The examination of cow's milk for the detection of pathogenic properties / by Sheridan Delépine.

THE EXAMINATION OF COW'S MILK FOR THE DETECTION OF PATHOGENIC PROPERTIES.

BY

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THE examination of milk as generally conducted for public health purposes has for chief objects the detection of impurities or alterations indicating clearly fraudulent admixtures or absence of the necessary care to prevent gross contaminations.

Comparatively simple, physical, and chemical methods have, with these objects, been used by public analysts.

After taking the reaction, the specific gravity, the amount of fat, and sometimes the total amount of solids other than fat, the chemist can generally tell whether water has been added, cream abstracted, or unfavourable fermentations have taken place. He may, by further examination, be able to recognise the presence of such substances as carbonate or chloride of sodium, carbonate of calcium, salicylic acid, boracic acid, glycerine, gum arabic, starch, etc., etc., when these have been added for some purpose or other.

By a microscopical examination some useful information may also be gained. Cells or granules, such as starch corpuscles, hairs, fungi, débris of food or litter, blood, pus corpuscles, colostrum corpuscles, etc., may easily be detected.

Such examinations make it possible to exclude from the market milk which does not come up to certain standards of purity ; exclusion which from the sanitary point of view, and quite apart from the legal aspect of the question, is very desirable. There can be no doubt that milk which has been adulterated or grossly contaminated has not only inferior feeding qualities, but is liable to produce disease. It is therefore unnecessary to insist upon the importance of ordinary methods of milk inspection and analysis.

It may be well, however, to consider whether such methods are sufficient to establish the absence of noxious properties. In other words, being admitted that when milk is shown by ordinary analysis to be of bad quality it should be condemned, is it certain that milk which is not thus condemned will be free from bad properties?

Numerous outbreaks of disease attributed to milk have been recorded during the last quarter of a century, and these seem to prove that milk is an important factor in the dissemination of disease, and that it is not easy by the methods of inspection at present in use to protect the public against this danger.

Mr Ernest Hart has published in 1881 and 1897 two series of articles on milk-borne disease, which show clearly the extent of the danger, and the interest taken in this question is well shown by the notoriety acquired by Power and Klein's investigations in connection with the Hendon outbreak.

Among the diseases said to be communicable by milk, it is enough to mention tuberculosis, diphtheria, scarlet fever, typhoid fever, cholera, aphthous stomatitis, various forms of gastro-enteritis, giving rise to diarrhœa, vomiting, and various other morbid manifestations. All these diseases are liable to occur in our country. It is unnecessary here to allude to forms of intoxications or infectious diseases which may occur elsewhere.

It would be a matter of great importance to discover how milk acquires the noxious properties which give rise to so much mischief. The cow has been blamed in a great number of cases, and on good grounds. If disease of the cow were the only cause of contamination of milk, it is evident that careful inspection of stables by competent veterinary inspectors should do much to stop the evil, and what has already been done in this direction has given, so far, results which justify our expectations.

But milk is exposed to many contaminations after milking, and it is important to discover also the share which these contaminations have in the production of disease.

For many years I wished to study this question, but as such a study requires the command of a large amount of material, and access to many sources of milk supply, I was able only to make now and again a few occasional observations. In the course of last year I was, however, suddenly supplied with the material I wanted.

Both in Manchester and Liverpool the Medical Officer of Health independently determined to enquire how far the milk supply of those towns was to blame for the spread of tuberculosis.

The object of the Medical Officers of Health of Liverpool and Manchester, was therefore to ascertain how much of the milk supplied in these towns was tuberculous.

Dr Hope dealt with the usual milk supply of cities so as to obtain a measure of the risks of infection from the mixed milk usually found on the market. He had altogether 167 samples of milk examined by Professor Hamilton, Professor Boyce, Dr Woodhead and myself, and the results of these examinations will be found in Dr W. E. Hope's report.[1]

In this communication I will confine my remarks to the specimens I have examined myself.

In Manchester the investigation was conducted in a different manner, and formed part, so to speak, of a sanitary inspection of the cowsheds. The chief object of the examination of milk was to

[1] Report of the Medical Officer of Health on Tuberculosis as affecting the milk supply of the city, Liverpool, 6th May 1897.

discover in what proportion of the cows found in a state of advanced tuberculosis the milk was capable of communicating tuberculosis. The result of this very important investigation will be found in Dr Niven's report.[1]

Before and since the beginning of these two enquiries I have also made a number of independent observations on milk infections of various kinds. Putting aside a number of examinations the results of which have yet to be submitted to Public Health Departments. I have as a basis for discussion, the results of some seventy examinations of milk, viz.:—

Thirty-six specimens of mixed milk from Liverpool, sent by Dr Hope.

Twenty-four specimens of unmixed milk from Manchester, sent by Dr Niven.

Ten specimens of unmixed milk from Manchester, collected by myself.

It will be convenient to consider separately the results obtained with the thirty-six mixed milks, and afterwards those found in the case of the thirty-four unmixed milks. Having already summed up in a report to Dr Hope the results which I have obtained with the mixed milks which he sent me from Liverpool, this report may with advantage be reproduced here.

I am much indebted to Dr Hope for kindly supplying me with information as to the sources from which the samples I had examined from him were derived. This information was given to me only after the issue of my report.

REPORT ON SPECIMENS OF MILK SENT BY THE LIVERPOOL MEDICAL OFFICER OF HEALTH FOR BACTERIOLOGICAL EXAMINATION.

4th April 1897

1. In submitting this report on specimens of milk which have been sent for investigation to me, by Dr Hope, I wish to explain briefly the methods which I have used.

In investigations of this kind the value of the report depends entirely on the methods used and on the care with which they have been carried out.

Too much care cannot be expended on researches the results of which may serve on the one hand to reveal a public danger, and, on the other hand, cause serious losses to certain members of the community.

To find whether a certain sample of milk is capable of causing tuberculosis two methods can be followed. (1st) The parasite causing the disease (the bacillus tuberculosis) may be looked for with the microscope in the milk; (2nd) The effect which this milk has on animals may be tested by direct experiments.

Young animals susceptible to tuberculosis may be fed on the milk in question, or small quantities of the same milk may be injected under the skin or into the peritoneum. When tubercle bacilli are present in the milk *in sufficient numbers to be a source of danger*,

[1] Report on the Manchester Cowsheds, etc., Manchester, 1897.

tuberculosis will be produced in the inoculated animal. As I have shown several years ago,[1] by inoculating guinea-pigs with very small quantities of tuberculous products under the skin of the hind leg, on the inner aspect of the thigh, distinct tuberculous lesions are produced within twelve days, and these become more and more distinct during the following week. Such lesions bear quite distinct relations to the seat of inoculation, so that it is possible to clearly recognise the lesions which are the result of the inoculation from those which might have been produced accidentally in some other way. The adjoining diagrams show the effects of subcutaneous inoculation of tuberculous matter of ordinary virulence (*e.g.*, tuberculous sputum) at various intervals after inoculation on the inner aspect of the leg at the level of the femoro-tibial articulation. They correspond to four stages which I have adopted for descriptive purposes: I. 1st stage, There is a local lesion at the seat of inoculation. At that date the popliteal inguinal, and even the sublumbar ganglia are usually also swollen and congested, and the first two may also show patches of necrosis. II. 2nd stage, All the lymphatic ganglia between the seat of the lesion and the diaphragm on the side inoculated are distinctly affected; the retrohepatic ganglion and the spleen are also involved. III. 3rd stage, The same organs as above are more diseased, and, in addition, the liver, the bronchial ganglia, and the lungs are affected, and several other lymphatic ganglia, *on both sides* of the body, above the level of the diaphragm. IV. 4th stage, Same organs as above, more diseased. In addition to them the lymphatic ganglia of the side *not inoculated*, and below the level of the diaphragm are beginning to be involved. In all these diagrams I have drawn the tuberculous lesions black. These diagrams indicate what can be recognised by means of a naked-eye examination only.

I have also demonstrated (by a series of several hundred experiments) that when proper precautions are taken (*isolation of animals, disinfection of cages, good ventilation of animal house, clean feeding, inoculation under strict aseptic precautions*) the most varied pathological products (other than tuberculous) can be introduced under the skin of the guinea-pig without producing lesions that can be mistaken for tuberculosis.

Many observers are still under the impression that intraperitoneal inoculation is preferable to subcutaneous inoculation; this is undoubtedly an error. The peritoneal cavity is not more sensitive to tuberculous infection than the subcutaneous tissue, the chances of accidents from other infections are greater, the relation of the lesions to the inoculations are not so clearly established, and by proper devices it is possible to introduce under the skin as large an amount of material as is necessary for the purposes of the test.

2. From the above it will be seen that I have special confidence in subcutaneous inoculation of guinea-pigs as a test for tuberculosis. This, however, has not prevented me from using other methods, for I thought they might furnish important additional information.

In order that the examination of the specimens should be as useful

[1] On the Value of Experimental Tuberculosis in Diagnosis. B. M. J., Sept. 1893, and several subsequent papers in the British Medical Journal and the Medical Chronicle, Manchester.

as possible, I have thought it also desirable in each case to apply a few tests showing the state in which the specimen was when I received it and, roughly, the quality of the milk. For this purpose I have, whenever possible, taken the reaction, the specific gravity, the amount of cream that could be separated in a quarter of an hour by means of the centrifugal machine (giving 3000 revolutions to the minute), the amount of sediment, the number of cells, and the total amount of bacteria present.

In all cases the specimens were examined the same day as I received them.

3. Even if tubercle bacilli were equally distributed through tuberculous milk (which they are not), a very large number of them would have to be present before one could expect to find one bacillus now and again in the few drops of milk used for microscopical examination.

To examine completely 1 ounce of milk microscopically it would be necessary to make at least 500 microscopical examinations, for not more than one drop can be examined at a time. If there were about 100 bacilli in that ounce of milk it would be necessary to examine on an average five drops of it before one bacillus was discovered. To prepare and examine each drop thoroughly does not take less than twenty or thirty minutes, so that the practicability of microscopical examination is much diminished by the length of time it requires and the considerable labour it involves when bacilli are very few.

Yet one bacillus in each five drops would correspond to a total of about 5000 bacilli in a single glass of milk, or 10,000 in a pint!

When the milk of a cow suffering from well-marked tuberculous udder, is examined unmixed with other milk it is often found that it contains small curdy masses in which many bacilli may be discovered, so that examining more specially these small masses it becomes possible to discover the bacilli within a reasonable time. In the case of mixed milk such as is obtained in our town dairies it is necessary to have recourse to some other device to obtain the bacilli in sufficient numbers for demonstration.

The simplest way is to cause the impurities contained in some ounces of milk to separate as a sediment, and then to examine a part of that sediment. As it is desirable not to allow the milk to stand exposed to various contaminations for any length of time, it is advisable, in order to obtain the sediment rapidly, to make use of the centrifugal machine.

The sediment obtained from 80 ccm. of milk (*i.e.* a little less than 3 ounces) is far too bulky to be examined entirely with the microscope. In most cases I have been able to examine only a small fraction of such a sediment. From this it will be evident that whenever tubercle bacilli are discovered by the microscopical method they must be in such abundance as to make the milk infectious in a very high degree. I may say at once that out of some forty specimens of *unmixed milk* obtained at various times from tuberculous cows I have found the bacilli abundant in microscopical preparations only in two cases, and in these cases the udder was much diseased, in two other cases tubercle bacilli were found in small numbers, and that, only after a very careful search. By the inoculation method 20 to 25 per cent. of

these milks were found to be tuberculous. In some eighty specimens of mixed milk which I have examined microscopically for tubercle bacilli I have not been able to demonstrate clearly this organism. I may say, however, that in no case have I examined more than four drops of sediment, and generally not more than two.

4. With regard to inoculations it is also necessary to keep a few points in mind.

It is not advisable to inject large quantities of milk into the peritoneal cavity and still less under the skin of guinea-pigs. Yet it is necessary that the number of bacilli injected should not be too small, or else the animal inoculated might resist the tuberculous invasion.

I have found that when more than 2 ccs. (about 30 minims) of milk are injected subcutaneously, a painful swelling is produced, often associated with necrosis of the tissues. 2 ccs. *of pure milk* can be injected without producing evidence of more discomfort than an ordinary injection of morphia would produce in man.

I have therefore invariably separated the sediment from about 3 ounces of milk, and injected half of that sediment mixed with 2 ccs. of the milk from which it had been separated under the skin of one guinea-pig, and the other half of the sediment mixed with 5 ccs. of the same milk into the peritoneal cavity of another guinea-pig.

The animals were then kept from the very beginning in separate disinfected cages in a warm, well-ventilated and well-lighted animal house. Under these conditions, in most cases, they seemed to suffer in no way from the injection, and a few minutes after the injection they looked as well as before. In a few cases, however, the milk proved highly virulent and killed the animals within forty-eight hours. Such milk when taken uncooked in large quantities would probably cause gastro-intestinal troubles. With the exception of the few that died thus, and of some others that died from accidental causes unconnected with the inoculations, all the other animals were kept three, four, and sometimes up to seven weeks. They were then killed and a careful *post-mortem* examination made.

5. In the case of the animals inoculated in the leg all the lymphatic glands situated on the lymphatic paths carrying the lymph from the inoculated part were examined ; so were the spleen and the liver and very often other viscera as well.

In the case of animals inoculated in the peritoneum, the mesenteric ganglia, the spleen, the liver, the diaphragmatic and pelvic lymphatics and the peritoneum itself were minutely examined.

Tuberculous lesions of some of these parts can be recognised easily with the naked eye from ten days to two weeks after inoculation, and after four weeks they are in all quite unmistakable. Yet to leave no room for doubt, whenever the lymphatic ganglia, the spleen, or the liver showed any signs of inflammation they were examined microscopically with a view to discover whether tubercle bacilli might be present against all expectations. In some cases I have also isolated the bacteria that had caused acute inflammatory lesions of the peritoneum, subcutaneous tissue, lymphatic ganglia, or spleen.

A bacillus resembling closely, if not identical with, the bacillus coli communis has been found to be the cause of rapidly fatal peritonitis in several cases, and seems to have been the cause of death in at least

one more case. In some other cases cocci and other micro-organisms have been found in the lesions.

6. All the inoculations and the *post-mortem* and bacteriological observations recorded in the tables appended in this report have been made by myself. Owing to the large accumulation of material which had to be examined microscopically I have been obliged to obtain some help for the staining and examination of many microscopical preparations, and I have to thank Dr F. C. Moore for the very able assistance he has given me in this respect. To Dr E. J. Sidebotham I am much indebted for the care with which he has submitted the various organisms I isolated from the lesions produced by the milk injections to numerous cultural tests. To obtain perfect uniformity in the results, however, I have examined myself all the preparations without exception, so that I am personally responsible for all the statements which are made in this report as well as for the methods used.

7. As an additional precaution to eliminate several sources of error, I have indicated in the table the number of hours which have elapsed between the time when the cows were milked and the time when I made the inoculations and examined the specimens. I have also separated from each other various lots of milk received at different periods, so that if there was anything in the way in which they had been collected that had caused them to become contaminated this would be at once apparent. I think there is only one lot in which the suspicion of such an accidental contamination of the sample might be entertained, and that is the last (specimens 400, 401, 402); yet in this group the milk had been kept a shorter time than in almost any other lot, and showed no special evidence of gross contamination.

8. Having given a general account of the procedure I adopted for the examination of a first series of thirty-six samples of mixed milk, and also explained the method followed in recording some of the results obtained, I may now, without entering into similar details again, give the results of other series of examinations conducted in exactly the same way.

Nine mixed milks received from Liverpool,[1] completing the first set of forty-five specimens, gave the following results.

Two samples produced well-marked tuberculosis, and in one case also severe septic lesions. Four (including the last) produced severe septic infection, more specially when injected into the peritoneum. Two of these specimens produced acute purulent peritonitis, fatal in twenty-four hours ; and one produced extensive necrosis and suppuration when injected subcutaneously. Two samples produced slight inflammatory lesions, indicating irritating properties. Only one was entirely free of any noxious properties.

As in the previous cases of rapidly fatal septic infection, it was possible to isolate from the peritoneal exudation and from the blood of the heart a bacillus to which further allusion will be made later on, and in one case the streptococcus pyogenes was also found in the

[1] These nine specimens are not included in the list of specimens given on the second page of this paper. If these nine specimens and a new series of thirty-two samples referred to later on be added to the first list, it will be found that the total number of samples is 111 instead of 70.

blood. In addition to these two organisms the local lesions several times contained the staphylococcus pyogenes aureus.

9. To sum up the points brought out by the examination of the series of forty-five mixed milks, the following statements may be made.

(1.) They were all more or less contaminated with extraneous matter, animal and vegetable, evidently derived from the cow, the stable, milk pails, cans, etc.

(2.) Cells from the udder were present in all cases, but sometimes they were very few in number, at other times they were excessively abundant. The milks which proved by inoculation to be tuberculous contained in all cases a large number of cells, but other milks containing many cells produced no tuberculosis, and sometimes even not more inflammation than milks containing very few cells.[1]

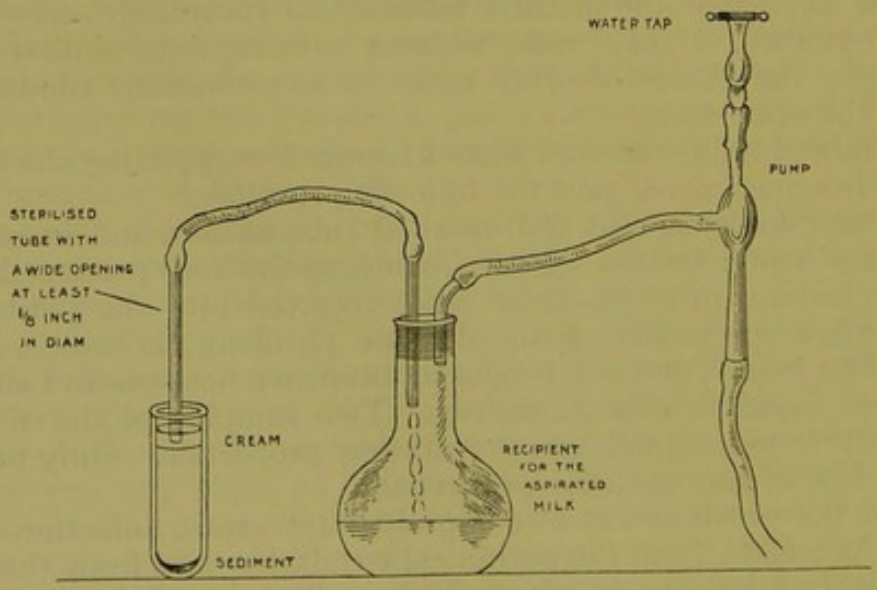
(3.) Twenty-nine out of the forty-five specimens contained a large number of bacteria of various kinds.[2]

[1] The cells present were chiefly large and small epithelial cells, vacuolated or not; debris of these cells were very frequent, and often resembled leucocytes. Leucocytes were present in a little more than half the cases, but were very seldom numerous. A few large epidermal scales were seen in about half the cases.

[2] As the organisms present were not isolated and cultivated it is difficult to speak positively regarding their identity, but I made careful microscopical examination in all cases, and the following types of microbes were found. (1) Staphylococci almost always. (2) Streptococci in less than half the cases. (3) Diplococci in about half the cases. (4) Tetrades or sarcinæ in about one-fifth of the cases. (5) A bacillus having all the morphological features of the bacillus lacticus was invariably present and sometimes excessively abundant; in five or six cases, however, it was very scanty. (6) Another short bacillus resembling the bacillus coli was present in many cases. (7) Long slender bacilli were not very frequent. (8) Thick long bacilli resembling the bacillus subtilis were found in nearly half the cases, but were seldom numerous. (9) Yeasts and Oidia were also found in a number of cases, but they were seldom abundant.

I have in the first part of this paper omitted accidentally to mention the method which I used in preparing specimens of milk for microscopical examination.

After the milk has been centrifugalised, the sediment is separated by removal of the supernatant fluid; this is done by aspirating the fluid by means of an air pump so as not to disturb the sediment. This may seem an unnecessary complication, but anybody who has tried to obtain a sediment free from cream after milk has been centrifugalised must have found that it is not at all easy to aspirate or syphon off the layer of cream, which is very dense and



resistant. Having exposed the sediment, a sample is taken with the sterilised platinum loop and spread over a cover-glass. The film is allowed to dry, and immediately after fixed in the flame of a Bunsen burner in the usual way. The cover-glass is then placed in a small covered capsule into which is poured about ¼ to ½ ounce of absolute ether and absolute alcohol (equal parts), in this it is left for from two hours (at least) to twenty-four hours, after which the covered capsule is placed in a shallow dish full of very hot water, so as to bring the ether to the boiling point or near it. After one-quarter to half-an-hour the cover-glasses are taken out, well washed with absolute

(4.) The reaction, notwithstanding the large number of bacteria present in the majority of specimens, was amphoterous in thirty-two cases; in six cases it was acid, and in seven it was alkaline. (As six out of these seven specimens were sent to the laboratory together, it is probable that the alkalinity was due to some of the alkali used in cleaning the bottles not having been entirely removed by subsequent washing. I may say, however, that all the specimens received in the laboratory had evidently been collected with great care and uniformity of method, and that the inspector whose duty it was to collect the samples had taken all the trouble necessary to obtain clean bottles.)

(5.) Notwithstanding the large number of bacteria often present in the milk, the sediment of seventeen samples could be injected into the subcutaneous tissue and the peritoneal cavity of guinea-pigs without producing any lesions beyond a slight temporary irritation not interfering with the general health of the animal; this was ascertained by careful daily observations and weighing in a number of cases.

In seventeen other cases marked inflammatory lesions were found at the seat of inoculation or in the lymphatic ganglia connected with the inflamed part, and in many instances the general health of the animal suffered, as indicated by a rapid loss of weight. This illness, however, was temporary, and the animals allowed to live long enough recovered. In a few cases, however, the lymphatic ganglia connected with the seat of inoculation remained large. These enlarged glands differed from tuberculous glands in that they contained no tubercle bacilli, were fleshy, had a brownish red colour, and showed no necrotic areas, clearly visible. A short bacillus could be demonstrated in several of these enlarged glands.

Eight of the milks produced intense septic lesions in the shape of acute fibrino-purulent peritonitis or extensive subcutaneous suppurations and necrosis.

In six of these cases the blood of the heart was examined bacteriologically, and a bacillus closely resembling the bacillus coli communis was found invariably present; in one of these cases the streptococcus pyogenes was also isolated. The same bacillus, associated or not with the streptococcus pyogenes and the staphylococcus pyogenes aureus, was also isolated from some of the local lesions. The bacillus, after being cultivated in a state of purity for several generations on agar, produced when injected into guinea-pigs a rapidly fatal septicæmia associated with peritonitis, just as when the milk had been injected.

alcohol, and stained in various ways. Tubercle bacilli can be beautifully stained by the Ziehl-Neelsen method, all the fat having been dissolved and washed out. The methylene blue brings out the cells and bacteria other than the tubercle bacillus. If such a film be stained directly with carbol fuchsin, the coagulated proteids occupying the space between the now dissolved fat globules, stain deeply, so that a permanent preparation showing the original size of the fat globules can be obtained. Such preparations are not suitable for the study of cells or micro-organisms. If, however, before staining with carbol fuchsin the specimen is left for a short time in 10 per cent. sulphuric acid, the proteid layer does not stain any more, and the cells and micro-organisms stain readily. Films of the sediment, cream, and milk half-way between the layer of cream and the sediment were repeatedly examined to ascertain whether the solid impurities were completely forced into the sediment. It was found that the sediment contained the bulk of these extraneous products, but many bacilli, cells, and sometimes even foreign bodies, such as hairs, were carried up by the cream. In two tuberculous cases tubercle bacilli though most abundant in the sediment were also easily demonstrated in the cream; the intermediate layer of separated milk was always freest from all impurities. Bacteria were naturally much more numerous in specimens which had taken a long time to reach the laboratory and in those that had been exposed to a high temperature than in the others. Specimens which had been kept for two or three days in winter often contained less bacteria than specimens examined in spring or summer.

The bacillus was present both in the local lesions and in the blood. I examined the blood of the heart of four guinea-pigs which had not shown sign of disease after being inoculated with milk, and also the blood of a guinea-pig that had become tuberculous, and in none of these cases could I obtain any organism, the tubes inoculated with large quantities of blood remaining absolutely sterile.

Finally, three of the samples examined produced tuberculosis on the animals inoculated.

(6.) None of the milks which produced tuberculosis contained enough bacilli to make their detection possible by microscopical examination of a reasonable duration. The bacilli could not be detected by the examination of two drops of sediment prepared in such a way that these organisms should have been easily recognised. The inoculation test proved, however, that in two of these specimens the bacilli were numerous enough in the sediment of 1½ ounce of milk to produce well-marked tuberculosis in forty-two days and eighty-six days respectively. In the third case the bacilli must have been very few, for only one of the two animals inoculated contracted the disease, and the lesions remained almost enterely localised in the lymphatic ganglia immediately connected with the part inoculated; the bacilli were very few in these lesions.

General Results of the Examination of Mixed Milks.—To conclude this part of the inquiry, the results may be tabulated as follows:[1]—

Properties of forty-five samples of mixed milks kept for twenty-four to fifty-six hours (one seventy-eight hours) after milking.

1. Samples producing no noxious effect or only trivial transient lesions	17
2. Samples producing local irritation, but no general infection	17
3. Samples producing intense local irritation, and general septic infection	8
4. Samples producing tuberculosis	3
Total . .	45

REPORT ON SPECIMENS OF UNMIXED MILKS COLLECTED IN MANCHESTER DIRECTLY FROM THE UDDER IN STERILISED VESSELS.

THE nature of the first Manchester investigation[2] differs considerably from that of the Liverpool one.

[1] In another series of thirty-two mixed milks examined for the health office in Manchester, I have obtained similar results, but as they have not been officially reported upon I will use them only with reference to the part of this communication which has no bearings on the official investigation.

[2] This part of the enquiry is based on the examination of the milk of thirty-one cows, most of which were tuberculous. With two exceptions all these cows were kept in Manchester or its suburbs. Twenty-four specimens were sent to me by Dr Niven in order that I should examine them for tuberculosis. Ten other specimens (from seven different cows) were collected specially for me. In three cases the milk of the same cow was examined both before and after the injection of tuberculin. The lungs and other organs of several of the tuberculous cows, the milk of which had been obtained previously, were examined in the laboratory after the animal had been slaughtered and the diagnosis made by the veterinary surgeons confirmed. The udders of seven tuberculous cows were also examined in the laboratory; two of these from which infective milk had been obtained were proved to be tuberculous both by microscopical examination and by inoculation experiments, five were either healthy or showed non-tuberculous lesions, and three of them on inoculation produced no effect.

The object which Dr Niven had in submitting specimens to me for bacteriological investigation was to ascertain to what extent the milk of cows found by the veterinary inspector in Manchester to be affected with advanced tuberculosis, was capable of producing tuberculosis.

Taking advantage of the material sent to me I determined to extend the investigation. Dr Niven who has, for a considerable time, paid much attention to the influence which milk has on the production of disease, gave me every facility to obtain the information I required.

Mr King, the Chief Veterinary Inspector, showed himself most willing to help me, and I have to thank him and his assistants (more especially Mr Holburn), for the trouble they have taken in this matter.

1. To collect the facts I wanted I had a form prepared in which all particulars regarding specimens sent to me were entered at the time of collecting the samples. The facsimile of this form will show the nature of the information which I thought desirable to obtain, and will indicate at the same time how the facts alluded to further on were obtained.

This label is to be fixed to each Sample of Milk sent to the Bacteriological Laboratory for Analysis, and all the information obtainable entered under the following heads.

Name of Farm — Lab. M. No.
Situation
Description of Cow
No. of Cows kept in same Byre
Byre:—Clean, Not Clean; Ventilated: Well, Badly; Lighted: Well, Badly.
Hands of milker:—Clean, Not Clean. Teats:—Clean, Not Clean.
Does the Cow show any evidence of Disease? Where?
Has the Cow been submitted to tuberculin test? Reaction.
Nature of food — General: Yes or No.
Quantity of Milk yielded — Local: Yes or No.
Date of Milking hour A.—P.M. Signature of
Bottling Sample *id* A.—P.M. Inspector
Bottle sterilised or not

N.B.—Bottle, which should hold 8 ozs., can be sterilised by boiling half-hour in water after cleaning. Corks should be also sterilised.

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2. The accompanying table will give the main results of the examination of twenty-seven samples of milk obtained from twenty-four cows. (The milk of seven other cows was also examined, but as the examination was not carried out in the same way, the results would not have been strictly comparable, and for this reason they have been excluded from the list, but the results obtained with them were quite in accordance with those I make use of here.)

From this table it appears that the milk of six out of the twenty-four cows was capable of producing tuberculosis. Seven specimens produced marked local irritation, in one case very severe but no general infection, no specimen produced rapidly fatal infection, eleven milks produced no marked irritation.

3. It is important to remember, in interpreting these results, that (1) all these cows were, with the exception of one, suspected of tuberculosis, (2) most of them were in an advanced state of tuberculosis, and (3) only seven of them had apparently healthy udders, and of the remainder ten had certainly diseased udders. It was intentionally that such a number of diseased cows had been selected, and it would not be correct to assume that the results obtained represent an average state of things; this being well understood the results may now be analysed.

4. The first question which suggests itself is, *What relation is there between the state of the udder and the pathogenic properties of the milk?*

The results of inoculations will I think show that, generally speaking, the milk from diseased udders was the only one capable of producing tuberculosis, but that with regard to common septic lesions the difference between the milk from diseased and healthy udders was not very striking; this will be shown by the following tables:

Pathogenic Properties of Milk from Diseased and Healthy Udders.

Milk from Udders.		*Producing Tuberculosis.*			*Producing marked Irritation.*			*Producing no marked Irritation.*		
		Actual No.	*Percentages.*		*Actual No.*	*Percentages.*		*Actual No.*	*Percentages.*	
A. Certainly diseased	10	5	50·0		3	30·0		2	20·0	
		6		31·5	5		25·30	8		42·08
B. Probably diseased	9	1	11·1		2	22·2		6	66·6	
C. Healthy	5	0	0	0	2	40·0	40·0	3	60·0	60·0
Total .	24	6			7			11		

Pathogenic Properties of Non-tuberculous Milk from Diseased and Healthy Udders.

Milk from Udders.		*Producing marked Irritation.*			*Producing no marked Irritation.*		
		Actual No.	*Percentages.*		*Actual No.*	*Percentages.*	
A. Certainly diseased	5	3	60·0		2	46·0	
	13	5		38·5	8		61·6
B. Probably diseased	8	2	25·0		6	75·0	
C. Healthy . .	5	2	40·0	40·0	3	60·0	60·0
Total .	18	7			11		

SAMPLES OF MILK COLLECTED IN STERILISED BOTTLES DIRECT FROM COWS SUSPECTED OF BEING TUBERCULOUS (WITH THE EXCEPTION OF ONE).

References.		Dates and Time.		State of Cow.				State of Milk when Received.					Results of Inoculation.			Remarks.
										Microscopical Examination.						
Lot.	*Inoculation No.*	*Date of Reception.*	*No. of Hours after Milking*	*Tuberculosis of Internal Organs diagnosed during Life.*	*Tuber-culin Reaction.*	*Udder found or not diseased during Life.*	*P.-M. Examination of Udder for Tuberculosis.*		*Reaction.*	*Sp. gr.*	*Amount of Cells in Sediment.*	*Amount of Microbes of all Sorts in Sediment.*	*Tubercle Bacilli found or not.*	*Length of Life.*	*Symptoms observed during Life and Lesions found P.-M.*	*Tubercle Bacilli in Lesions.*	
							Microscopic.	*Inoculation.*									
A	325	18 VII 96	76 *	Advanced	—	Doubtful	—	—	Strongly acid	—	—	Very abundant	Not found	—	—	—	Milk coagulated; experiment failed
	326 A1	14	66	14	—	14	—	—	14	—	—	14	14	110	Suppuration—recovery	0	Milk coagulated
	A2													67	14	0	
	327 A	14	66	14	—	14	—	—	14	—	—	14	14	73	Suppuration, diarrhœa—recovery	0	Milk coagulated
B	337 A	11 IX 96	72	Advanced (?)	—	Yes	—	—	Strongly acid	—	Abundant	Abundant	14	71	Suppuration—recovery	0	Milk coagulated
	4103 (2283)	14	14	Advanced	—	Yes	—	—	14	—	Very abundant	Very abundant	Many	No	inoculation	—	14
C	335 A	7 X 96	24	Yes	—	Doubtful	No tubercle found	—	Amphot.	—	—	Moderate	0	71	Slight inflammation—recovery	0	Slight local induration at seat of inoculation
	(337) A													74	14	—	
	(336) B													46	—	—	
D	338	12 X 96	72	Yes	—	Not	—	—	Amphoterous	1032	Very few	Moderate	0	77	Slight local inflammation—recovery	—	Notwithstanding the great length of time this milk had been kept it was quite sweet
E	339 A1	12 X 96	39	Lung found tuberculous by inoculation	0	Not	No tubercle found	0	Amphot.	1029*	Very few	Few	0	32	Slight local inflammation—recovery	—	Milk taken just before tuberculin test
	339 A2	14	25						14	1035*	14	14	0	77	14	—	14 14 hours after injection of tuberculin
	340 A1	14	39	Lung found tuberculous by inoculation	+	Not	14	0	Slightly acid	1028*	Very few	Moderate	0	20	Suppuration—partial recovery	—	Before injection of tuberculin
	340 A2	14	25						Amphot.	1031*	14	Very few	0	32	—	—	14 hours after tuberculin
	341 A1	14	39	Yes	+	Not	14	0	Amphot.	1030*	Very few	Many	0	22	Local inflammation well-marked	—	Before injection of tuberculin
	341 A2	14	25						Amphot.	1027*	14	Moderate	0	42	Local inflammation—recovery	—	14 hours after tuberculin
F	351	4 XI 96	24	Yes	—	—	—	—	Amphot.	1030	Very few	Few	0	58	No distinct lesion	—	
G	393 A	26 I 97	19	Advanced	—	Doubtful	—	—	Slightly alkaline	1026	Excessively abundant	Moderate	About 50 or 60 in about 3 milligrammes of sediment	12	Well-marked tuberculosis	Many	External evidences of tuberculosis were clear 9 days after inoculation of animals
	B													15	14	Abundant	
H	404 A	9 II 97	10	Advanced	+	—	—	—	Slightly alkaline	1026	Very few	Very few	0	37	No distinct lesion	—	
	B													37	Slight local lesion	0	
I	405 A	13 II 97	Under 30	Advanced	+	Yes	—	—	Amphot.	1024	Excessively abundant	Many	Very few	32	Advanced tuberculosis	Abundant	The calf of this cow was killed on the seventh day, and was found to have tuberculous lesions of the mesenteric glands, liver, and lungs. All these lesions were examined in the laboratory. The cow when slaughtered was found in a state of advanced tuberculosis, thoracic and abdominal, and one of the uterine cornua was distinctly tuberculous
	B												2 or 3 in about 3 milligrammes of sediment	12	Inflammation of peritoneum and mesenteric glands	Not found	
	406 A	14	14	Advanced	+	Well-marked	Tubercular lesion of mucous membrane of cistern	—	Slightly alkaline	1030	Excessively abundant	Moderate	Very few	32	Advanced tuberculosis	Moderate	Milk taken during the tuberculin reaction
	B													32	14	14	
	407 A	14	14	Advanced	+	Yes	—	—	Alkaline	1028	Abundant	Moderate	0	32	Slight local inflammation and glandular enlargement	0	Milk taken during the tuberculin reaction
	B													32	—	—	
	408 A	14	14	Yes	+	Well-marked	—	—	14	1027	14	Moderate	0	16	Very slight glandular enlargement	0	Milk taken during the tuberculin reaction
	B													25	—	—	
K	409 A	24 II 97	24	Doubtful	—	Well-marked	—	—	Amphot.	1025	Excessively abundant	Abundant (chiefly streptococci)	0	23	Marked local suppuration and glandular enlargement	0	
L	410 A	24 II 97	12	Yes	+	Well-marked	Tubercular lesions well-marked	Produced tuberculosis by inoculation	Milk squeezed from the diseased udder				—	18	Tuberculosis beginning and suppuration	Few	Milk collected in the cistern, opened aseptically, only 2 ccs. of milk obtained
	411 A	14	14	Yes	+ (?)	14	14	14	Milk squeezed from the diseased udder				—	18	Well-marked tuberculosis	Many	14
M	423 A	23 III 97	90	Yes	+	Well-marked	—	—	Alkaline	1019	Abundant	Abundant	0	6	Local suppuration	0	
	B													90	Nothing found, P.M.	—	
N	424 A	24 III 97	Under 24	Yes	+	Doubtful	—	—	Slightly alkaline	1026	Abundant	Moderate	0	90	0	—	
	425 A	14	14	Doubtful	0	Nodular	—	—	Alkaline	1021	Excessively abundant	Few	0	90	Slight local lesion—recovery	—	
	426 A	14	14	Yes	+	Doubtful	—	—	Slightly alkaline	1030	Few	Very few	0	27	0	—	
	427 A	14	14	0	0	0	—	—	14	1023	Moderate	Very few	0	46	0	—	

In this table + means a positive result obtained by actual observation; 0, a negative result tested in the same way; —, a negative result, not tested, because there was no reason to suspect anything else, or absence of direct observation. These samples have been collected in thirteen different byres.

* Samples 339, 340, 341 were collected on two different occasions, one specimen being taken in the evening just before the injection of tuberculin, the other sample being taken fourteen hours afterwards, whilst the cows were under the influence of tuberculin.

It will be noticed that the sp. gr. of the milk was in each of these cases 6° or 7° higher during the reaction than before.

In comparing the results of inoculation of milk showing distinct evidence of disease of the udder by the presence of a large number of cells and more especially pus corpuscles, with the effects of inoculation of milk showing no such evidence of disease, similarly ambiguous results are obtained with regard to non-tuberculous milks.

Pathogenic Properties of Milk, Rich and Poor in Cells from the Udder.

	Producing Tuberculosis.		*Producing marked Irritation.*		*Producing no marked Irritation.*	
	Actual No.	*Per cent.*	*Actual No.*	*Per cent.*	*Actual No.*	*Per cent.*
Cells abundant or very abundant	6	46·2	3	23·1	4	30·8
Cells scanty or in moderate numbers	0	0	2	25·0	6	75·0

This table would seem to show that about half of the milks containing such a large number of cells that the existence of mastitis could hardly be doubted were tuberculous, but of the remaining half the majority had no noxious properties whatever.

Of the milks which did not exhibit any trace of disease of the udder none were found tuberculous, and one-quarter were capable of producing irritation. If we exclude tuberculosis the influence of disease of the udder on the production of irritative lesions will be more exactly ascertained.

Pathogenic Properties of Non-tuberculous Milk.

	Producing marked Irritation.		*Producing no marked Irritation.*	
	Actual No.	*Per cent.*	*Actual No.*	*Per cent.*
Cells abundant . .	3	42·9	4	56·2
Cells scanty or moderate	2	25·0	6	75·0

Disease of the udder seems therefore to account for the noxious properties of certain samples of milks, yet when other possible factors are considered, it will be evident that other causes are at work, and to determine exactly the possible share taken by disease of the udder (apart from tuberculosis) as a source of human disease, it will be necessary to compare only specimens which have been examined immediately after their being drawn from the udder.

5. Further doubts are thrown upon the primary importance of non-tuberculous disease of the udder by a comparison of the effects of mixed and unmixed milks. If these results be tabulated we get the following figures.

Mixed Milks from the Town Market, and Unmixed Milks from Diseased Cows Compared.

Milk Producing.	*Mixed Milks.*		*Unmixed Milks.*	
	Actual No.	*Percentage.*	*Actual No.*	*Percentage.*
No marked irritation . . .	17	37·74	11	45·76
Marked local irritation, but no general infection	17	37·74	7	29·12
Intense local irritation and general infection	8	17·76	0	0
Tuberculosis	3	6·66	6	24·96
Total .	45	99·90	24	99·84

The most striking outcome of this comparison is that even *the milk of markedly diseased cows is much less often capable of producing irritation than the ordinary mixed milk supplied for consumption*, and whilst 17 per cent. of the latter are capable of producing severe infection, rapidly fatal, none of the milks obtained direct from the udder, in sterilised vessels, produced any affection of comparable intensity.

Even with regard to the minor forms of irritation, such as local abscesses, inflammatory enlargement of lymphatic ganglia, mixed milk proved inferior in quality to unmixed milk from diseased cows.

The same difference is also shown by the comparatively small number of mixed milks which were free from all noxious properties, for whilst only 37·74 per cent. of the mixed milks proved perfectly innocuous, 45·76 (*i.e.* 8 per cent. more) of the unmixed milks showed the same quality. If it be remembered that twenty-three out of the twenty-four of the latter had been taken from tuberculous cows, and that more than one-half of these cows had diseased udders, it will be evident that to get a proper idea of the relative noxiousness of mixed and unmixed milks on the basis of the results quoted above, the tuberculous milks should be excluded.

If this be done there remain forty-two specimens of non-tuberculous mixed milks, and eighteen of non-tuberculous unmixed milks. The pathogenic properties of which were as follows.

	Not Irritating.	*Irritating locally.*	*Highly Virulent.*
	Per cent.	*Per cent.*	*Per cent.*
Mixed milks . . .	40·8	40·8	19·2
Unmixed milks . .	60·5	38·5	0

The obvious conclusion from this is that, *apart from tuberculous mastitis, disease of the udder cannot be the chief source of the pathogenic properties of bad milk.*

6. On the other hand, it is equally evident that something must occur

to the *milk after it has left the udder* to account for the marked difference in the properties of mixed and unmixed milks. Comparing the fate of samples which had been collected straight from the udder and of those which had been collected at railway stations, or in town dairies, it is at once evident that, after leaving the udder, the former had come in contact with nothing but sterilised bottles; in addition to this, it was ascertained in each case that the teats of the cow and the hands of the milker were clean.

In the case of the mixed milks nothing is known of the chances of contamination due to dirty udders, hands, pails, cans, etc. From the cans they were taken with due precaution to avoid any further contamination, but nothing more could be done. These specimens of mixed milks were, when collected, exactly in the state in which they would have reached households, but they had not suffered from any of the additional contaminations which might have occurred at the hands of the retailer, or in the households of the consumers.

Many of the samples were kept in the sterilised bottles for many hours; but this occurred equally in the case of the mixed and of the unmixed milks, so that any differences which were detected in the laboratory were due less to the changes taking place after the taking of the samples than to what was already present in the milk when the samples were taken.

7. It is well known that milk obtained at the farm, in the usual way, is always more or less contaminated with microbes, and that these multiply very rapidly at the ordinary temperature all the year round, much more rapidly, of course, during warm than during cold weather.

We have numerous observations recorded giving the number of microbes which may be found in ordinary dairy milk collected in the usual way.

Gösta Grotenfelt[1] gives several figures which will prove interesting in connection with the points.

Samples taken in a pasture on a fresh somewhat damp summer morning:—

No. of Bacteria in 1 *cc.*

Immediately after Milking.	*Half-hour after.*	*Two Hours after.*
10	88	1530

Samples taken in a farm at the end of the winter and under favourable conditions:—

No. of Bacteria in 1 *cc.*

Immediately after Milking.	*Half-hour after.*	*Two Hours after.*
106	980	3655

[1] The Principles of Dairy Practice from a Bacteriological Point of View. Translation by F. W. Woll, 1894. New York, p. 83 and following.

Samples taken in byres where conditions were less favourable :—

No. of Bacteria in 1 *cc. Half-hour after Milking.*

4,100
2,450
1,890
14,670
830
3,030
5,430
21,700
1,030

Other observers have given much higher figures. Cnopf and Escherich in Munich report having found in a sample of milk recently drawn under precautions said to be of great cleanliness 60,000 to 100,000 bacteria per cc.; but I doubt whether much importance can be attatched to these numbers, because undoubtedly some unsuspected source of bacterial contamination must have existed in that case.

De Freudenreich has given very interesting results showing the rapid multiplication of bacteria in milk at various temperatures. The following table gives the results of examination made at Rütti, and is quoted by Grotenfelt.

Increase in Bacterial Contents in Milk.

	When kept at 15° *C.*	*When kept at* 25° *C.*	*When kept at* 35° *C.*
On arrival at lab.	9·300 per cc.	9·300 per cc.	9·300 per cc.
3 hours later .	1·06 times more	2·0 times more	4·0 times more
6 hours later .	2·5 times more	18·5 times more	1·290 times more
9 hours later .	5·0 times more	107·5 times more	3·794 times more
24 hours later .	163·0 times more	62,097·0 times more	5·376 times more

To have some terms of comparison as to the original bacterial contents of milk obtained in Manchester under the condition of my experiments, I had milk collected from two cows, one kept in a town byre and the other in a suburban farm. The milk was drawn from the udder directly into sterilised beakers and brought at once to the laboratory; both specimens were collected on the 12th of December 1896. The cowsheds, cows, and milkers were clean, and the cows healthy.

	Time after Milking.	*Temp. of Milk.*	*Sp. Gr.*	*Reaction.*	*Microscopical Examinations.**		*No. of Bacteria in* 1 *cc. Plate Culture.*
					Cells.	*Microbes.*	
Town byre milk	20 mins.	30° C.	1025	Amphot.	Very few	Very few*	2·929
Suburban byre	7 hours	17° C.	1026	Amphot.	id	Few*	90·216

* These terms correspond to the terms used in the tables to indicate the number of microbes ecognised by microscopical examination.

When milk is collected in filthy, dark, and altogether insanitary byres the number of bacteria becomes excessive; in one case Grotenfelt found in 1 cc. of milk, three-quarters of an hour after milking, 67,000 bacteria.

I might quote many other figures, some of which are still more remarkable, but I have thought it better to mention only those which, being in accordance with my own observations, seem *to me* the most reliable.

I might mention in connection with this that by keeping milk at a temperature of about 4° C. I have been able to satisfy myself of the fact that instead of an increase there is, generally, a decrease in the number of bacteria, which gets more marked as the milk is kept longer.

From all this it seems most probable that (1) dirty byres, cowkeepers, cows, milk pails and cans; (2) keeping the milk in hot places and during hot weather; (3) keeping the milk for a long time before use, are the chief causes of the noxious properties too frequently acquired by that fluid.

8. I have already explained that in the case of the mixed milks it is impossible to obtain accurate data regarding the first group of causes.

With regard to twenty of the cases of unmixed milks which I examined, I was able to obtain from the veterinary inspectors accurate data as to the state of the byres and cows from which the milk had been drawn. Some of the byres were badly lighted and ventilated, but *in every case* the byre, hands of the milker, and teats, were clean, so that I could find nothing in that direction to account for the marked differences observed in the results of the inoculations.

That all the milks get infected with germs at the time of milking is certain, but no special source of gross contamination could be detected in any of the cases under examination, therefore there remained only the two factors, temperature and time, which could be investigated with any chance of obtaining a clue to the source of the irritating properties of some samples.

A few noxious germs introduced at the time of milking would, possibly, not be numerous enough to produce any pathogenic effect at first, but if allowed to multiply they might become capable of doing much harm.

This line of investigation was also indicated by the marked differences between specimens collected in summer and in winter.

In the following tables I have arranged all the specimens examined, and which had not proved tuberculous, in three groups; the innocuous, the irritating, and the virulent, in the same way as I have done in the previous tables. In each case I give also the length of time the specimens have been kept from the time of milking to that of examination, and the external temperature observed in Manchester during that time.

The recorded temperature which has seemed to me of most importance for this purpose was that observed in the shade, for such temperature would affect equally both the mixed and unmixed specimens. It is probable that many of the mixed specimens had been exposed to higher temperatures during their transit by rail, for, whether conveyed in covered vans or open trucks, they would suffer from the effects of sunshine; I found it, however, impossible to take an accurate account of this difference.

The mean between the maximum and minimum temperature in the shade has been taken for each day. When the specimen had been kept only ten hours the temperature of the day on which the examination was made is given. When kept twenty-four hours, the temperature of the previous day. When forty-eight hours, the average temperature of the two previous days. When seventy-two hours, that of the three previous days.

In every case the recorded temperature has been taken from the very complete and useful table published by Dr Niven in the "Weekly Return of the Medical Officer of Health" Manchester.

Unmixed Milks which did not produce Tuberculosis.

	No. of Reference.	*Date when Examined.*	*How long Kept.*	*Temp. in the Shade.*	*Reaction of Milk.*
			Hours.	*Average.*	
I. Milks causing no lesion, or only trivial irritation, at the seat of inoculation or near it, in one or both of the animals inoculated.	335	7 X 96	24	44·2	Amphot.
	338	12 X 96	72	46·5	,,
	339A1	12 X 96	39	43·8	,,
	339A2	12 X 96	25	38·0	,,
	340A2	12 X 96	25	38·0	,,
	341A2	12 X 96	25	38·0	,,
	351	4 XI 96	24	39·3	,,
	404	9 II 97	10	44·0	Slightly alkaline
	407	13 II 97	under 30	39·8	Alkaline
	408	13 II 97	30	39·8	,,
	424	24 III 97	24	50·7	Slightly alkaline
	425	,,	under 24	50·7	,,
	426	,,	,, 24	50·7	,,
	427	,,	,, 24	50·7	,,
Averages		October November February March	28	43·8	Amphot.
II. Milks producing marked local irritation with no general infection.	326	13 VII 96	66	64·4	Strongly acid
	327	,,	66	64·4	,,
	357	11 IX 96	72	62·5	,,
	340A1	12 X 96	39	43·8	Slightly acid
	341A1	,,	39	43·8	Amphot.
	409	24 II 97	24	49·9	,,
In one of the two animals death on the sixth day, but only local suppuration found *P.-M.*	423	23 III 97	90	50·0	Alkaline
Averages		July September October February March	56	47·0	Acid

Note 1.—The milk of three cows—339, 340, and 341—having been examined twice at various intervals after milking, the results of the two examinations have been entered separately. It will be noticed that in two of these cases—340 and 341—the samples kept thirty-nine hours proved more noxious than those kept twenty-five hours.

Note 2.—The milk in case 423 came from a very diseased udder, as proved by the examination of the veterinary surgeon and the examination of the sample of milk by myself; this sample was crowded with cells, and its specific gravity was only 1019.

Mixed Milks which did not produce Tuberculosis.

	No. of Reference.	*Date when Examined.*	*How long Kept.*	*Temp. in the Shade.*	*Reaction of Milk.*
			Hours.	*Average.*	
I. Milks causing no lesion, or only trivial inflammation at or near the seat of inoculation.	360	23 XII 96	52	35·5	Amphoterous
	361	,,	78	35·3	,,
	362	,,	53	35·5	,,
	374	31 XII 96	29	43·1	(?) ,,
	375	,,	29	43·1	,,
	378	16 I 97	42	35·9	,,
	380	,,	40	35·9	,,
	381	,,	42	35·9	,,
	382	,,	50	35·9	Slightly acid
	385	23 I 97	48	34·4	Slightly alkaline
	386	,,	50	34·4	,,
	388	,,	50	34·4	,,
	389	,,	40	34·4	,,
	390	,,	50	34·4	,,
	395	28 I 97	42	31·9	,,
	398	,,	40	31·9	Amphot.
	449	4 V 97	45	47·4	,,
Averages		December January	46	36·4	Amphot.
II. Milks producing marked local irritation, but no general infection.	359	23 XII 96	54	35·5	Amphot.
	363	,,	53	35·5	,,
	365	,,	27 (No certain data.)	35·5	,,
	366	,,	27 (No certain data.)	35·5	,,
	368	,,	27 (No certain data.)	35·5	,,
	371	31 XII 96	28	43·1	,,
	373	,,	30	43·1	,,
	376	,,	30	43·1	,,
	377	16 I 97	41	35·9	,,
	379	,,	40	35·9	,,
	387	23 I 97	51	34·4	Slightly alkaline
	394	30 I 97	49	33·2	Amphot.
	396	,,	49	33·2	Acid
	397	,,	42	33·2	Amphot.
	401	,,	28	33·2	,,
	446	5 V 97	56	46·7	Acid
	447	,,	45	46·5	Amphot.
Averages		December January	42	37·6	Amphot.
III. Milk producing intense local inflammation and general infection rapidly fatal.	364	23 XII 96	53	35·5	Amphot.
	367	,,	27? Not certain.	35·5	,,
	400	2 II 97	27	36·7	,,
	402	,,	29	36·7	,,
	445	5 V 97	56	46·7	,,
	448	,,	45	46·5	,,
	481	18 V 97	32	56·5	,,
	483	,,	32	56·5	Acid
Averages		February May	39	43·8	Amphot.

Second Series of Mixed Milks examined in Manchester (this Series has not been included in any of the previous Tables).

	No of Reference.	*Date when Examined.*	*How long Kept.*	*Temp. in the Shade.*	*Reaction of Milk.*
			Hours.	*Average.*	
I and II. Cases producing no general infection rapidly fatal.	470	2 VI 97	31	58·5	?
	471	,,	31	58·5	?
	472	,,	31	58·5	?
	473	,,	31	58·5	?
	495	7 VII 97	9.30	53·7	Slightly alkaline
	497	14 VII 97	8	64·3	Alkaline
	498	,,	8	64·3	Amphot.
	500	,,	8	64·3	Slightly acid
	507	28 VII 97	8.20	59·7	Amphot.
	508	,,	8.35	59·7	,,
	509	,,	9.30	59·7	,,
	510	,,	9.45	59·7	,,
	515	4 VIII 97	8.50	75·3	,,
	516	,,	9.20	75·3	,,
	520	11 VIII 97	8.40	60·7	,,
	521	,,	8.20	60·7	,,
	522	,,	9	60·7	,,
	523	,,	9.20	60·7	,,
	525	18 VIII 97	9.35	60·3	,,
	526	,,	9.55	60·3	,,
	527	,,	10	60·3	,,
	528	,,	10.15	60·3	,,
Averages			13	62·0	Amphot.
III. Cases producing intense local lesions and general infection.	441	23 IV 97	over 36	55·3	Slightly alkaline
	502	21 VII 97	17	61·8	Slightly acid
	503	,,	17	61·8	Slightly alkaline
	504	,,	17	61·8	Slightly acid
	505	,,	17	61·8	Acid
	517	4 VIII 97	8	75·3	Amphot.
	518	,,	8	75·3	,,
Averages			17	64·7	Acid or alkaline

[TABLES—*continued*

These tables may be summarised as follows:—

I. *Mixed Milks coming from a distance of generally over 40 miles, and generally kept for from twenty-four to sixty hours, and even more in a few cases. (Tuberculous samples excluded.)*

Mean Temperature in the Shade (Manchester) during Time the Specimens were kept.	*Specimens producing no Noxious Effects.*	*Noxious Specimens.*	*Totals.*	*Percentage of Good Specimens.*
30° to 35	7	5	12	58·0
35 to 40	7	11	18	38·5
40 to 45	2	3	5	40·0
45 to 50	1	4	5	20·0
50 to 55	—	—	—	—
55 to 60	0	2	2	0·0
	17	25	42	39·0

II. *Mixed Milks coming from a short distance (generally under 20 miles), most of them kept for less than ten hours (with the exception of five out of the seven bad specimens, and four out of the twenty-two good specimens). (Tuberculous samples excluded.)*

Mean Temperature in the Shade (Manchester) during Time the Specimens were kept.	*Specimens producing no Noxious Effects.*	*Noxious Specimens.*	*Totals.*	*Percentage of Good Specimens.*
50° to 55	1	0	1	100·0
55 to 60	8	1	9	88·8
60 to 65	11	4	15	73·2
65 to 70	—	—	—	—
70 to 75	2	2	4	50·0
	22	7	29	75·68

III. *Unmixed Milks kept for various lengths of time, but collected from the Udder in Sterilised vessels. (Tuberculous samples excluded.)*

Mean Temperatare in the Shade (Manchester) during Time the Specimens were kept.	*Specimens producing no Noxious Effects.*	*Noxious Specimens.*	*Totals.*	*Percentage of Good Specimens.*
35° to 40	6	0	6	100·0
40 to 45	3	2	5	60·0
45 to 50	5	2	7	71·5
50 to 55	—	—	—	—
55 to 60	—	—	—	—
60 to 65	0	3	3	0·0
	14	7	21	67·2

The influence of time is well shown by the number of specimens remaining good even at high temperature when the milk had been kept only half-a-day.

On the other hand, the influence of temperature is still more evident, for in every category the number of good specimens is almost inversely proportional to the height of the temperature.

When the clear relation existing between time of keeping *plus* temperature and the noxious properties of a certain number of samples of milk is contrasted with the ambiguous results obtained when an attempt is made to connect these noxious properties with disease of the udder, it is difficult not to feel convinced that *infection of the milk outside the udder and the conditions under which it is kept are the most important factors causing it to acquire at times pathogenic properties.*

9. I have been able to collect some facts during the last few years in connection with outbreaks of epidemic diarrhœa due to the consumption of milk. I have found a close resemblance between the infection which I have produced in guinea-pigs by the inoculation of milk which had given rise to one of these outbreaks and that produced in guinea-pigs by the injection of several samples of mixed milks obtained on the market. This has led me to suspect that milk must be one of the most potent causes of the summer diarrhœa of children, an opinion which is also held by several well-known authorities. My belief has been strengthened by the fact that from all the cases of fatal septicæmia due to milk injections I have been able to isolate a bacillus identical with that which I have obtained twice from milk causing intense diarrhœa in children and a few adults. Other organisms have been present in a few specimens, but the only organism constantly present has been this bacillus. This microbe retains its virulent properties even after being cultivated for several generations outside the body. It resembles closely the bacillus coli communis in its pathogenic action, its mode of growth on gelatine, agar, potato, milk, lactose agar, glucose gelatine; its size and shape; its motility. The differences are so much within the limits of variations observable in that organism, that I am still uncertain whether the differences I have observed are enough to allow of its being considered a different species. It does not either correspond exactly to any of the bacilli resembling closely the bacillus coli. It is possibly only a pathogenic variety, but for the present it will be enough to say that it is *a bacillus of pathogenic milk.* In a further communication I will deal more in detail with this bacillus, and discuss the probable share which milk has in the production of human disease.

10. One of the outcomes of this investigation is that the ordinary methods of examination used for the detection of bad milk do not fulfil adequately their purpose.

An examination of the tables I have given in this communication will show that the Sp. gr., the reaction, the quantity of cream, the presence of cells or of microbes, taken separately or together, are insufficient indices of the pathogenic properties of milk.

An excessive number of cells is undoubtedly a very suspicious sign, especially when many of these cells are leucocytes. About one-half of the specimens very rich in cells were found to be capable of producing

tuberculosis, but, tuberculous specimens excluded, there were some poor in cells which were exceedingly noxious. The number of microbes is a still less certain guide.

Unless I am greatly mistaken in my conclusions, it would seem that if cowsheds and the distribution of milk were under proper supervision, a very small amount of bad milk, if any at all, should reach the consumer. The chief disease to be dreaded, as communicable by milk, is tuberculosis, and there can be no doubt that usually it is only the milk of tuberculous cows with tuberculous udders which is capable of producing tuberculosis experimentally.[1] Surely such a danger could well be eliminated by general, systematic, and frequent inspection of *all* cowsheds by *competent* veterinary surgeons. The effects of this system of inspections should be periodically tested by investigations such as the one so successfully conducted in Manchester by Dr Niven, to whose valuable report I must refer the reader for much information not alluded to in this communication.

Work of this kind should be done under the direction of Public Health Authorities. Any other course would cause great loss of time and money. I am not, in saying this, overlooking the importance of the agricultural interests, but as the protection of human life is an object of paramount importance, I am convinced that more good will be obtained by harmonious joint action of the veterinary and medical professions than by independent action of either.[2]

As to the other sources of pathogenic properties, they could also be checked by proper inspection, having for object to obtain, (1) cleanliness in the byre, and of milk vessels. (2) Rapid transit of milk. (3) The keeping of the milk at as low a temperature as possible. I do

[1] It must be well understood that I do not mean to infer that the most common source of human tuberculosis is to be found in the ingestion of tuberculous milk. Undoubtedly in the great majority of cases infection takes place through the lungs. But because infection through the bowel is not the most common source of tuberculosis, this is not a reason why it should be neglected. It is easier to protect children against this danger than against inhalation of tuberculous dust, and it would be madness not to attempt to combat the lesser evil because there is a greater one more difficult to contend with. Some doubt regarding the value of experiments on guinea-pigs might be entertained. It may be justly argued that guinea-pigs are more liable to tuberculosis than man, and also that injection of tuberculous matter into the tissues is more likely to produce tuberculosis than ingestion of the same material. This is all very true, but it must not be forgotten that the difference between man and guinea-pigs, between subcutaneous inoculation and ingestion, are only matters of degree; they depend on the quantity of bacilli present. The same milk which is capable of producing tuberculosis in the guinea-pig will be capable of producing it in man wherever the number of bacilli is great. It must be remembered that young children are very susceptible to tuberculosis. Young animals, such as rabbits, guinea-pigs, cats, pigs, calves, can be easily rendered tuberculous by feeding on tuberculous milk.

To meet the objections that might be based on quantity, I have inoculated guinea-pigs with the sediment of 1 ounce and a half of milk. If that quantity of milk can produce tuberculosis in a guinea-pig weighing 10 ounces, it may be assumed as a possibility that 1 pint of the same milk would contain enough bacilli to produce, under conditions favourable to infection, tuberculosis in a child, not weighing more than 8 or 10 lbs. A single tuberculous cow yielding (as in one of the Manchester tuberculous cases) 36 pints of tuberculous milk, might produce enough milk in one day to infect a large number of children. Fortunately the conditions favouring infection do not occur frequently, and I speak here only of a potential danger. Yet if we take the case of an infant entirely fed on the milk of a single tuberculous cow it must be owned that the child runs considerable risks of becoming infected when the udder of the cow becomes tuberculous. Under similar conditions, kittens, young rabbits, pigs, and calves, would readily become infected.

[2] It might be urged that I attach too much importance to the lesions produced in the lower animals by inoculation of milk. I hope it will be well understood that I do not suggest that because a specimen of milk is capable of producing lesions in the guinea-pig, it would necessarily have caused disease in man. But, if I have proved that milk from healthy cows, and well kept, can be injected in large quantities without affecting the health of the animal, and, that milk of inferior quality is capable of producing various lesions, I am justified in saying that *milk causing these lesions is bad milk*, and that, whether it would cause disease in man, or not, *it is not right that it should be allowed in the market*. It is quite easy to obtain milk free from noxious properties, and why should one tolerate the existence of a public danger?

not offer these as new suggestions, but as well recognised desiderata, put forth by many authorities, and which the present investigations show in a remarkably clear manner to be of great importance. I hope to be able to allude to the work of some of my predecessors on a future occasion.

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