PATHOGENIC MICROBES IN MILK.

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Milk, as every bacteriologist knows, is not only a universal and excellent food-stuff for human beings, but a medium admirably adapted for the growth and multiplication of microbes. For the latter reason milk deserves every attention at the hands of the hygienist, it being incontestably established that it may serve as a vehicle of disease agents. How well natural milk is adapted to this purpose, viz. to serve as nutritive medium for bacteria, is clear from its alkaline condition, and from its containing all ingredients required for the growth and multiplication of bacteria: proteid, fat, carbohydrate, and a large percentage of the essential mineral matters. It is a matter of common experience that all milk, however carefully it may be collected, however clean and aseptic may be the vessels into which it is received, will on standing, or after being handled in the way usual between collection and distribution, be found to teem with various kinds of bacteria. This fact is confirmed by the bacteriological examination of the milk sold in London shops, milk which, normal though it may be in appearance, chemical analysis, and taste, is usually found to contain hundreds of thousands of bacteria per cubic centimeter; bacteria which belong to various species and some of which when grown separately in sterile milk cause rapid changes and alter profoundly the character of the milk, *e. g. Bacillus lactis*, *Proteus vulgaris*, *Bacillus coli*, *Bacillus mesentericus*, spores of *Bacillus enteritidis*, etc. If allowed to stand, the milk containing the above mixture of bacteria exhibits even at ordinary temperatures, but in a more marked degree at temperatures of 70° F. and above, those profound changes which are popularly expressed as "going bad," changes caused by the rapid multiplication of one or other of the above microbes. Thus, for instance, if different samples of

milk received and brought in a sterile vessel from a shop be placed in the incubator at 37° C. the next day, or at the latest, after two days it may be completely clotted and sour, due to the growth and activity of *Bacillus coli*, or it may be decomposed by *Proteus* or *Bacillus mesentericus*, or it may be full of gas, clotted with a large amount of clear whey caused by the growth of the anaerobic *Bacillus enteritidis sporogenes*—the layer of cream on the top of the milk insuring something approaching anaerobiosis.

The enormous number and nature of bacteria present in ordinary seemingly perfectly normal and wholesome milk prove how easily milk becomes the receptacle of extraneous bacteria derived from dust and utensils, and how readily these multiply therein. When one considers the conditions under which milk is received from the udder, the nature and amount of handling it is subject to before it reaches the consumer, further, that the methods used in these manipulations are far from preventing—if anything the reverse is the case—the milk receiving extraneous matters abounding in microorganisms, we cannot wonder that milk as a rule does contain such multitudes of bacteria. Nor can we wonder that milk readily becomes a vehicle for infectious diseases like typhoid, diphtheria, and scarlet fever, if in the course of the long way between the cow and the consumer access is given to it for the specific microbes of these diseases.

Not only as a receptacle of extraneous microbes, both pathogenic and non-pathogenic, but also as a receptacle of microbes derived direct from the cow or the cow's udder, does milk deserve special attention, and in this article I will limit myself to certain pathogenic microbes which were found in samples of milk collected and analysed at the instance of the Medical Officer of the London County Council during the first months of last year. These samples were taken by an inspector in sterile glass-stoppered bottles from milk churns sent from country farms to the principal stations in London, before being handed over to the agents. Immediately after filling, the bottles were carefully stoppered, sealed, tied and brought directly to the laboratory. The bacteriological analysis was undertaken chiefly with the view of seeing whether or not any sample of the milk contained the tubercle bacillus, but in the course of the inquiry some other microbes were detected now and again, which on account of their specific pathogenicity to animals, at any rate, deserve consideration.

The Bacillus tuberculosis.

The statements by different observers as to the percentage of occurrence of the tubercle bacillus in cows' milk are of so divergent a character that it is impossible to explain them by different methods used in the analysis, or by faulty diagnosis. I am rather inclined to assume that the cows were less frequently affected with tuberculosis when the milk yielded a low percentage, and more affected where a high percentage was obtained. I think this is the more likely because no one amongst those observers who have published their analyses could be assumed not to have undertaken all and every test necessary for a reliable diagnosis, and I would therefore refuse to admit the suggestion that has been made[1] that some of the published high percentages probably include samples which did not produce real tubercle in the experimental animals but produced pseudo-tuberculosis. If such an explanation were a good one it would imply that the observer omitted some of the most important tests for his diagnosis, viz. the demonstration of the real tubercle bacillus in the deposits of the animals experimented upon. In this I am assuming that the microscopic specimens (cover-film specimens) of the deposits have been suitably prepared. Under suitably prepared specimens I understand not merely that the films made from the deposit were stained in fuchsin and treated with dilute mineral acid, and after this counter-stained with methyl-blue, thus showing bacilli which retained the pink coloration; for these are manipulations which admit of great variations, variations which may, and which as a matter of fact do, affect the result. By suitable preparations I understand that the cover-films are placed in carbolfuchsin solution (Ziehl) and heated over the flame till the stain boils; the films are now washed in water to remove the excess stain, then washed thoroughly in 33 p.c. nitric acid; a treatment of 10—15 seconds being sufficient to remove all red as far as naked eye inspection is concerned; then washed in water, whereby a little of the red tint reappears. Now the films are placed in methyl-blue anilin-water for $\frac{1}{4}$ of a minute, washed well in water, dried, and mounted in balsam. If real tubercle bacilli are present they appear as bright pink, slender bacilli of a distinctly cylindrical shape, and showing the well-known segregation of their protoplasm.

I am not aware of any bacilli belonging to the acid-resisting forms

[1] Annett, *Thompson-Yates Laboratories Reports*, Vol. II. p. 32, 1898–1899.

hitherto described as capable of simulating the tubercle bacilli of tubercular deposits, which under this mode of staining present the above well-pronounced acid-resisting qualities and morphological characters. Too weak acid, insufficient time in the acid, or insufficient counter-staining may bring forth a picture simulating acid-resisting bacilli, but I have never found yet that washing for 10—15 seconds in 33 p.c. nitric acid and counter-staining for ¼ minute in methyl-blue anilin-water did not reveal and differentiate the true tubercle bacilli; and if under this treatment the films show the well-known slender cylindrical bacilli with segregated protoplasm they can be relied upon to be the true tubercle bacilli.

A no less important item in framing the diagnosis is that of culture. I have not found the least difficulty in obtaining the characteristic colonies of the tubercle bacilli on the slanting surface of solidified horses' serum if this surface is inoculated with a fair quantity—of course under the usual precautions—of the caseous or purulent deposits of the omentum, pancreas, lymph glands or spleen of the experimental animal. By the end of 8—10 or 12 days the first indications of growth are noticed, and the developing colonies can after several more days be used for the preparation of films and for experiments on animals.

Besides these tests, the nature and progress of the disease in the inoculated guinea-pigs are of importance, as also the histological character of the tubercular deposits in the viscera of the experimental animal. As in most cases time is an important factor, I invariably inoculate a large amount of the sediment of the milk sample into two guinea-pigs: Animal I. receives subcutaneously into the groin half of the sediment of about 250 c.c. of the original milk distributed in a few c.c. of the milk, and Animal II. receives the other half intraperitoneally. By inoculating the two animals in different ways the test is more apt to lead to a successful result, it having frequently been observed that more especially the animals which are inoculated subcutaneously may die of acute septicaemia. It might be added that not one of 120 samples which I used for the inoculation (240 animals) produced acute death in both guinea-pigs. Had I relied upon the result of the subcutaneous inoculation of a single guinea-pig a considerable percentage of the tests would have failed.

The various statements as to the percentage of true tubercle bacilli in the milk of proved tubercular cows as demonstrated by animal experiment vary between 14 and over 71·4 p.c.; Bang 14 p.c.; Hirschberger over 50 p.c.; Ernst 28·5 p.c.; Rabinowitsch and Kempner 71·4 p.c.;

Boyce found 6—8 p.c. of 'town' milk and 17 p.c. of 'country' milk to contain tubercle bacilli.

Out of 100 samples of 'country' milk which I analysed, seven proved to contain the true tubercle bacillus. Amongst the 93 remaining samples there was one which was derived from a cow that, according to the veterinary inspector, was affected with tuberculosis, but its udder was free from disease. The milk of this animal did not contain the tubercle bacillus. The proof in the above seven cases was furnished (*a*) by the result of animal experiment: the disease—inoculation tuberculosis—was quite typical in its progress and pathology, and the deposits contained an abundance of typical tubercle bacilli—typical as regards aspect, size and staining; and (*b*) by culture on horses' serum, the culture being obtained from the deposits of the experimental animal. Tubercle bacilli could only be detected in one of the seven samples, films having been prepared in the usual manner from the milk sediment. On the other hand, the intraperitoneally as also subcutaneously injected guinea-pigs developed characteristic lesions of the lymph glands and viscera in the course of 3—5 weeks.

An important series of observations which were carried out for the Local Government Board proved that tubercle bacilli grow well in milk kept at 37° C. When sterilised milk is inoculated with tubercle bacilli derived from a culture on serum or from a tubercular deposit of the omentum, spleen, or lymph gland of a guinea-pig it shows, after a fortnight and later, a good growth of tubercle bacilli in the deeper layers, the milk and layer of cream remaining macroscopically unchanged. When a little of the bottom layer is removed by means of a capillary pipette great numbers of small and large clumps of typical (cylindrical slender 'granular') tubercle bacilli are found, these clumps being composed, just like the colonies on the surface of serum, of wavy, branching and reuniting strands and festoons of the tubercle bacilli. After four to six weeks the number of small and large clumps of bacilli present in the deeper layers is of course greatly increased. Such milk cultures prove to be highly virulent on inoculation into guinea-pigs, distinctly more virulent than the original materials (as shown by control experiments) with which the milk was inoculated. This increase of virulence through cultivation in milk is strikingly shown by inoculating sterilised milk from a glycerine-agar culture which has lost its virulence as the result of cultivation through many generations upon glycerine-agar. I possess glycerine-agar sub-cultures which have been carried on from generation to generation on this medium for over ten years.

The growth is very rapid and characteristic, *i.e.* folded crinkled membrane on the surface of the fluid of condensation and on the slanting surface of the glycerine-agar; and by staining, the culture can be shown to be composed of typical acid fast tubercle bacilli. When transferred to serum (slanting surface) the culture forms characteristic colonies of tubercle bacilli. But when the above glycerine-agar cultures are tested on the guinea-pig it is found that even large quantities—one-third to one-half of a culture—(the whole slanting surface being covered by the growth) injected subcutaneously or intraperitoneally fail to produce any effect, not even a local one. If, however, sterilised milk be inoculated from such a non-pathogenic glycerine-agar culture, it is found that after incubation of even a week good growth has taken place, better still after a fortnight. If then from such a milk culture, say after two, three, or more weeks, guinea-pigs are inoculated subcutaneously or intraperitoneally each with several drops of the milk, the result is in a large percentage positive. Some animals do not show any result, but the majority develop tubercles which are crowded with tubercle bacilli. Animals injected subcutaneously in the groin develop, in the majority of instances in the course of a month, distinct swelling and caseo-purulent tubercles of the inguinal glands; those injected intraperitoneally show caseo-purulent tubercles in the omentum and pancreas, as also in the spleen; a small number of guinea-pigs developed general and fatal inoculation tuberculosis in the course of two, three or more months. The tubercles in all the positive cases show in stained film specimen crowds—chiefly in clumps—of acid fast, typical tubercle bacilli, and culture on serum, which was practised in all positive instances, yielded readily copious and pure growths of the tubercle bacilli. From this I think there can be no doubt that by growing even highly attenuated tubercle bacilli in milk the pathogenic action can to a large extent be restored, though it must be added that in the majority of instances the inoculation of such milk culture produces only local tubercle, and further that only in a small percentage did it lead, after long periods, to general fatal tuberculosis.

Pseudo-tubercle.

Amongst the hundred samples of country milk analysed, as above mentioned, eight contained the *Bacillus pseudo-tuberculosis* as proved by the experimental results. The guinea-pigs injected subcutaneously or intraperitoneally with the sediment of these eight samples developed,

in the course of three to four weeks, caseo-purulent nodules in the inguinal lymph glands (subcutaneous injection), caseo-purulent nodules in the omentum and pancreas (intraperitoneal injection), caseous or purulent nodules in the spleen, pelvic lymph glands, liver, and besides, in several instances, in the lungs. The caseous and purulent matter of the above lesions did not contain the acid fast tubercle bacilli or any other acid fast microbes, but contained an abundance of the relatively thick, rounded, short, oval bacilli (lying often within the pus cells and contained in abundance in the necrotic tissues) which in their cultural character, distribution and action are identical with the classical *Bacillus pseudo-tuberculosis* first cultivated by A. Pfeiffer[1], and carefully investigated by Preisz[2] and others. I myself have described them[3] as occurring in sewage, and in water polluted with sewage.

The *Bacillus pseudo-tuberculosis* resembles the *Bacillus coli* in size and shape. On gelatine and agar the colonies resemble and grow nearly as fast as those of *B. coli* or colilike microbes, though the resemblance ceases here, for in broth, sugar gelatine, milk, and on potato, the characters are altogether different from those of *B. coli* or colilike microbes. Milk remains unaltered, the *B. pseudo-tuberculosis* forming no acid, on the contrary it forms alkali. It forms no indol. Its action on the guinea-pig, rabbit and mouse is definite, both on subcutaneous and intraperitoneal infection. By feeding guinea-pigs with the culture it produced caseous purulent deposits in Peyer's glands, the mesenteric glands, omentum, pelvic glands, spleen, liver and lungs. The action of recent cultures is far more rapid than that of the tubercle bacillus.

It is generally recognised that the *Bacillus pseudo-tuberculosis* of A. Pfeiffer represents a well-defined species, well-defined by its morphology, cultural characters and action. It seems therefore greatly to be regretted that some observers apply the name of *Bacillus pseudo-tuberculosis* to an altogether different species—different in morphology, culture and experiment—of bacilli. Petri, Rabinowitsch, Möller and others have described certain acid fast bacilli occurring in milk and butter which, on injection into guinea-pigs, cause disseminated caseous deposits in the viscera, and which they describe as pseudo-tuberculosis, a process as slow as that of true tubercle and only limited to the guinea-pig. The same applies to Dr Annett[4], who follows Rabinowitsch in

[1] Ueber die bacilläre Pseudotuberculose bei Nagethieren, Leipzig, 1889.

[2] *Annales de l'Institut Pasteur*, 1894, No. 4.

[3] *Centralbl. f. Bakteriologie*, XXVI. No. 9.

[4] *Thompson-Yates Laboratories Reports*, 1898–1899, Vol. II. p. 33.

accepting the acid fast non-tubercle bacilli of milk and butter as *Bacillus pseudo-tuberculosis*, and their action on the guinea-pig (subcutaneous or intraperitoneal) as pseudo-tuberculosis. This can only lead to confusion, and I think the name of *Bacillus pseudo-tuberculosis* should be reserved to the organism described by A. Pfeiffer and others.

It will then be understood that the pseudo-tuberculosis and the *Bacillus pseudo-tuberculosis* which I mentioned as having been met with by me in eight of the one hundred samples of country milk is the non-acid fast microbe which was first isolated and described by A. Pfeiffer, and which I met with also in sewage and in sewage polluted water; which is pathogenic to guinea-pig, rabbits and mice, and which on inoculation and feeding produces in the guinea-pigs more rapidly than the true tubercle the above-mentioned caseo-purulent deposits in the lymph glands, in the abdominal, and further in the thoracic viscera[1].

Bacillus diphtheriae.

Amongst the 100 samples of country milk referred to above, one produced on subcutaneous injection into the groin of the guinea-pig a swelling of the inguinal lymph glands—the intraperitoneally injected guinea-pig remaining quite well. By the fifth day the inguinal glands of the first guinea-pig were found swollen to about the size of a filbert and surrounded by soft oedematous tissue. It presented the following appearances at autopsy: about the seat of inoculation the subcutaneous tissue was oedematous and streaked with blood. The inguinal glands were enlarged, firm and deeply congested. Film specimens which were made of the juice of the incised gland, and stained, showed numerous bacilli closely resembling the diphtheria bacilli in size and shape. Cultures made on agar and ascites-agar brought forth numerous colonies of a pure culture of the *Bacillus diphtheriae*. A broth culture was made from one of these colonies and after 48 hours' incubation at 37° C. showed the characters of a diphtheria culture, forming acid. One quarter of a cubic centimeter was injected subcutaneously into the groin of a medium-sized guinea-pig with the result that the animal died in 36 hours with haemorrhagic tumour in the groin and deep congestion of the viscera. Films and cultures made from the fluid of the tumour showed the diphtheria bacilli in pure culture. Stained

[1] *Reports of the Medical Officer of the Local Government Board*, 1899—1900; and *Centralbl. f. Bakt. und Infekt.* Vol. xxvi. No. 9.

according to Neisser's method the bacilli gave a positive result like true diphtheria bacilli.

A final proof that we were dealing with the true diphtheria bacilli was furnished by the following experiment:

Of a 48 hours' old broth culture, $\frac{1}{4}$ c.c. was injected into the groin of a medium-sized guinea-pig *a* (weight 306 grammes); another medium-sized guinea-pig *b* (weight 302 grammes) received a mixture of $\frac{1}{4}$ c.c. of the same broth culture and $\frac{1}{10}$ c.c. of Burroughes and Welcome's diphtheria antitoxin. The result was striking: guinea-pig *a* was dead in 36 hours with the characteristic tumour, guinea-pig *b* had no tumour at any time and remained lively and well.

From these observations it is justifiable to conclude that the bacilli in question obtained from the above sample of milk were the true diphtheria bacilli.

After these results had been obtained inquiry was set on foot as to the derivation and distribution of the milk. This could be done readily because the farm from which the milk was derived was known. The dealer who received the milk and the locality in which the milk, of course mixed with other milk, had been distributed were known. But nothing suspicious could be found; the farm and its employees were in all sanitary respects correct, and no case of diphtheria could be discovered amongst the houses to which the milk was delivered, either directly from that farm or after mixing with other milk.

Whether owing to the small number of diphtheria bacilli originally present in the milk or perhaps to their lesser virulence, or owing to the possibility that the consumers of the milk had all healthy throats and therefore were less susceptible to infection, no cases of diphtheria could be referred to that milk, must remain undetermined; the fact remains, that the sediment of the milk produced, by subcutaneous injection into the guinea-pig, a disease which could only be regarded as diphtheria of a somewhat subnormal type, considering that it took the better part of a week to develop; this would also point to the number of diphtheria bacilli originally present in the milk being very small. I need scarcely say that any accidental contamination with diphtheria bacilli in the laboratory either of the milk treated or the instruments used, is altogether excluded, there having been no diphtheria work done for a considerable time, certainly for more than half a year previously.

Bacterium diphtheroides.

The secretion of an indurated quarter of the udder of a cow was collected and the milk was submitted to bacterioscopic analysis. The induration was of a chronic nature and the secretion was of the nature of thick creamy pus. The veterinary inspector declared the induration to be of the nature of tuberculosis, but neither the microscopic examination of the pus nor the experiments of injection into guinea-pigs confirmed the diagnosis. The microscopic examination of the secretion revealed the presence of a conspicuous number of bacilli, singly, but more frequently in larger and smaller clumps, which had a certain resemblance in their shape and size to diphtheria bacilli; amongst them there were clubbed forms. By injection into the peritoneal cavity or subcutaneously into the groin, sub-acute abscess was produced, containing thick yellowish-white 'granular' grumous pus. This abounded with large and small masses of the microbe.

The pure culture of the microbe injected in small quantities subcutaneously or intraperitoneally causes local abscess in the course of from one to two weeks. This abscess after subcutaneous injection into the groin comprises the inguinal glands and the surrounding tissue, and reaches after three weeks the size of a pigeon's egg. After intraperitoneal injection abscesses are produced on the omentum, the pancreas or around the kidney.

Pure cultures were easily obtained both from the original cow-secretion as also from the pus of the abscesses in the guinea-pig. In the latter case, as mentioned above, the microbe abounds to an enormous extent, so much so that the 'granules' of the pus are almost entirely made up of the bacterium. The microbe does not stain readily in the ordinary dyes, but it stains easily and well by means of Gram's method: 1 minute gentian violet, 4 minutes iodine iodide of potassium.

Although in shape and size this bacillus belongs to the group of the diphtheria bacillus its cultural characters readily differentiate it from the latter and from the known diphtherioid bacilli, *e.g.* bacillus of Hoffmann, and the group of Xerosis bacilli.

In the first place it does not grow on gelatine at 21° C., it does not grow below 25° C., it shows very little or no growth in ordinary nutrient bouillon at 37° C. On agar and glycerine agar at 37° C. its growth is very slow and limited, the colonies do not appear before the third day, and then are small grey dots, which on subsequent days enlarge to

circular plates with a thick, dark, granular centre and a greyish, thin translucent margin, which latter is somewhat irregular and angular.

In stab agar there is no growth in the depth, only on the surface of the stab is there a small, flat, circumscribed, grey plate. The growth of the microbe in milk and solidified blood serum is, however, very characteristic, and by it our microbe is easily distinguished from all other known diphtherioid bacilli; viz., it coagulates milk at 37° C. and forms acid: litmus-milk becoming red; beginning with the third day the milk (as also the litmus-milk) separates into the top cream, a chief middle layer of clear whey and at the bottom the white coagulated casein. On the slanting surface of solidified blood serum the microbe grows as small, round, granular colonies; these make their appearance on the third day and are recognisable by the depression (liquefaction) of the serum; on the third and fourth day the surface of the serum is uniformly pitted, each pit being a depression (liquefaction) with a small colony in its depth. Gradually and slowly, as growth proceeds, the serum becomes liquefied.

Comparatively speaking the microbe dies off rapidly on agar and glycerine-agar, but retains its vitality longest on serum; I have succeeded in obtaining good cultures on this medium, even after several weeks' transference.

As mentioned above, owing to its shape, I have called the microbe *Bacterium diphtheroides*[1], it is, however, in respect of staining, in its cultural characters and in its action on the guinea-pig easily differentiated from the known diphtherioid bacilli. Formation of local abscess occurred in all guinea-pigs subcutaneously injected, whereas only about half of the animals develop abscesses of the abdominal viscera after intraperitoneal injection.

Streptococcus radiatus (*pyogenes*).

A large number of observations have been reported regarding the occurrence of streptococci in the diseased udder of cows. I have myself found and isolated from purulent secretions of the udder of different milch cows: *Streptococcus pyogenes*, *Streptococcus brevis*, and *Streptococcus longus*; in these cases the streptococci were present abundantly in the purulent matter and in masses, particularly in some of the purulent matter *Streptococcus pyogenes* and *Streptococcus longus* occurred in great numbers and in aggregated masses.

[1] *Centralbl. f. Bakt. und Parasit.* Vol. XXVIII. Nos. 14, 15.

But there have been also described as *Streptococcus mastitidis*, a specific microbe causing a specific contagious purulent inflammation of the udder. Nocard and Mollereau[1] described and isolated this microbe first and proved by inoculation in cows and goats that it is capable of producing mastitis. In Germany the disease is known as 'Gelber Galt,' and the streptococcus of the French observers was isolated in this affection by Eisenberg, Adametz, Zschokke and others.

Amongst the secretions of diseased udders submitted to me for bacterioscopic analysis (with the object of testing whether they contained tubercle bacilli) there was one which was not of the character of purulent matter, but was a thin serous exudation with fibrin and blood. Injected into the subcutaneous tissue of the groin or into the peritoneal cavity of the guinea-pig it caused acute purulent inflammation. The serous fibrinous exudation of the udder, and more especially the purulent exudation in the guinea-pig, contained streptococci, which in culture proved to belong to one and the same species, and to possess characters not coinciding with those of hitherto described species. In the purulent exudation of the guinea-pig our streptococcus occurs in very large numbers, as shorter or longer chains, isolated or in small aggregations or forming dense convolutions and big clumps. A small amount of the culture injected into the groin of the guinea-pig produces in a few days, in the great majority of instances, abscess. The streptococcus stains easily in ordinary dyes; it stains well by Gram's method; it measures 0·6—0·8 μ.

The microbe grows in a characteristic manner on the surface of gelatine: after a few days' incubation it forms grey, translucent, round colonies; these show a thicker, dark, granular centre, from which radiate densely aggregated fine striæ to, and also here and there beyond, the margin, whereby the outline is slightly crenate and toothed. This character on gelatine distinguishes it from *Streptococcus pyogenes* and for this reason I proposed the name of *Streptococcus radiatus* (*pyogenes*). The gelatine is at no time liquefied—which character distinguishes it at once from the *Streptococcus radiatus* (non-pathogenic) of Flügge. On the surface of agar our microbe forms round flat discs with thicker centre and translucent periphery; the margin is also here and there irregular and crenate. It grows well in the stab both in gelatine and in agar, the line of inoculation being marked by a row of dark (white in reflected, brownish in transmitted light) separate granular colonies; on the surface of the stab there is very little growth.

[1] *Annales de l'Institut Pasteur*, I. p. 109. 1887.

In milk (at 37° C.) it grows well, the milk remaining fluid (unlike the *Streptococcus mastitidis*), though the use of litmus-milk shows that acid is formed. In alkaline broth it grows well at 37° C., and in this medium it is readily distinguished from *Streptococcus pyogenes*, the broth remaining clear, but at the bottom are formed greyish-white, flocculent masses, just like those produced in broth by *Streptococcus conglomeratus scarlatinæ*.

On solidified serum the growth is very rapid and resembles that on agar, except that on serum the contrast between dark centre and translucent broad margin is more pronounced; the serum is not liquefied.

The cultures lose their vitality rapidly, so that before the end of the week new transference has to be made in order to keep the cultures going. The microbe lives longest in gelatine stab-culture. The characters described, particularly those exhibited on the surface of gelatine, in broth and on serum, indicate, that our *Streptococcus radiatus* differs markedly from *Streptococcus pyogenes*, as also from those hitherto described of the diseased udder; its pyogenic action on the guinea-pig distinguishes it also from the *Streptococcus mastitidis* of Nocard and Mollereau. Although in broth it resembles *Streptococcus conglomeratus scarlatinæ*, it differs from this latter by the character of the colonies on gelatine, agar and blood serum, and by the fact that it does not curdle milk.

The two pathogenic microbes, *Bacterium diphtherioides* and *Streptococcus radiatus pyogenes*, mentioned in the foregoing pages although obtained from secretions of diseased udders, may and probably do find access to the milk obtained from the rest of the udder, since it is the usual practice not to discard the milk of the three apparently sound quarters if one quarter appears to be diseased, and for these reasons, I think, these microbes deserve a place amongst the pathogenic microbes in milk.

Pathogenic Yeast in Milk.

I now propose to describe a microbe which was obtained from a sample of country milk which in all respects appeared normal, but which on subcutaneous injection into the guinea-pig produced a chronic and peculiar pathological condition.

The history of the disease in the guinea-pig is as follows: with the sediment of a sample of 'country' milk (this being one of the samples

delivered by the Inspector of the London County Council) two guinea-pigs were injected: one subcutaneously, the other intraperitoneally. After three weeks both animals were killed. The intraperitoneally injected guinea-pig was found at autopsy to be quite normal, the omentum, pancreas and all viscera being free of any disease.

The subcutaneously injected guinea-pig showed a big tumour in the groin of the inoculated side, this tumour included the swollen hyperæmic lymph-glands; when cut into, a quantity of thick, greyish, viscid fluid was obtained, which on microscopic examination showed a few red blood corpuscles, numerous pus-cells and crowds of yeast-cells of different sizes: some not larger than a red blood corpuscle, others twice and thrice as big; there were also present numerous longer and shorter moniliform cylinders, in which the varicosities corresponded to the outlines of individual yeast-cells; in some of these cylinders the terminal element was much enlarged, pear-shaped or club-shaped. The yeast-cells were met with singly and more frequently in masses held together by a gelatinous interstitial substance; on staining they showed a thick homogeneous capsule. In the fresh state many of the large yeast-cells showed within the membrane a clear marginal plasma, in the centre a mass of granular substance. By the ordinary aniline dyes the cells and cylinders stained very easily. Most of the yeast-cells are spherical, some, the larger ones, oval or pear-shaped. There was no difficulty in finding such as showed distinctly the process of gemmation. The tumour did not contain any bacteria and no tubercle bacilli could be detected.

Cultivations made on agar and glycerine-agar (at 37° C.) and on gelatine (at 20° C.) proved that the juice of the above tumour contained only yeast cells, these forming innumerable colonies; there were no colonies of bacteria.

Inoculations were made of a number of guinea-pigs and rabbits with the juice of the above tumour, and subsequently many inoculations were made with the sub-cultures of the above yeast-cells, and I will here give a summary of the results both of the animal experiments, as also of the cultural characters of the yeast.

First as to the animal experiments:

(*a*) Subcutaneous injection in the groin with the matter of the tumour from the above guinea-pig or from other subsequently inoculated animals causes tumour of the inguinal lymph-glands of the injected side. This tumour shows itself by the end of the week as a soft nodule about the seat of inoculation. By the end of two weeks several nodules

are noticed, some in the groin, others, larger ones, extending towards the back—sacral region. The animals either die about the end of the second week or the tumours change into abscesses. In the first case the autopsy shows that the tumour consists of a mass of firm, gelatinous tissue with more or less haemorrhage in it; in the other case the abscess contains thick, viscid grumous purulent matter. But in all instances the matter of the tumour or of the abscess is crowded with yeast-cells of exactly the same description as in the first case: viz. spherical cells varying in size from that of a red blood corpuscle to that twice or thrice as big; the great majority are spherical, some few large ones are oval or pear-shaped, while others are moniliform cylinders.

Some of the subcutaneously injected guinea-pigs developed in the course of three weeks an enormous tumour—as large as a hen's egg—in the groin and extending on to the thigh and sacral region; the animals died between the 19th—25th day. The tumour on cutting into it looked like blood-streaked bacon in the peripheral part, like a semi-fluid jelly in the central part. In all parts continuous masses of yeast-cells were found.

(*b*) After intraperitoneal injection the guinea-pigs as a rule die about the end of two or three weeks (14—20 days) seldom later; at autopsy numerous small and large whitish nodules are observable in the omentum, pancreas, and sometimes also in the spleen. The mucous membrane of the stomach and large intestines shows numerous whitish spots surrounded by haemorrhages; the haemorrhages and whitish spots are particularly conspicuous in the peritoneum around the ovary in females, and the testis and epididymis in males. But what is very remarkable is the circumstance that in many such animals the stomach and large intestine are enormously distended by gas; the lungs show petechiae and look almost emphysematous and full of closely placed gas-bubbles.

All the above whitish nodules contain besides leucocytes great numbers of the yeast-cells as is shown by cover-film specimens and culture, and sections through the organs demonstrate the presence of the yeast. Here also amongst the single and aggregated yeast-cells there occur the moniliform cylinders above mentioned.

In addition to the above studies upon the distribution of the yeast-cells in the diseased organs, cultivations were made also of the heart's blood, both of subcutaneously and intraperitoneally injected guinea-pigs that died spontaneously, and it was found that yeast-cells

were present also in the blood; in some cases the culture was negative, in others a drop of blood yielded three, in another eight, and in one case as many as 28 colonies.

Mice are susceptible to infection. After subcutaneous injection with culture into two mice, one died within 48 hours; all the viscera were found on autopsy to be deeply congested, and the heart's blood yielded colonies of yeast-cells on cultivation. The second mouse was ill after two days; being quiet, cuddled up with curved back; coat rough, eyes closed, and not feeding; it remained in this condition off and on for about a fortnight. Then it became again lively, fed well and completely recovered.

The only experiments on rabbits hitherto made consisted in the intravenous injection of two animals (Nos. 1 and 2) with salt emulsion of an agar-culture of the yeast. The animals appeared quite well and fed well for the first fortnight; then they became quiet and refused food; by the end of 24 days both animals showed great weakness in the hind limbs; one rabbit, when trying to walk, dragged the hind limbs after it; the breathing was laboured. The other developed the paraplegia a week later. Rabbit No. 1 died after 31 days, the other, No. 2, after 39 days. In both cases the bladder and intestines were found at autopsy to be much distended. The chief changes, however, were in the cord: the lower dorsal and lumbar cord being greatly congested both in its substance and membranes; yeast-cells were found in these regions, both in the cord and its membranes.

The cultural characters are these: The microbe grows well on alkaline gelatine at 20° C., on alkaline agar at 37° and on blood serum, in milk at 37° C., whereas it grows feebly in ordinary alkaline bouillon. It grows much faster and more copiously on grape-sugar-gelatine, on grape-sugar-agar and in grape-sugar-bouillon. It grows better and more vigorously on alkaline than on neutral or acid media; it grows well on the surface of solid media, but shows only feeble or no growth in the depth (stabcultures). It does not produce fermentation (gas) in any medium, be it growing on the surface or in the depth, be the medium gelatine, agar, or bouillon, to which grape-sugar has been added. It does not produce any fermentation in beerwort-gelatine.

The colonies on ordinary alkaline nutrient gelatine are thick and rounded, moist looking and raised in the centre; white in reflected, brown and granular in transmitted light; on sugar-gelatine the colonies grow more rapidly, are larger and thicker, and with time assume a light

yellow colour and slowly liquefy the gelatine into a thick, turbid, syrupy mass; such liquefaction does not occur at all or only after many weeks' growth on ordinary gelatine. On glycerine-agar and on sugar-agar the microbe forms in a few days a thick, smeary, viscid growth, gradually assuming a yellowish colour; on ordinary nutrient agar the growth is less copious and whitish in reflected light. In sugar-broth the microbe forms a white powdery sediment leaving the broth clear; the same is the case in ordinary bouillon, but to a much more limited degree. In milk and litmus-milk the microbe grows well at 37° C., the milk remaining fluid and unchanged, the litmus-milk remaining fluid and blue. The growth on all solid media is of a peculiar viscid mucoid character, so much so that it is difficult to make an emulsion of it, the growth on shaking in salt-solution or bouillon can at most be separated into flocculi. This, as the microscope shows, is due to the presence of a viscid, gelatinous, interstitial substance by which the individual yeast-cells are agglutinated.

All cultures, gelatine, agar, sugar-gelatine, sugar-agar, glycerine-agar prove pathogenic when injected subcutaneously or intraperitoneally. Feeding experiments of guinea-pigs made with milk-culture, or with sugar-gelatine or sugar-agar culture remained entirely negative.

The microbe obtained from cultures stains well within its capsule, and except for differences in the size of the spherical cells is morphologically pure yeast; there are at no time found in the cultures those moniliform cylindrical threads which are fairly common in the tissues of the infected guinea-pigs.

We have then here a distinctly pathogenic yeast, belonging to the group of pathogenic blastomycetes to which the researches of Sanfelice, Plimmer and others, in connection with cancer, have drawn attention. From the published reports of these authors, however, our milk yeast in its cultural characters and its pathogenic action on the guinea-pig and rabbit seems distinctly different from those found in cancer.

Conclusions.

To sum up the following are the experimental results of the bacteriological examination of the milk samples and secretions of diseased udders:

(1) 7 p.c. of the samples of "country" milk produced typical true tubercle in the guinea-pig.

(2) 8 p.c. of the samples of "country" milk produced typical pseudo-tuberculosis (non-acid fast bacillus of pseudo-tuberculosis A. Pfeiffer).

(3) 1 p.c. of milk samples produced diphtheria in the guinea-pig, yielding the typical true *B. diphtheriae.*

(4) 1 p.c. of milk samples caused a chronic disease (in most cases with fatal results) due to a pathogenic torula apparently differing in cultural and physiological characteristics from the torula (pathogenic blastomycetes) obtained by Sanfelice, Plimmer and others from human cancer.

(5) Out of the secretions of the cow's udder two pyogenic microbes were obtained: *B. diphtherioides* and *Streptococcus radiatus* (*pyogenes*).

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