12
AR Chicago	Union	Milw. R.R.	Am Hiawatha	2:40 PM	9/12

Rates from Chicago to Seattle, Washington

TOUR NO. 1

Round-trip rail fare $115.00 plus $11.50 tax, total $126.50

Round-trip sleeping car fares Roomette, $64.68; Double Bedroom, $101.54; Drawing Room, $175.56

TOUR NO. 2

Round-trip rail fare $126.45 plus $12.65 tax, total $139.10

Round-trip sleeping car fares Roomette, $79.97; Double Bedroom, $125.63; Drawing Room, $217.14

TOUR NO. 2-A

Round-trip rail fare $126.45 plus $12.65 tax, total $139.10

Round-trip sleeping car fares Roomette, $73.48*; Double Bedroom, $115.90*; Drawing Room, $198.66*

*Includes seat charge of $1.00 plus $0.10 tax from Porland to San Francisco on Shasta Daylight

TOUR NO. 3

Round-trip rail fare $115.00 plus $11.50 tax, total $126.50

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