SOME BACTERIOLOGICAL ASPECTS OF MASTITIS DIAGNOSIS AND CONTROL

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SUMMARY

A brief review is given of some of the data available in the literature that are pertinent to the planning of programmes of research on bacteriological aspects of bovine mastitis in New Zealand. Emphasis has been placed on staphylococcal infections and attempts will be made to define conditions of stress and to elucidate variables in the host-parasite relationship that may directly or indirectly result in inflammation of the udder.

MUNCH-PETERSEN'S (1938) survey of the literature, summarizing the work of 1,400 authors from 1814 to 1935, illustrates that the problem of bovine mastitis has long been the subject of intensive investigation. Many specialist disciplines, including those of bovine nutrition, genetics, physiology, microbiology, hygiene, pathology, machine and herd management, can all contribute relevant information and experimental methods. It is not implied that the aspects considered below are more important, over-all, than those not considered. This paper is presented simply to describe the work being carried out at Wallaceville and to invite discussion on the implications of this work in relation to current approaches to the diagnosis and control of mastitis.

DEFINITION OF THE PROBLEM

SOURCE OF INFORMATION

The data at present available for the definition of the problem of mastitis in New Zealand and on which opinions can be based regarding the wastage involved, the need for, and planning of, further investigations and for the design of control measures, come primarily from two sources: the diagnostic examination of milks taken from clinical or suspect cases, and the three surveys conducted by, or in conjunction with, the New Zealand Dairy Board in 1943–5, in 1947–8 and 1957–8. The bacteriological information from these sources does not represent the cattle population as a whole. Also, that obtained from the Dairy Board surveys is not strictly comparable from one survey to another because of variations in field and laboratory techniques.

Data provided on the incidence, distribution and sequelae of any disease complex decreases in usefulness if it is provided by non-comparable procedures at irregular intervals.

In conjunction with the Animal Health Division and the Biometrics Section of the Department of Agriculture, a survey is currently in progress at Wallaceville in which composite milk samples are being taken from each of 10 cows in 500 herds randomly distributed throughout the country. By taking great care to standardize laboratory and field techniques, it is hoped to establish a standard and reproducible procedure with which it will be possible to define the bacterial flora, and associated factors, in the udders of a random part of the cattle population. Results from the 1965–6 survey will be compared with those obtained from future surveys, so giving information which will be continually relevant and which will reveal trends suggesting change in the incidence of particular micro-organisms.

Micro-organisms Associated with the Bovine Udder

Wilson (1963) has reviewed the information available on the infection rate of milks in the United Kingdom and has stressed the prevalence and pathogenic importance of four species: *Staphylococcus pyogenes, Streptococcus agalactiae, Str. dysgalactiae* and *Str. uberis.*

Klastrup (1963) has reviewed comparative data available in Denmark and has noted the relatively high incidence of *Streptococcus* group L in the udders of cattle in that country. As group L streptococci are often carried in the genital tract of swine, it is suggested that the Danish practice of housing cattle and pigs together may account for the relative prevalence of this micro-organism.

In order to define the incidence and distribution of bacteria in the flora of cow udders in New Zealand, the methods being used in the initial survey are somewhat laborious. What data there are already indicate that the majority of clinical cases are associated with staphylococci and with *Str. agalactiae.* They strongly suggest also that staphylococci are the bacteria most likely to pose the greatest problems in the control of mastitis. These latter data can be conveniently grouped under three headings:

(1) *Prevalence*: Examination of Table 1, in which the results of cultures from milks taken from clinical quarters in the first and third Dairy Board surveys are compared, suggests that in 1958 cases of clinical mas-

titis were more often associated with staphylococci and less often associated with streptococci than was the case *circa* 1942–5. A similar trend has been noted in the United Kingdom by Wilson (1963).

TABLE 1: INCIDENCE IN TERMS OF AETIOLOGY

Locality and Year of Survey		*Clinical Quarters*	*% Strep.*	*% Staph.*
Canterbury	1942–3	9	56	33
	1943–4	58	57	28
	1944–5	71	59	23
Manawatu	1942–3	167	63	24
	1943–4	126	64	17
	1944–5	195	65	18
New Zealand	1957–8	1,156	22.4	44.1

(2) *Difficulties of Control*: *Streptococcus agalactiae* has been shown to be largely confined to the udder and it has been demonstrated that infection due to this genus can be eradicated (Stableforth *et al.*, 1949; Agric. Res. Council, 1955) or at least largely eliminated from a herd (Anim. Res. Div., 1950, 1953). Davidson (1961) has demonstrated that staphylococci are not confined to the bovine udder and that eradication of this infection from a herd can consequently be expected to be more difficult. In an experiment at the National Institute for Research in Dairying, at Reading (1963), designed to show that, by strict hygiene and management practices, the udder flora can be markedly reduced and thus maintained, a marked reduction in the incidence of streptococci was obtained, but the difference in total new staphylococcal infections between control and experimental groups was far less pronounced.

(3) *Penicillin Resistance*: Since penicillin became available in New Zealand (1948), *Str. agalactiae* has been shown to be readily susceptible whereas penicillin resistance is common in staphylococci associated with clinical cases of mastitis.

In the current survey, in which composite samples from 2,254 cows have so far been examined, 43% of the samples contained coagulase-positive, beta-

haemolytic staphylococci. Of those samples which contained coagulase-positive stapyhlococci, 60% contained staphylococci resistant to at least 10 units of penicillin.

For reasons such as these, emphasis at Wallaceville is now concentrated primarily on the staphylococci.

As this genus embraces a large variety of micro-organisms, and the exact classification of the species is still a matter of dispute, studies on the epidemiology and therapy of staphylococcal mastitis are impossible without the means of specifying, as exactly as possible, a particular strain. It is necessary, therefore, to establish a routine set of "phages" which will allow the majority of staphylococci of bovine origin in New Zealand to be differentiated. (Phage-typing is a technique in which a particular strain of staphylococcus is identified by its pattern of susceptibility to a standard set of bacterial viruses or bacteriophages.) These studies can then be followed by investigations, in a small experimental herd, of the epidemiology of staphylococcal mastistis under typical New Zealand conditions of management.

Incidence and Sequelae

In Table 2, taken from the results of the Dairy Board surveys, the number of cows affected by clinical mastitis, and the percentages, in different years, of those that had to be culled during the acute stage, or for subsequent low production, are compared.

Table 2: Incidence and Sequelae

Season	*Number of Cows*	*Incidence %*	*Cows Culled for Mastitis, %*
1943–4	92,339	11.8	40
1957–8	25,954	12.2	11.7

Penicillin first became available in New Zealand for the treatment of mastitis *circa* 1948. It can be seen that whereas the incidence of clinical mastitis differed little between 1944 and 1958, a dramatic change took place in the sequelae.

This comparison, if still valid in 1966, can be interpreted to suggest that there is now a system of "control-by-therapy". While the advent of antibiotics has proved to be of great value, this system of control has not reduced the incidence of mastitis, and it carries with it the inherent disadvantages of the cost of the antibiotics; of antibiotic

residues, causing manufacturing problems and constituting a potential danger to sensitized humans; and of antibiotic resistance in populations of micro-organisms, particularly in staphylococci.

THE CONCEPT OF "SUB-CLINICAL MASTITIS"

In efforts to reduce the incidence of clinical mastitis, *i.e.*, to achieve prevention, rather than to rely only on cure of an established infection, much research activity has been concentrated on the definition of "sub-clinical mastitis".

This form of mastitis is usually defined as that in which there are no obvious symptoms of inflammation of the udder, and no macroscopic changes in the milk, but the milk can be shown to have an increased cell-content and to contain recognized pathogens (Brit. Vet. Assoc., 1965).

Because of the difficulties involved in making large numbers of reproducible cell counts from milk samples, considerable attention has been given to "indirect tests" in which the cellular content of a milk sample is estimated by adding a reagent (*e.g.*, an anionic surface-active agent, as in the California Mastitis Test (C.M.T.) of Schalm and Noorlander (1957) and assessing or measuring resultant changes in viscosity. Many such tests, some designed for use in the cowshed, have been developed (*e.g.*, Michigan Mastitis Test, Wisconsin Mastitis Test, Modified Whiteside Test, Negretti Field Test, Brabant Mastitis Reaction, etc.).

There is sometimes a tendency, perhaps a temptation, to over-simplify the issues involved in the relationships between micro-organisms and the bovine udder. This tendency can lead to control schemes based largely on detecting the individual cow with sub-clinical mastitis, and/or to short-cuts in which only one parameter, *i.e.*, culture tests only, or cell-content (or a substitute indirect test) only, is measured. Because of this, it is probably worth while looking more closely at the concept that milk samples containing "pathogens" always tend to contain some evidence of the effects of irritation.

THE PRESENCE OF STAPHYLOCOCCI AND THE CELL COUNT

Murphy (1956) notes that of 1,154 streptococcus-free milks, which contained over 200 staphylococci/ml, 49.3% contained over 500,000 leucocytes/ml. It can be assumed that 50.7% of these samples contained under 500,000/ml.

Simon *et al.* (1963) reported that of 1,032 quarters associated with coagulase-positive staphylococci, 44% showed a leucocytosis in the milk; 55% did not.

Galton *et al.* (1961) observed that of 609 milk samples containing coagulase-positive staphylococci, 16% gave a positive C.M.T. Of 212 samples containing coagulase-negative staphylococci, 7% were C.M.T.-positive.

In a comparison of C.M.T. readings and bacteriological culture results (staphylococci, streptococci, and staphylococci and streptococci), Rude (1963) recorded the data shown in Table 3.

TABLE 3: CORRELATION OF C.M.T. READINGS AND BACTERIAL RESULTS

C.M.T. Readings	*3*		*2*		*1*		*0*	
	Pos.	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*
Bacteriological results on tested quarters	158	98	38	59	25	73	11	105
Percentage of quarters positive	61.7		39.1		25.5		9.5	

Rude concluded that "the bacteriological results indicated that the prevalence of mastitis-producing micro-organisms in the milk had a relationship with the C.M.T. readings in that the percentage of organisms recovered increased as the C.M.T.-positive readings increased".

It can be seen from Table 3, however, that 9.5% of those milks reading C.M.T. 0 did contain bacteria and that 38.3% of the milks showing the maximum C.M.T. reading did not contain bacteria.

In an evaluation of the California Mastitis Test and the Negretti Field Test as indicators of sub-clinical bovine mastitis, Ewbank (1962) found little relationship between the indirect tests and the occurrence of bacteria. Using a "percentage agreement" figure to calculate the agreement between the indirect tests and the cell counts of the same samples, he concluded that a positive reading means that the cell count of the milk is most probably over 100,000 cells per ml, and a doubtful or negative reading suggests that the cell count is less than 250,000 per ml.

If, however, using the same data, the type I errors (milks with low cell count being falsely picked as positive) and type II errors (positives being "missed") are calculated separately, it is possible to obtain a clearer picture of how

efficiently the C.M.T. read as negative those milks containing less than 100,000 cells per ml, or as positive those milks containing more than 100,000 cells per ml. This is shown in Fig. 1. Regarding C.M.T. results 0 and "doubtful" as negative, and readings of 1, 2, and 3 as positive, it can be seen that the C.M.T., in this instance, showed a Type I error of 1.7% and a type II error of 37.7%.

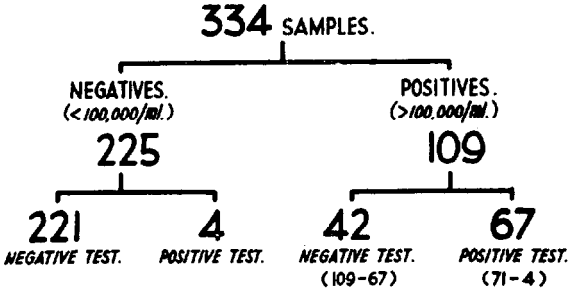

Fig. 1: California Mastitis Test and cell counts.

Using the same data, the relationships observed in this experiment between cell count and bacteria are shown in Fig. 2. It can be seen that, of 110 samples containing bacteria classed as pathogens, 49 or 44.5% contained more than 100,000 cells/ml and 61 or 55.4% contained less than 100,000/ml. Sixty of 224 samples containing no pathogens (26.3%) contained over 100,000 cells/ml.

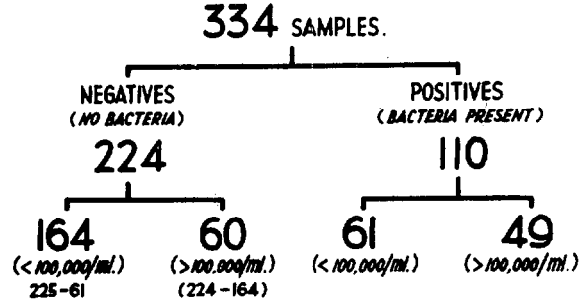

Fig. 2: Comparison of cell counts and cultural results.

PATHOGENICITY OF STAPHYLOCOCCI FOR THE MAMMARY GLAND

Although the pathogenic significance of some micro-organisms found in the udder is still a matter of dispute, it is generally accepted that staphylococci which can coagulate human or rabbit plasma (coagulase positive) can be considered as pathogens. Strains which can produce coagulase usually produce a beta-haemolysin, the effects of which can be easily seen around a culture on a blood agar plate.

However, recent bacteriological surveys of milking herds are tending to stress the ubiquity of coagulase-positive staphylococci in milk samples and to show that the relationship between the cow and the micro-organisms may vary over the range from the carriage of "pathogenic" coagulase-positive staphylococci, without any associated clinical or sub-clinical signs whatsoever, to an acute gangrenous mastitis with toxaemia and perhaps death.

Marshall (1964) cultured milks from 143 cows in three herds in the South Island and found a 65% carriage rate of coagulase-positive staphylococci.

In a comparison of cultural findings and C.M.T. readings of a large number of milk samples in New Zealand, Brookbanks (1965) found that in those cases which showed staphylococci only (1,699), 9.0% were C.M.T. 0, 13.2% were C.M.T. 1, 22.7% C.M.T. 2, 25.1% C.M.T. 3, and 30% C.M.T. 4. Thus the C.M.T. in this study (reading 0 and 1 as "negative", and 2, 3 and 4 as "positive") showed a Type II error of 22.2% (*i.e.*, 22% of the milks containing staphylococci were missed) and a Type I error of 35.8% (*i.e.*, over a third of the clean samples were C.M.T. 2 or more).

In the current survey at Wallaceville, 43% of 2,254 composite samples have been shown to contain coagulase-positive staphylococci. The over-all incidence of cows in the total sample showing signs of clinical mastitis was 1%. In all, only 18% of all samples were negative on culture. Galton *et al.* (1961), in a study of a herd of 262 cows over six months in the United States of America, record that 71% of 1,010 samples contained coagulase-positive staphylococci. The incidence of clinical mastitis associated with 609 samples containing coagulase-positive staphylococci was 11%; the incidence of clinical mastitis associated with 212 samples containing coagulase-negative staphylococci was 7%.

White and McDonald (1961) describe a problem herd of 80 cows, in Scotland, in which 214 cases of clinical mastitis, from which coagulase-positive staphylococci were isolated,

occurred over a two-year period. In 1957, 5,859 lb of milk from this herd had to be discarded as unfit for sale, but in 1958, only 450 lb had to be rejected. The fall in the number of clinical cases of mastitis after 1957 was not accompanied by any appreciable fall in the incidence of coagulase-positive staphylococci isolated from milk samples.

The ubiquity of the staphylococci diminishes the significance of the isolation of these bacteria from the individual cow.

CONCLUSIONS

From the examination of the results of some of the experiments quoted, it appears that there are a number of observations which should be kept in mind:

(1) A wide range of micro-organisms, including many considered routinely as non-pathogenic, have been isolated from milks from udders showing varying degrees of inflammation. On the other hand, organisms associated with inflammatory changes in a large number of cases can be cultured from milks showing no abnormality. Crossman *et al.* (1950) stated that "one type of organism may infect more than one quarter of the same udder, causing little or no irritation in one gland and marked abnormality in the other".

Rather than being a constant property of a particular genus of micro-organisms, pathogenicity seems more likely to be related to the particular relationship between a particular micro-organism and its surrounding microclimate of host tissue at a particular time under particular conditions.

Rather than listing some bacteria found in the udder as pathogenic, while ignoring others as non-pathogenic, Wilson (1963) in the United Kingdom divides organisms into four categories according to the frequency with which they can be isolated and to the conditions they apparently require to initiate inflammatory changes. Klastrup (1963) in Denmark has found it useful to consider mastitis under the two headings "occupational" and "contagious". A commission set up recently by the International Dairy Federation (1965) has used the phrase "udder infection" rather than "pathogen" in defining sub-clinical mastitis, and notes that all micro-organisms which infect the udder may cause pathological changes in milk "where a predisposition occurs or appears".

(2) The cell-content of milk may well increase for reasons other than as a response to bacterial irritation alone. H. C. Hovmand and B. Sorenson (1964, pers. comm.) have noted in Denmark that stresses such as those posed by bad weather and changes in personnel and technique are often reflected in higher cell counts in both bulk and individual milk samples. Indirect tests can become more reliable tools for measuring the effects of bacterial irritation in a particular quarter only if the limits of non-bacterial variation in milk-cell counts can be more precisely defined. These variations could conceivably be due to physiological factors, environmental stress or to the reaction of the mammary gland to stresses produced by diseases other than mastitis itself.

(3) Schemes in which indirect tests are used infrequently, and without ancillary tests (*i.e.*, culture) as often as practicable, could prove to be expensive methods of control if "positive" individuals were to be culled or treated. Aynsley and Buol (1965) in England have described the use of indirect tests at frequent intervals as a herd-screening procedure and have stressed that the results of single tests on particular cows are unreliable.

(4) The concept of a particular "threshold value", cell counts below which can be considered normal, while those above which are abnormal, is one that may prove of limited value. A commission of the International Dairy Federation (1965) has suggested that a threshold value of 300,000 cells/ml of milk from individual cows would be a suitable criterion for accepting or rejecting milks on the basis of quality.

Guallini and Vallis (1957) found that cell counts below 80,000/ml were associated with milks from healthy udders, and that counts above 1,000,000/ml were primarily associated with udders showing abnormalities, and that counts between these limits could be associated with either condition. It would not be illogical, in using indirect tests such as the C.M.T., to interpret results on the basis of a low "pass" mark, and a high "fail" mark, separated by a "retest" or "no-opinion" interval.

(5) The veterinarian faced with a herd mastitis problem may find the classifications of "occupational" and "contagious" mastitis useful in predicting what effort will

be required to control a particular outbreak. It appears that very few organisms cultured from milks can be summarily dismissed as of "no pathogenic significance".

If *Str. agalactiae* is the organism predominantly associated with the clinical abnormalities, it may be possible to tailor an eradication or "near-eradication" scheme to the needs of the farm and farmer concerned. Some eradication schemes reported in Australia and New Zealand (C.S.I.R. Bull., 1940; Animal Res. Div., 1953; Frost and Sanderson, 1965), have proved less dramatic than those reported from the United Kingdom, but worthwhile control can be achieved.

Cultural tests suggesting a herd diagnosis of staphylococcal mastitis place the onus on the practitioner of establishing whether any gross variations in machine function or usage, shed hygiene, management, sources of contamination, or other factors can be found that could conceivably have conferred an advantage on the parasite over the host. The available knowledge on the control of mastitis has been summarized by a mastitis subcommittee of the British Veterinary Association (1965) and a practical hygiene programme in New Zealand has been evaluated by Brookbanks (1966).

(6) Schalm (1962) expressed the opinion that "the entire programme of management of the dairy cow must be studied and situations producing stress within the mammary gland corrected. Mastitis prevention based on improved management has a better chance for success than programmes which consider only the bacteriological aspects of the problem".

From the point of view of bacteriological research, especially with regard to staphylococcal mastitis, there is a crucial need to attempt to detail the specific alterations occurring in the relationship between the host and the parasite that make for a change from an innocuous-carrier state to a state in which symptoms or signs of inflammation occur. This question suggests collaborative experiments with those in the specialist discipline of machine design and function. It could suggest studies on the interactions between the different genera of micro-organisms in the udder flora, studies on the relationship between staphylococci and host cellular and humoral defence mechanisms, on the interactions between staphylococcal exotoxins and the microclimate of host tissue and studies on the specific

growth requirements of staphylococci in relation to the substrates and/or inhibitors in the surrounding tissues of the udder.

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DISCUSSION OF PAPERS ON MASTITIS

DR W. G. WHITTLESTONE (COMMENT): In Mr Brookbanks' paper, it was shown that there was a high correlation between the bulk C.M.T. reading and the incidence of infection, as well as with the percentage of strong C.M.T. reactions by individual cows. There is little doubt that the use of bulk C.M.T. tests would be a means of locating "problem" herds.

Several important points arise from Mr Elliott's paper. The increase in the percentage of clinical mastitis due to staphylococcal infection compared with streptococcal infection is ominous. Even more so is the fact that the current survey carried out at Wallaceville shows 43% of samples to contain coagulase positive beta-haemolytic staphylococci, 60% of which are penicillin resistant.

Another important observation is the comparison between 1944 and 1958 showing that, whereas the incidence of clinical disease in the former year was 11.8% with a culling rate of 40%, in the latter year the incidence of clinical cases was 12.2% with a culling rate of 11.7%. As Mr Elliott aptly put it, there has been "control by therapy", a system to which there are substantial objections, namely: there has been no reduction in the incidence of the disease; there is a high cost of drugs; milk contaminated with antibiotics presents manufacturing problems; there is a danger to sensitized humans, and, furthermore, there is an active encouragement of the development of antibiotic resistance.

In recent years, increasing emphasis has been placed on the importance of subclinical mastitis. This form of the disease shows no obvious inflammation and no macroscopic milk changes. There is, however, an increased cell count and recognizable pathogens may be isolated. This change in emphasis has given rise to the development and application of indirect tests for leucocytes in milk, in particular the California Mastitis Test which is now widely used in a number of modifications. Such tests are of great importance but their limitations must be clearly understood. As Mr Elliott has pointed out, using Ewbank's data, it can be shown that the C.M.T. in this case had a type 1 error (milks with low cell count being falsely picked as positive) of 1.7% and a type 2 error (positives being missed) of 37.7%.

There are other aspects of the diagnosis of the disease which are equally confusing. The fact that non-pathogens have been isolated from inflamed udders and apparently normal udders found to harbour known pathogens indicates that pathogenicity is probably an "ecological" phenomenon determined as much by the environment as by the nature of the organism.

The use of the cell count or its equivalent for the diagnosis of udder disease raises some fundamental difficulties. The cell count is a physiological response to a challenge by trauma or infection. Furthermore, it is a response to stress, physiological or even psychological. The cow which responds readily to a challenge by an abrupt rise in leucocytes will more effectively defend herself against invasion. However, such a cow would be classified as having subclinical mastitis, while a cow incapable of an adequate leucocyte response and so likely to become clinical would be regarded as clean. This raises real difficultes in defining the threshold above which a cow may be regarded as normal. Mr Elliott has suggested that counts above one million should be regarded as positive, negatives being those below eighty thousand with the zone in between

regarded as intermediate. In my opinion, this is a very sound approach; the intermediate zone may be regarded as "the range of control" within which one will find fluctuations in leucocyte count in response to challenges from a normal environment.

PROFESSOR M. C. LANCASTER: *Is phage typing of staphylococci being studied?*

R. E. W. ELLIOTT: Yes. One of our major lines of activity is an effort to determine which of the internationally recognized phages are best suited to the typing of staphylococci of bovine origin in New Zealand. A large collection of staphylococci isolated from a random sample of bovine milk is being built up from isolations made in the present survey. This collection will be used for the phage-typing investigation. We realize that a useful set of typing phages must be established as a pre-requisite to epidemiological studies.

A. R. AQUINSO: *In Australia, mastitis has been produced experimentally in sheep due to* Actinobacillus lignieresi. *Has this organism been associated with mastitis in cows like streptococcal or staphylococcal mastitis?*

MR ELLIOTT: The type of organisms one finds tend to some extent to depend on what one is actually looking for. In a trial preceeding the present survey, we used a wide range of bacteriological techniques on a group of herd bulk milk samples in an effort to determine which were necessary and which gave too little return for the effort expended. Actinobacillus-like organisms were found in some of these samples, but very few have been found in the survey milks themselves so far. To the best of my knowledge, *Actinobacillus lignieresi* organisms have not been found in New Zealand in association with clinical or suspect cases of mastitis.

PROFESSOR E. D. FIELDEN (COMMENT): Mr Elliott has stressed the increasing importance of penicillin-resistant staphylococci. It may be of interest that of 103 clinical cases of mastitis (encountered in the Massey University practice) from which staphylococci were isolated, 60% of these isolations were found resistant to penicillin as judged by *in vitro* sensitivity tests.

The errors arising when comparisons were made of C.M.T. findings and bacteriological recoveries are not all necessarily associated with deficiencies in the C.M.T. technique; faults in bacteriological technique in regard to efficiency of recovery could equally apply.

MR ELLIOTT: This is a valid point, but, from a bacteriological point of view, such errors should be reducible or at least definable. The more important problem in the evaluation of the C.M.T. is the difficulty of deciding which organisms in the particular sample can be classed as "pathogens" and which can be dismissed as non-pathogens. The question of a "normal udder flora", of opportunist pathogens, and variations in host susceptibility have not been sufficiently explored. On the other hand, and assuming that the indirect tests give an approximation of the cell count, we have the problem of assessing the significance of a particular cell count, other than of those counts which are very high or very low.

Thus, there are considerable difficulties in testing the concept of subclinical mastitis itself other than by accepting (*i.e.*, by agreement rather than by experimentation) definitions of "pathogenicity"

and of normal, threshold, or abnormal levels of cell count. These difficulties are not likely to be resolved by comparing more cell counts with more culture results.

Problems of this nature naturally lead the investigator to attempts to measure the concept of subclinical mastitis as a whole against other parameters not themselves contained within the definition. The most attractive measurement is obviously that of yield, but here we run into the very difficult problem of how to compare what a cow has produced with what she should or might have produced. At the same time, internal and external environmental effects have to be minimized and the "subclinical" changes under examination have to be separated from clinical changes. Of course, the observation that clinical mastitis is usually associated with reduced yields is not under dispute.