Editorial.

THE GRADING OF MILK.

In an Editorial in the February number of the *Journal* we referred to the recommendations of the Committee on Cattle Diseases of the Economic Advisory Council on the grading of milk. The Committee recommended that only four grades of milk should be allowed to be sold: (1) Certified milk derived from tuberculosis-free herds; (2) Pasteurized milk; (3) Sterilized milk; and (4) Milk (uncertified), i.e. milk which has not been heat-treated and has not been derived from tuberculosis-free herds, but which attains a certain hygienic standard. In respect of cleanliness a standard should be prescribed with which all milk should be required to comply.

The designation Grade A should not be continued, as if the herds are subjected to routine clinical inspection, and the milk reaches a certain standard of cleanliness, the fourth grade suggested will not differ materially from what is now called Grade A milk.

The following bacteriological standards were prescribed by the Milk (Special Designations) Order, 1923. Certified milk and Grade A (Pasteurized) milk must not contain more than 30,000 organisms per cubic centimetre and must not contain the coliform bacillus in one-tenth cubic centimetre. Grade A (Tuberculin Tested) milk and Grade A milk must not contain more than 200,000 organisms per cubic centimetre and must not contain the coliform bacillus in one-hundredth of a cubic centimetre. Pasteurized milk must not contain more than 100,000 organisms per cubic centimetre.

The Order contained definite instructions for the medium for plates, dilutions, counting of colonies and coli tests, etc.

Recent work by Professor G. S. Wilson and his co-workers has shown the unreliability of the plate count and the coli test except under definite and restricted circumstances. They advocate a reduction test of methylene blue for the general sampling of milk.

It is important to realize that milk collected under aseptic conditions from apparently healthy udders invariably contains bacteria derived from the milk ducts. Bacteria such as the *Staphylococcus aureus* appear to be often present in milk from normal udders.

The work of many observers has shown that the main sources of contamination after the milk has left the udder are unsterilized utensils. The bacteria gaining access to milk from the air and from dust are negligible compared with the microbes derived from pails, cans, coolers, strainers and bottle fillers which have not been sterilized by steam.

Imperfect cooling is probably the cause of the presence of large numbers of bacteria in milk. These are mainly saprophytic in character and lead to a rapid deterioration in the keeping quality of milk. A specimen of milk collected under insanitary conditions if properly cooled may contain fewer

bacteria than a clean milk produced under excellent conditions which has not been so treated.

The term "cleanliness" expressed by numbers of bacteria is too ambiguous, as it does not distinguish between the bacteria present in milk from these two sources.

Wilson and his co-workers have made a long series of experiments on the bacteriological grading of milk, and their report was published by the Medical Research Council in 1935. (Special Report Series, No. 206.)

They consider that what is required for the bacteriological grading of milk is a simple inexpensive test, with a small experimental error, which can be used on a large scale by inexperienced workers. They discuss the various tests that have been used, and point out how these fail to secure the end in view.

The sedimentation test is declared to have a very limited sphere of usefulness. Its main value is educational in demonstrating to the farmer the dirtiness of his methods.

The Breed smear method, in which 0·01 millilitre of whole milk is spread over one square centimetre, the fat removed with xylol, and over-stained with methylene blue, has been much used in the United States for counting the bacteria in milk, and so enabling samples to be rapidly graded. Within a few minutes unsatisfactory samples can be picked out, and consequently the test is of great service in collecting stations where the milk from individual farms is mixed. The test unfortunately requires the use of a microscope and skilled assistants.

The coliform count and the coli-aerogenes rates are of great value for water examination, but in the case of milk are not so useful as an index of excretal pollution. In water 92 per cent of presumptive coli tests are found to be due to coli of excretal origin ; but in milk 50 to 70 per cent of the coli are of the indefinite or cloacæ-aerogenes type, which are derived from soil and grain.

The true coli types found in milk are derived from cow dung or unsterilized utensils, and have not the same significance as those from human excreta which may indicate the presence of organisms pathogenic to man.

The *B. coli* also does not multiply in water, but does so rapidly in milk kept above 50° F.

As a method of assessing the cleanliness of production of ordinary market milk neither the coliform test nor the coli-aerogenes ratio appears suitable. In the control of pasteurization the coli test may be of wider use. The majority of coliform organisms are killed by the heat of pasteurization. A certain number, however, are heat-resistant, and these are mainly the type which is predominant in cow dung. If no *B. coli* are found after pasteurization it may be concluded that the process has been properly performed and that the tubercle bacillus will have been killed as its thermal death-point lies between the death-point of the non-heat resistant and the heat-resistant *B. coli*.

If the coli test is used as a test of the efficacy of pasteurization it should be carried out on milk from the holding tank before re-contamination or multiplication of the surviving organisms has had time to occur.

Wilson's experiments show clearly that the plate-count is most unsatisfactory for the grading of milk supplies. The plate-count does not measure the real numbers of bacteria in milk; many of the bacteria are in groups, and colonies on plates do not represent individual organisms, but aggregates which may vary in size and numbers. Clumps may disintegrate during dilution to a variable extent leading in extreme cases to errors of 1,000 per cent. Even if the technique were standardized it would be necessary to allow a margin of ± 50 to 90 per cent on the result of any one milk, depending on the number of plates made for each dilution.

The modified methylene blue reduction test is recommended by Wilson and is performed as follows: A test tube containing ten millilitres of milk and one millilitre of standard methylene blue solution is fitted with a sterile rubber cork; the tube is inverted once or twice to mix the methylene blue with the milk, and then incubated at a constant temperature of 37° to 38° C. in complete darkness. Every half-hour the tube is inverted once in order the keep the fat globules and the bacteria homogeneously distributed. The end point is taken when the dye is completely decolourized to within five millimetres of the surface.

The essential point is the half-hourly inversion of the tube, which prevents the separation of the cream and the formation of irregular zones of reduction.

The decolourization of the methylene blue has been supposed by Demeter to be due mainly to the activity of bacteria capable of forming acid, but Wilson and his co-workers have found that very few aerobic bacteria are incapable of causing the reduction when grown in a medium containing even a very weak reducing system. The predominant organisms at the time of reduction in the tubes incubated at 37° C. were very much the same types in both certified and raw churn milk, viz. micrococci, staphylococci, coliform bacilli, and streptococci.

The reduction test applied to certified milk has been found most satisfactory: it has shown a higher correlation with the keeping quality of the milk than has the plate count. It is considered to be well adapted for the examination of all raw milk, whether of high or low grade, provided it is not used for the milk of individual animals. As regards pasteurized milk, it is thought better to withhold judgment on the value of the test until more data have been obtained.

The reduction test is inexpensive and has a small experimental error: it can be carried out by relatively unskilled workers on a large number of samples; it can classify milk on the basis of cleanliness into the maximum number of grades desirable. It also affords a good index of the keeping qualities of the milk.

Wilson considers that the test has two great advantages over the plate

count. The first is that it is not affected by the aggregation of bacteria; whether arranged in small or large clumps, the reduction time is a measure of their total metabolic activity. The second is that it is considerably more sensitive to the growth of bacteria than is the plate count. After the production of milk there is a lag phase during which, if the temperature is favourable, there is a marked growth in the size of the bacteria, but no division and consequent increase in numbers. During this phase the plate count remains stationary, but since the bacteria are in active growth there is a considerable fall in the reduction time of the methylene blue.

Obviously the keeping quality of the milk is much less at the end than at the beginning of the lag phase. The reduction test is the only one having the power of showing this early growth of the bacteria, and is therefore fitted for gauging the keeping quality of milk.

Wilson's observations have been confirmed by workers in Canada and the United States, who consider that the methylene blue reduction test is the best measure of the keeping quality of milk yet available.

Wilson suggests as a test that 75 per cent of samples of morning milk from any given farmer left at atmospheric temperature for twelve hours after milking, and subsequently refrigerated over night, should have a reduction time of over five and a half hours in the summer and of over six and a half hours in the winter. The same standard should apply to evening milk left at atmospheric temperature for eighteen hours and examined directly.

By means of the methylene blue test, Wilson thinks it should be possible to examine the milk of every farmer at weekly or fortnightly intervals throughout the year at a fraction of the cost of that of the plate count. He insists on the importance of frequent examinations of the raw milk. He considers that the condemnation of a producer or distributor on the result of single samples is most unjustifiable, and should be avoided by public health officials.

The new Milk (Special Designations) Order, No. 356 of 1936, was issued by the Minister of Health on the 18th April and came into operation on the 1st June.

Under this Order, the special designations which may be used in relation to milk will be "Tuberculin Tested," "Accredited" and "Pasteurized."

In order to obtain a licence for the designation "Tuberculin Tested," the producer has to have every animal in the herd submitted to a tuberculin test at an interval of not less than two and not more than six months after the last preceding test of such animal and every animal born or bred in the herd must be tested before it reaches the age of 12 months. No animal can be added to the herd unless it has passed the tuberculin test within fourteen days of being so added.

Every animal in the herd must be examined by a veterinary surgeon at intervals of not more than six months and every animal showing the

existence of disease likely to affect the milk must be segregated or removed from the herd.

All dealers in milk, whether producers or not, must not treat the milk at any stage by heat unless a licence to use the designation "Pasteurized" has been granted.

Until December 31, 1936, the milk, if not pasteurized, must not contain more than 200,000 bacteria per millilitre and there must be no coliform bacillus in one-hundredth of a millilitre.

On and after January 1, 1937, milk, if not pasteurized, must satisfy a methylene blue reduction test and there must be no coliform bacillus in one-hundredth of a millilitre. The tests must be carried out in a manner directed by the Minister of Health. If the milk is pasteurized a sample taken before delivery must not contain more than 30,000 bacteria per millilitre.

In order to obtain a licence for "Accredited" milk a producer must arrange to have every milch cow belonging to the herd examined once in every three months by a veterinary surgeon. If an animal is certified as showing evidence of any disease likely to affect the milk injuriously it must be segregated or removed from the herd. The herd must not at any time contain any animal which was known to have previously reacted to the tuberculin test.

As in the case of the "Tuberculin Tested" milk an "Accredited" milk must not be treated by heat. Until December 31, 1936, this milk must not contain more than 200,000 bacteria per millilitre and there must be no coliform bacillus in one-hundredth of a millilitre.

After January 1, 1937, "Accredited" milk must satisfy the methylene blue reduction test and must contain no coliform bacillus in one-hundredth of a millilitre. The tests to be carried out as the Minister of Health may direct.

"Pasteurized" milk must not contain more than 100,000 bacteria per millilitre and the number of bacteria will be determined in such a manner as the Minister may direct. Savage points out that the legal standard of 100,000 bacteria per millilitre is of no value as regards the efficiency of pasteurization. It simply refers to the quality of the milk as delivered to the consumer, and for this purpose it should be taken as near to the consumer as possible and not at the pasteurizing plant. He considers that the coli test is the best bacteriological test and a sample of twenty cubic centimetres taken at the holder should contain no coliform organisms.

Savage has found the phosphatase test recommended by Kay and Graham to be a reliable test of the efficiency of pasteurization. The test has been used regularly in the Somerset County Laboratory during the past six months and has been found reliable and most valuable.

It is a quantitative test and depends on the ability of phosphatase to liberate free phenol from disodium phenyl phosphate. The phenol is estimated colorimetrically. Kay and Graham consider that 2·3 Lovibond

blue units should be the maximum colour allowable in properly pasteurized milk.

There is a short test which can be performed in half an hour; the milk tubes under test are heated to 47° C. in a water bath for ten minutes. If the test is properly carried out a colour greater than 2·3 Lovibond blue units means that the milk has not been properly pasteurized.

There is also a longer test in which the milk tubes to be tested are warmed to 37°-38° C. and maintained at this temperature for twenty-four hours.

A full description of the methods is given in the *Journal of Dairy Research*, May, 1935.

In a great many experiments carried out with commercial milk in the laboratory Kay and Graham found that if the milk had been heated at 145° F. for thirty minutes and then cooled rapidly the Lovibond blue units did not exceed 2·2. It was possible to detect the addition of small quantities of raw milk to milk pasteurized at 145° F. for thirty minutes; as little as 0·2 per cent of raw milk raised the blue units to 2·5, and 0·5 per cent of raw milk gave 3·7 and 4·2 blue units in different samples. Very occasionally the milk of individual cows, usually in early stages of lactation, had a low content of phosphatase so that heating to 142·5° F. was enough to reduce the blue colour to below the standard of 2·3 units. This did not occur with the mixed milk of a herd.

They consider that taken together the two tests form an efficient method for the laboratory diagnosis of faulty pasteurization.

The short test will tell us in a few minutes if a sample of milk has been treated sufficiently to destroy pathogenic organisms. If it is negative the milk must have been heated and the only possibility of pathogenic organisms being present would be if raw unheated milk had entered the main bulk after pasteurization.

The long test can be used quantitatively by estimation of blue units under standard conditions, and will reveal small deficiencies in pasteurization technique. A large reaction in urban pasteurized milk suggests that living pathogenic organisms may be getting into the milk and a guinea-pig test will probably reveal the presence of the tubercle bacillus.