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# TEXT BOOK

#### $\mathbf{OF}$

# MILK HYGIENE

BΥ

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# AUTHORIZED TRANSLATION WITH ANNOTATIONS AND REVISIONS

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# With 29 Illustrations and 5 Colored Plates

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# Translator's Preface

THE importance of food hygiene in the protection and preservation of public health is now generally recognized. Milk constitutes one of the most important foods for the human race, and since its composition and wholesomeness are entirely dependent upon its proper handling, the necessity for a strict supervision and control is obvious.

The problem of milk hygiene is very complex and must embody all phases of milk control from the time the milk is produced until it reaches the consumer. In all stages it may be subjected to wilful adulteration and to contamination with injurious and obnoxious substances. Furthermore, the danger threatens this most valuable food not only from outside sources but also from internal influences, as the milk may leave the animal in the condition of a dangerous product, a carrier of pathogenic microbes. Various kinds of infection of the udder are frequently important factors in the contamination of milk, which would render it dangerous to Thus in recent years numerous outbreaks of inthe consumers. fectious sore throats have been caused by such conditions. It is therefore apparent that in the proper control of the milk supply it is necessary to be familiar with all conditions which may be responsible for an injurious or unwholesome product. The subject is one in which every sanitarian should be thoroughly qualified.

Although there are numerous splendid publications available on this subject, they are either too voluminous to be used as textbooks or they fail to contain the more recent very important developments made in this branch of public hygiene. The excellent German publication of Dr. Ernst entitled "Milk Hygiene" meets the requirement of a concise, up-to-date work on that subject, and it is with pleasure that in response to requests from various sources we have accepted the preparation of an English edition of this publication. We did not lose sight of the fact that it should meet with the conditions prevailing in this country and accordingly we have included much valuable information from the reports of the various Milk Commissions, and other sources. For this reason Chapter X dealing with German laws and regulations has been replaced by Chapter XI which deals solely with the conditions and standards existing in this country.

We cannot refrain from expressing our sincere thanks to Dr. H. J. Washburn for his most valued suggestions and assistance in proofreading the manuscript; also to the publisher, Mr. Alexander Eger, for his interest and courtesy during the preparation of this volume.

JOHN R. MOHLER, ADOLPH EICHHORN.

Washington, D. C., July 1, 1914.

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# Author's Preface

THE increased importance of milk as human food demands . more and more the application of modern accomplishments and experiences achieved by science and practice, in order to elevate the milk industry to the desired high standard.

The principal stress must be laid upon production, which constitutes a special field of the milk industry, and which is most generally in need of elevation and improvement. The product will be without reproach only when the conditions of production correspond to the value of this food.

In the field of production, veterinarians are the proper experts who must stand by the side of the producers and give them the necessary advice and instruction. Only by the active and expert aid of veterinarians can it be hoped to improve the good-will of the producers; provided, at the same time, other points of milk hygiene which possess bad features—in spite of the active progress of milk control and sanitary methods which have been noted for many decades—also receive proper attention.

In order to be able to offer expert advice a thorough knowledge of milk, its formation, procurement and characteristics, is necessary; likewise, a knowledge of conditions which have an influence upon milk while still in the animal body, and the factors which change this food after its procurement. These points have received the principal consideration in the following chapters.

In the plan which I have followed, those questions which treat of the judgment of milk as human food in relation to its chemical contents, were given less prominence. Certain points of this subject have been mentioned only to an extent that was considered advisable for the general comprehension of the subject. More specific questions, as for instance, the preparation of certain milk mixtures for the feeding of infants, the advantages and disadvantages of feeding cows' milk to infants, the action of a milk diet in the treatment of adults, etc., are subjects for the physician. A special chapter on the preparation of infants' milk, or certified milk, has been omitted, since the sanitarian can not make any distinction in his judgment of milk as food, but must remember that milk which is consumed by children of the masses should also come up to the requirements established for any food product from a hygienic standpoint.

The chemical and physical properties of milk are only discussed to an extent deemed necessary to instruct the veterinary experts in court cases in judging physiological, pathological and external influences. Since the chemical examination of milk should be placed in the hands of the food chemist, I have eliminated the analytical examination of milk and the examination for preservatives. For this information I would recommend the numerous publications which have appeared during recent times, as for instance, the works of Grimmer and Sommerfeld, Teichert, Utz and Barthel. Only those methods have been described which may be undertaken by the veterinarian and which are sufficient for a thorough preliminary test of milk for adulterations.

The illustrations are taken partly from the known works of my previous teacher, Professor Dr. med. Th. Kitt (Pathological Anatomy) and from Friedberger and Fröhner's Methods of Clinical Examination; some were drawn by myself. The illustrations of apparatuses have been avoided, as they appear in all commercial catalogues.

In dividing the subject into individual chapters repetitions, of course, could not be avoided.

With the preparation of this small work I desire to show to my colleagues the road which they must follow in order to cöoperate from a milk inspection standpoint in accordance with the call made upon their profession.

A difficult point of milk hygiene lies in the changing conditions of production and not in the control of milk consumption or in the supervision of milk transportation.

Munich, January, 1913.

W. ERNST.

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# CHAPTER I.

# ANATOMY, PATHOLOGY AND HISTOLOGY OF THE MAMMARY GLAND.

# Development and Gross Anatomical Structure.

In the lowest form of mammalian life a group of glandular ducts becomes differentiated from the glands of the skin in the median abdominal region. These ducts exude their lacteal secretions upon tufts of hair of the mammary region, from which it is either licked or sucked by the young (duckbill, Ornithorhynchus paradoxus).

One of the land duckbills, the spiny anteater (Echidna hystrix), has lacteal ducts opening within an abdominal pouch formed by a fold of skin of the mammary region in the shape of a pocket, in which the young are protected and nourished during their development. This abdominal pouch is not identical with the tegumentary wall from which is developed the teats of higher mammals, but it may be taken as the point of origin of the different forms of teats. In higher marsupial animals the glandular ducts are united into a complex gland with teats which constitute the orifices of the confluent lacteal ducts. In other still higher species the most varied kinds of gland structures are observed with various forms of teat development.

Among the higher mammalian forms the evolution of these anatomical structures may be followed during embryonic life.

On both sides of the body, between the anterior limb-bud and the inguinal fold, the milk-ridge develops from a linear thickening of the ectoblast in the form of a ledgelike elevation of the epidermis. Along this milk-ridge a series of at first spindle-shaped, then round enlargements appear, which are separated by absorption of the intervening portions of the ridge. These enlargements consist of masses of epithelial cells, which correspond to the anlage, primordium or point of origin of the true mammary gland of the lowest mammalia. This anlage sinks into the underlying mesoblastic tissue and becomes surrounded by a proliferating integument, which forms an investment for the growing epithelial mass. From this mammary envelope which becomes more or less flattended the fibrous and muscular tissue of the areola and teat are derived. At its base, solid epithelial sprouts grow out from the sides of the conical epidermal plug, later becoming the lactiferous ducts, while the club-shaped thickened extremities in the further course of their development, form the milk sinus. Subsequently, the central part of the ectoblastic ingrowth undergoes degeneration and what at first was an elevation, now be-

comes a depression. From the middle of this depressed area there appears an elevation that later becomes the teat.

In cattle a single excretory canal enters from the bottom of the mammary envelope (point of the teat), into the tissue (the milk duct), the end of which, the milk cistern, breaks up into the secondary lactiferous ducts. The lower opening of the teat contains unstriped muscle fibres which act as a sphincter to prevent the escape of milk. (Meckel, Kölliker, Langer, Bonnet, Profé, Schwalbe, Huss, Gegenbauer, Klaatsch.)

According to the number of the glandular organs there are distinguished the oligomasts and the polymasts. Cows are normally tetramasts, and usually possess four distinctly separated glandular masses, commonly termed the quarters, from each of which protrudes a long teat. The four quarters are united together in pairs and are arranged symmetrically. Between their bases and the yellow abdominal fascia they have a rich layer of fat.

The udder is attached along the linea alba to the yellow abdominal fascia, and to the tendons of the abdominal muscles, by two layers of elastic tissue, the suspensory ligament (ligamentum suspensorium mammarum) which penetrates the udder between the two halves.

Although the quarters situated on one side show no visible anatomical separation, injection tests with colored gelatin, and observations in cases of inflammation of the udder in natural and artificial infections have proven that the secretory canal systems of the anterior and posterior quarters are separated in the same way as those of the opposite quarters.

These canal systems collect into excretory ducts and terminal tubules and finally empty into the milk cistern, which in its upper part is greatly dilated and in its lower part is more constricted. Each quarter possesses a teat (6 to 10 cm. in length) from the milk sinus of which, the duct of the teat (ductus lactifera) of about 8 mm. in length, passes to the outside. The entire udder is covered by fine, slightly hairy skin, which extends posteriorly and superiorly into the escutcheon or so-called milk mirror.

The size of the udder varies in the different breeds and individuals.

In the sheep and the goat there are two milk glands, each possessing a teat which stands out in a divergent direction from the one opposite. Each teat has one excretory duct. While the teats of the sheep are finely haired, those of the goat are hairless.

The blood vessels of the udder are derived from the branches of the external pudic artery and anastomose with the various venous branches, through which the blood flows posteriorly through the perineal vein into the internal pudic vein and finally into the obturator vein. The greatest part of the venous blood flows laterally into the external pudic vein and anteriorly into the subcutaneous abdominal vein, which forms the immediate continuation of the external pudic vein and which is known as the milk

vein. It runs bilaterally of the median line, penetrates posteriorly and laterally to the xiphoid cartilage of the breast bone into the deeper parts and then empties into the internal thoracic vein.

The lymph vessels which are very numerous enter two large lymph glands which lie bilaterally in a depression at the posteriosuperior portion of the udder and are known as the supramamary lymph glands. The lymph passes thence to the lumbar glands and into the thoracic duct.

The nerves originate from the lumbar plexus. The udder is supplied by the internal branch of the ilio-hypogastric nerve, the external branch of the lumbo-inguinal nerve, and the external spermatic nerve. In the goat the external spermatic nerve divides in the abdominal cavity into three branches, of which the median and the caudal branches pass through the inguinal ring to the udder. The cephalic branch passes to the abdominal muscles. The caudal branch (inferior) is purely a vascular branch. The median branch passes to the udder, and ramifies to the milk ducts and the teats.

### Pathological Anatomy of the Udder.

Of the pathological processes which are of importance from a practical standpoint, the inflammations and changes which have more or less influence on the quality of the milk are of special interest. The other anomalies will be mentioned only briefly.

Not infrequently there may be present in cows supernumerary teats, or supernumerary milk glands, which may be considered as a reversion to early stages in the evolution of cattle. Usually two rudimentary formations occur which are generally situated behind the posterior normal glands and normal teats. These may at times yield milk (Burkart, Dauberton). These accessory glands may also occur between the normal teats. In several instances as many as four supernumerary teats were observed.

If the udder is abnormally small in its development or is entirely absent, it constitutes **hypoplasia** or **aplasia** of the udder. According to Bosetti the absence of the mammary gland was observed in a cow two and a half years old. Although there were four small teats on the skin, no milk was secreted even after the birth of a healthy calf. The milk veins were well developed on both sides.

The opposite condition, hypertrophy of the udder, with or without secretion, is most conspicuous in male animals. Pusch observed a buck which produced 70 gm. of colostrum-like milk daily, and which possessed nipples 7 to 9 cm. in length. Gurlt has reported that the udder of a steer was as strongly developed as in a cow, and produced daily  $1\frac{1}{2}$  liters of secretion.

It is known that newly born kids and suckling colts occasionally secrete milk for several days (Gurlt, Martin, Hess, Ibel).

Schmidt, of Dresden, reported a giant udder with an entirely

normal milk secretion, (16 liters). A functional hyperemia in the beginning of the lactation period increased the four quarters uniformly to such an extent that a day after parturition the udder touched the ground with its central surfaces.

Before and after parturition an abnormal amount of hyperemia occurs physiologically in the udder (hyperemia congestiva). In inflammations the same condition may be present, the capillaries are abnormally dilated, and greatly distended with blood. This condition may result in the exuding of fluid and the solid constituents of blood. These are known as capillary hemorrhages. In larger extensions of these hemorrhages they are spoken of as suggilations, and when the blood is contained in a sac-like cavity or swelling it is known as hematoma uberis.

If in the congestive hyperemia the fluid constituents of the blood pass into the tissue of the udder, it results in **edema of the udder**. The same condition may develop as a result of hydremia, as for instance after changing from dry to sloppy foods (Bang), or as a result of multiple emboli of the blood vessels, or from a **varicose** condition of the veins of the udder.

Edema of the udder manifests itself as a soft or tense swelling of the tissue, which retains the impression of the finger. While the teats usually remain normal on account of their slight but dense connective tissue, quantities of fluid collect in the front of the udder and between its glandular substance and the skin.

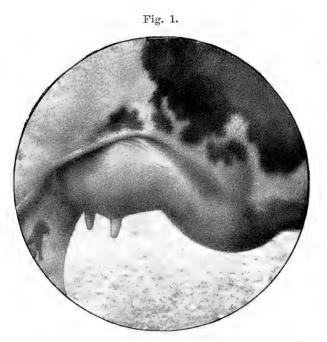
The edema frequently extends posteriorly to the udder and up to the vulva. Infections of wounds with the bacillus of malignant edema may result in edema of the udder.

To those engaged in milk hygiene the most important of all pathological conditions of the udder are the inflammations which result from a reaction of the glandular tissue to any inflammatory irritant. In most instances the inflammations of the udder are produced by microbian infections of various kinds, particularly by poly-bacterial infections. The bacteria penetrate the udder either by way of the blood circulation or from the outside through the orifices of the milk ducts. In such cases it is spoken of as a hematogenic or galactogenic mode of infection. If the infection results from a mixture of bacteria, and is not caused by one kind alone, the affection is a mixed infection. The infection may result from traumatic conditions when injuries extending into the parenchyma of the glands make the infection possible, or from galactiferous-traumatic causes when the infectious material enters the milk cisterns upon milking tubes or straws. The infection may take place also through simple contact of the orifice of the teat with the infectious material. Thus the different forms of mastitis, the peracute, acute or chronic inflammations of the udder may arise, depending upon the character of the infectious material and upon special accessory conditions.

The possibility of galactiferous infection was first experimentally proven by Frank. The character and the varieties of inflammations of the udder were further established by the work of Kitt, Nocard and Mollereau, Lucet, Bang, Hess and Borgeaud, Guillebeau, Zschokke, Sven Wall, and others.

The principal producers of mastitis are the colon-paratyphoid group, staphylococci, streptococci, *Bacillus pyogenes bovis*, *Bacillus tuberculosis*, and the actinomyces.

Colon infection and severe mixed infections usually result from galactiferous contact, or after the introduction of milking tubes, straws, quills, cat-guts, and hairpins. Highly acute, inflammatory conditions develop in the affected quarters, whether affected throughout or only partially with parenchymatous mastitis. Hot, painful swellings of the quarters, with collateral edema

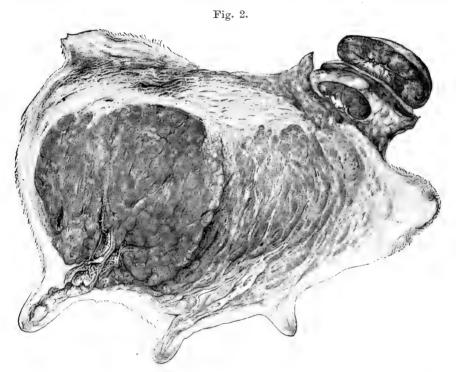


Acute inflammation of the right forequarter with collateral edema. (After Kitt.)

of the surrounding tissues, are the associating symptoms of this form of inflammation, which either results in recovery with atrophy of the affected parts of the udder, or with regeneration of the epithelia destroyed by the disease or on the other hand the disease becomes chronic and may even terminate with complete gangrenous and ichorous destruction of the affected part of the udder.

In the infectious forms of mastitis the supramammary lymph glands may swell to fist-sized nodes.

If the process becomes chronic a suppurative softening of the affected parts of the tissue, or a suppurative demarcation of necrotic parts of the tissue results. These conditions are designated as **suppurative** and **purulent mastitis** respectively. The acute forms of mastitis interest those engaged in milk hygiene but little, since noticeable changes in the milk quickly follow the commencement of the inflammation, and the animals soon stop their secretion. On the other hand the hidden forms of inflammation are of the greatest importance because the milk is frequently almost unchanged, and does not always indicate its inedible condition. Such conditions of the udder may vary from a **simple catarrh** to a **purulent inflammation**. The manifestations of these forms of inflammation vary to a great extent, and the symptoms may be only slightly pronounced, so that a single clin-

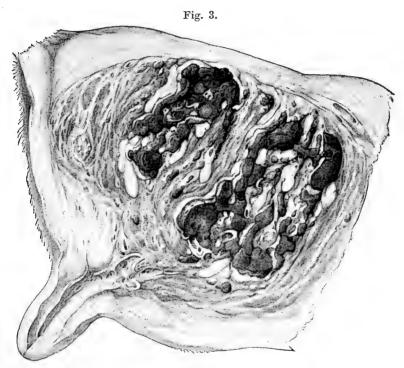


Fibrinous form of parenchymatous mastitis; separation of quarters plainly visible. (After Kitt.)

ical examination may cause a suspicion, but a positive diagnosis cannot always be established.

Literature shows that slightly marked swelling of the affected quarters, increased local temperature, nodular formation of the parenchyma, and inducation of the glandular tissue, may appear in the most varied forms, sometimes with and sometimes without general symptoms. At the beginning it may be localized around the base of the teats, but the hardening of the glands then progresses forward, upward and backward (Sven Wall).

The examination of the milk ducts should not be neglected. The mucous membrane of the cistern may have become inflamed, resulting in ulcerations, scar formations or polypoid proliferations, which are difficult to recognize. Sometimes such changes of the teats are characterized by cicatricial contractions (strictures). The udder, which usually becomes affected in the individual quarters, may remain either normally soft, or may become somewhat harder in consistence. The yellowish-red, normal color of the cross-section disappears, and changes into a grayish-orange or brownish-gray tinge. The parts which are of a harder and tougher consistence show an increase of connective tissue; the interstitial connective tissue changes into a bluish-white thickened network.



Purulent mastitis showing necrotic foci. (After Kitt.)

The edema of the skin which develops at the beginning of the inflammation results sometimes in extensive sclerosis, even the parenchyma of the glands being sometimes dislodged by the proliferating connective tissue causing the quarter to atrophy and harden.

Tuberculosis although almost invariably resulting from a hematogenous infection, appears either in the form of a single focus (tuberculosis uberis circumscripta), or it may be disseminated over the entire parenchyma (tuberculosis embolica disseminata), or the tissue may be diffusely affected, becoming infiltrated throughout almost its entire extent (tuberculosis diffusa). These

forms of the disease may be present in combination in the same udder. During the tuberculous invasion nodular inducations of the tissue develop, which hypertrophy and become tense, hard and knotty. The lymph glands usually manifest hard, painless, thickening, and nodular formations. Caverns may also develop in tuberculosis of the udder.

Actinomycosis which commonly develops from the penetration of actinomycotic barley beards, or particles of straw into the tissue, or more rarely by embolic infection, may also be produced experimentally by the injection of solutions containing actinomyces through the milk ducts. Actinomycosis of the udder has been observed in cows by Peterson, Rasmussen, Bang, Harms, and Jensen. Nodular formations, connective tissue proliferations and softening of the tissues, localized or in larger areas, are also observed in this disease.

Botryomycosis and glanders enter into consideration only so far as the udders of mares are concerned.

For completeness, various growths may also be mentioned as anomalies of the udder, such as fibroma, adenoma, adenofibroma, adenocarcinoma, chondrofibroma, chondroma, lipoma, sarcoma, angioma, etc., which are dependent on the tissue elements and the character of the tissues of which they are composed. Cystic formations have also been observed.

Not infrequently the connective tissue and the subcutis of the udder of cows may show bone formations in the form of bony hooks and plates, (ossificatio plana or racemosa). Parasites have also been found in the udders of cows, namely echinococci (Behmert and Steuding). For further information see Kitt, Pathol. Anatomy, 1910, Vol. 1, page 280.

The author once concluded that a goat affected with adenoma papilliferum uberis was troubled with mastitis, basing this decision upon an examination of the milk, although the secretion contained no specific inflammatory agents. The continually increasing quantity of milk was remarkable. Postmortem and histological examination finally revealed the adenoma in the udder.

#### Structure of the Tissue

The external skin of the teats possesses neither hair nor sebaceous or sweat glands, and continues as cutaneous mucous membrane into the milk ducts, which it lines up to the cistern. The mucous membrane has no glands, possesses fine folds running lengthwise, and is covered by pavement epithelium which is supported upon a well developed papillary base, and is firm and horny next to the lumen. The papillæ are extraordinarily long; they apparently branch near the base, and slant towards the orifice of the teats. This cutaneous mucous membrane of the milk ducts continues without demarcation, with the mucous membrane of the milk cistern, which is covered by several layers of cylindrical epithelium, and possesses accessory glands which are lodged in the connective tissue. The wall of the teats contains bundles of involuntary muscles running lengthwise and crosswise, forming a strong and elastic encasement around the canal of the teats.

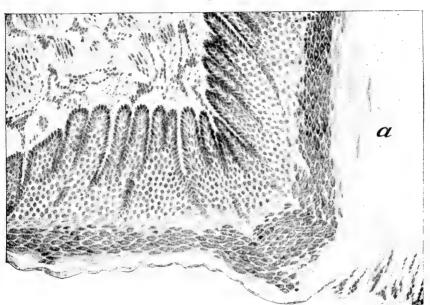
The supporting structure is penetrated by numerous blood

vessels and lymph vessels. Numerous and strong elastic fibres strengthen the dense fibrillar connective tissue of the teats.

In order to describe the finer structure of the parenchyma of the udder it is necessary at first to touch on the further development of the organ from birth until the moment of the appearance of the secretion.

#### (a) Normal Appearance.

The milk gland is an organ which performs increased functions only at certain times. It does not secrete during the entire



Vertical section through the lower end of the teat canal which is closed by a horny plug (a).

life but only when the newly born offspring is to be nourished by the milk. The udder of a virgin animal does not correspond even in its finer structure, with the appearance of a fully secreting udder, and this again varies in its finer structure from a gland which is at the beginning or at the end of the lactation period; even this is not all, since the microscopical appearance changes in accordance with the condition of activity, where a lobule or only a part of the lobule may be found on examination, depending whether the cell-complex is just forming the secretion or has already discharged its secreted product.

The gland of a newly born calf shows but relatively few cell tubes and cell buds, imbedded in connective tissue rich in fat and branching in all directions. These practically form the basis of the glandular ducts and are without alveoli. The end of the tubes is frequently somewhat dilated, or thickened in the form of a club.

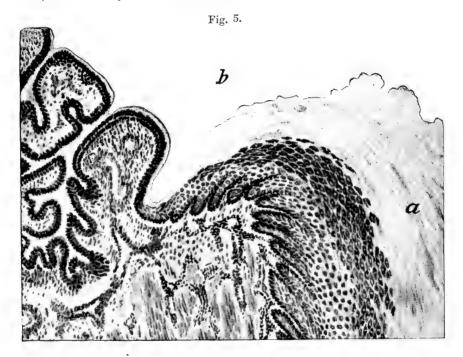
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Fig. 4.

With puberty the alveoli appear in the cow surrounded by strong connective tissue. In older virgin individuals they sometimes show a slight amount of secretion.

A considerable increase of the glandular tubes appears only after the first conception. The tubes become more dilated and branch more and more, forming alveoli, from which other ducts bud out.

Although indications of secretions in the cells may not yet be visible, the cavities contain a homogenous or fine granular mass of cells or cell fragments. The gland prepares for the secretion, growing at the expense of the atrophying or expanding connective tissue, until ready to commence its secretion.

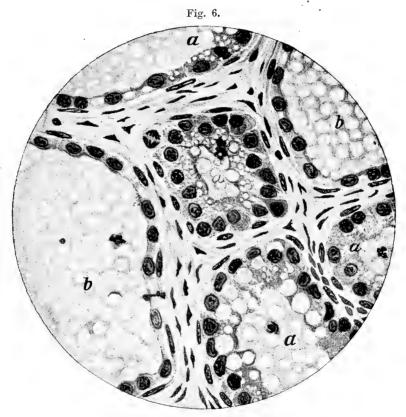


Superior portion of the teat canal (a) with a reflection of the cutaneous mucous membrane of the cistern (b).

The **cell lining** of the larger glandular ducts is of double layers, as in the cistern, while that of the smaller ducts and alveoli is composed of only a single layer. The epithelium of the latter appears cubical or flat, while the upper layer of the former is cylindrical; in the deep layer the cells are more cubical and rounded, partly wedged in between the bases of the superficial cylindrical cells. The borders of the cells are sharp and the protoplasma is clear. The nuclei of the epithelia frequently show mitosis, that is, division and multiplying forms. The cells rest on the so-called basket cells and the membrana propria. The basket cells

should be considered, according to the investigations of Benda and Bertkau, as involuntary muscle cells because of their appearance and their staining qualities. They probably play a part in the emptying of the glandular ducts and the milk secretion.

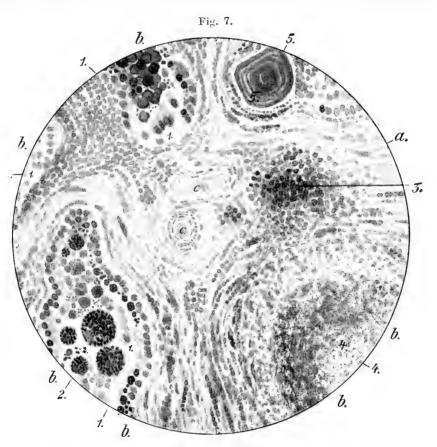
Blood capillaries, lymph vessels and nerves run in the inter-and intra-lobular connective tissue, which is strengthened by elastic fibres, and contains involuntary muscle cells. Therefore, the same tissue elements are represented as in the teats, with the exception of the many-layered pavement epithelium.



Structure of the mammary gland in secretion, Hematoxylin. 1 × 800. (a) Secreting glandular alveoli. (b) Alveoli with dormant cells.

At the end of pregnancy the picture again changes considerably. The protoplasm of the previously clear epithelial cells of the secretory system becomes cloudy, the nuclei larger, their chromatin collects in flakes on the periphery of the nuclei, the borders of the cell become indistinct, the cells become swollen, the nucleus lies in the center, and the indications of the division by indirect fission of the nucleus appear relatively in groups. Some epithelial cells show two nuclei at this stage; towards the alveoli fat globules appear. Leucocytes with which a few eosinophiles are mixed, collect beneath the epithelial cells and penetrating the epithelial layer, separate themselves from the epithelial cells and enter the alveoli, which at this stage contain fatty secretions, leucocytes and epithelial cells in all stages of degeneration.

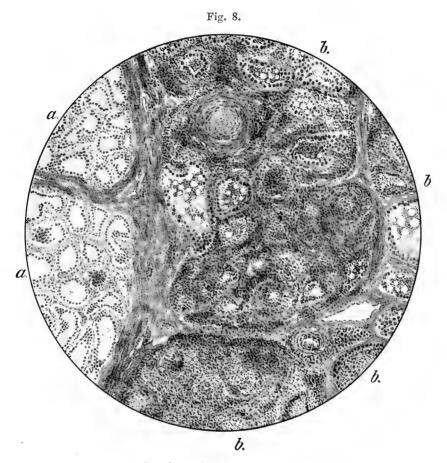
With these manifestations the gland cell commences its function. The desquamation of epithelial cells and the cell degeneration disappear; the cellular infiltration of the connective tissue recedes until it is very slight between the now greatly di-



Chronic mastitis of cow. 1 × 800. (a) Thickened interstitial tissue. (b) Alveoli. (c) Blood vessels. (1) Epithelial desquamation. (2) Colostral bodies. (3) Cellular infiltration. (4) Fatty degeneration and necrosis. (5) Milk concrement.

lated and distended glandular ducts. The cells are finely granular on the basilar border, and at times show striation, that is, fine streaks running in parallel directions (bioplasts according to Altmann).

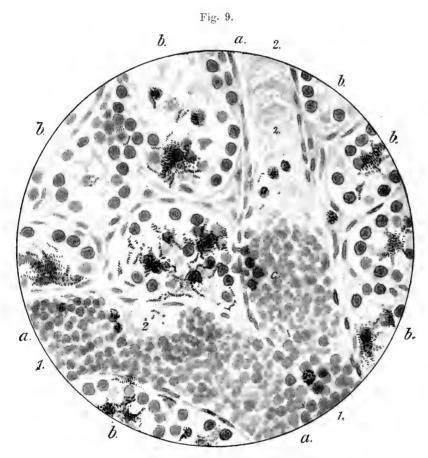
The nucleus is large and vesicular in shape; the upper part of the cell is granulated and shows large and small fat globules. This granulation and streaking may be seen, according to Steinhaus and Duklert, at each act of secretion. The fine fat globules collect into larger ones, which are only separated from the lumen by fine protoplasm, or having been expelled have already entered the alveoli. With the collection of the secretion these dilate, the cell becomes flattened during the expulsion of its products, and the part lying towards the lumen appears indistinctly bordered as if shredded after the expulsion of the fat. They soon become smooth again, and by the pressure of the alveolar contents and the dilation



Chronic mastitis of cow.  $1 \times 90$ . (a) Healthy portion. (b) Glandular portion with chronic mastitis.

of the alveoli, the cells sink and become so flat that the nuclei not infrequently appear bulged out towards the lumen. After the expulsion of the secretion the formation of additional secretion again commences in the cell, the protoplasm again becomes cloudy and granular, and so on, a continuous change of the form of the cell taking place.

During the entire lactation period, but more so in the later stages, manifestations of atrophy of the gland appear, at first commencing at the base of the gland, and finally during the end of lactation in the entire udder. Epithelial cells are thrown off, the alveoli become fewer, smaller, and irregularly distended, the connective tissue increases, and cellular infiltration starts under and between the epithelial layers. The epithelium contains no fat globules, it is sharply bordered towards the alveoli and the protoplasm becomes pale. Finally the last remains of the secretions



Acute streptococcic mastitis of sheep. 1 × 1000. (a-1) Blood capillaries. 2. Thrombosis by disseminated streptococci. (b) Glandular alveoli, with elumps of streptococci. (c) Migration of leucocytes into the infected alveoli.

disappear, the plasma cells and leucocytes taking care of the resorption.

At the end of this process the gland is at rest, and the cow is dry.

Of course these processes are not always so schematically uniform as they have been described. During the entire lactation period, colostrum-forming, and retrogressing lobules may be observed; likewise certain parts of the udder may remain in secretion during retrogression until storing of the secretion, leucocytic resorption activity and connective tissue proliferation cause them to cease their activity.

## (b) Pathological Appearance.

Any kind of irritation of the gland, such as stasis of the milk, especially in chronic catarrhs and inflammations, may result in the most varied kind of pathological conditions, either in mixed form or individually. The manifestations vary, depending upon whether degeneration and destruction of the tissue, or reparation and recovery gain the predominance.

Sometimes desquamation of epithelium, with or without fatty degeneration, occurs together with cellular infiltration of the interstitial connective tissue and capillary engorgement as the only indications of inflammation; or, on the other hand, the changes in the interstitial parts may be very pronounced, while the changes of the parenchyma may be less prominent. The inter- and intralobular connective tissue extends forming thick inducations, from which the separated epithelium is compressed to small necrotic nests. In other stages of inflammation the cellular infiltration of the tissue predominates. The alveoli and the milk ducts are plugged up thickly with leucocytes, and dilated with the pus. In highly acute inflammations the rapid breaking down of cells, destruction of epithelium, serous and cellular infiltration of the tissues even to their dissolution, are the principal manifestations. The ducts and the alveoli are inundated with serous, bloody coagulated masses.

In stasis of the milk, and in all inflammatory manifestations, especially of the acute form, the alveoli contain hyalin and concrement arranged in layers, in addition to inflammatory cells and broken down cellular products.

# CHAPTER II.

# PHYSIOLOGY OF LACTATION AND CHARACTERISTICS OF MILK IN GENERAL.

As already mentioned the udder secretes only in certain lactation periods between births. The lactation lasts under natural conditions in healthy animals as long as the young needs the glandular secretion for its nourishment, and stimulates the lactation by the irritation of the intermittent suckling. Shortly before parturition, or at the time of parturition, the glandular tissue terminates its increase in development, and the milk secretion starts and becomes actively established.

The causes of the increased cell production during pregnancy, and for the secretion after this time, are variously explained. Nervous irritation from the genitals to the milk glands may by means of reflex action stimulate the glands into activity.

That such reflexes on the genitals may originate from the milk gland is proven (Pfaundler). Reflex actions in the opposite way, however, have not been proved (Halbau).

It has been impossible either experimentally (extirpation of the lumbar cord) or by accident (fracture of the spine), to produce a complete "nervous isolation," since as emphasized by Pfaundler, there are still remaining the nervous connections through the vasomotors. However, the re-section of nerves, operations on the spinal cord, transplantation experiments, etc., by Eckhardt, Rohrig, Sinety, Busch, Mirnow, Pfister, Ribbert, Golts and Ewald would suggest that besides the nervous influences, which undoubtedly exist, there must be some other agent which stimulates continuous growth during pregnancy, terminates the same with the end of parturition, and inaugurates the secretion.

Hematogenic influences may be readily accepted, as they may be led to exert their action either by the quantity or by the quality of the blood.

After parturition the body and the milk gland have at their command great quantities of blood which was previously utilized by the gravid uterus. The plethora which appears at this time may be held responsible for the inauguration of the secretion, after the udder has been rendered ready for action by the increase of its growth through nervous influences. On the other hand it has been observed that in other conditions, in which there exist also a diversion of great quantities of blood from the genital parts for the supply of other organs, as for instance after operation on very large tumors in the region of the genital organs, no secretion appears even when the udder is prepared for the secretion.

As a matter of fact the secretion may commence before birth, and even in early abortions, or if the fetus dies. At times when the uterus is only so slightly distended that the quantity of blood set free after abortion is hardly sufficient for an effective hyperemia of the milk gland, the secretion of milk may result (Sinety, Kreidl, Mandl). Therefore the explanation that the quantitative influences of the blood may give rise to a stimulation of the milk secretion (Freund), can scarcely be accepted. Consequently the qualitative changes of the blood must be considered as more probable factors.

Authors have diversified opinions upon this question.

While some accept the view that substances are eliminated from the impregnated organs, or by the fetus itself into the blood of the mother by internal secretions, and that these act as stimulants on the milk glands, others believe that the factors causing lactation lie in the assimilation of certain nutritive substances.

The supporters of the theories of "stimulation substances" (Sinety, Halban, Starling) take the stand that stimulating substances which cannot be utilized for the cellular growth and cellular activity, contrary to the nutritive substances, cause the development of the gland during pregnancy, and at the same time prevent it from secreting (stimulines, hormones [I stimulate], substances of pregnancy). Development of the gland and prevention of secretion may, of course, be the action of one and the same substance (Hildebrand, Starling), or its development, as long as the growth continues, may retard secretion. With birth the stimulation of growth and development ceases, and secretion commences.

Contrary to this, the theories of nutritive substances emphasize the fact that the glands at times may start the specific activity without the presence of certain stimulines, probably through nutritive substances which are present in the blood at various times.

Rauber attempts to explain the activity of the gland after birth by declaring that after the expulsion of the fetus a nutritive material becomes available, which has served prior to birth for the preparation of nutriment for the offspring. While the explanation of the author that the lymph cells play the most important part in this can no longer be considered, still it furnishes the basis for all new theories relating to the action of nutritive substances.

These views were strengthened in 1908 by Schein by the statement that during pregnancy the mother animal, in order to meet the requirements of the fetus and of the impregnated organs, en-

riches her blood with the so-called "milk producing substances." Pfaundler recommends the designation "offspring nutritive producing substances." Since during pregnancy the continuously developing placenta utilizes and consumes these substances for use in the nourishment of the young, there remain for the milk gland only slight remnants, just sufficient to result in the necessary stimulation for the cellular increase in the gland. After parturition when the activity of the placenta is completed, the milk gland takes up the released nutritive substances for its own use (specific affinity of the substances to the cells of the milk gland), and is stimulated to secretion by the quantity of the disposable material. Schein's milk producing substances in the blood constitute the initial material for the formation of specific components of the milk, milk sugar, casein and milk fat.

The material acquired by the mother, through placental contact with the fetus, while aiding in the development of the latter is also of benefit to the activity of the milk gland, whose product adapts itself exactly to the requirements of the young, as far as it concerns the material which the young uses for the growth of its body.

If conception again takes place the developing placenta of the new fetus enters into competition with the lactating gland, and draws from it milk producing substances for its own use, whereby the secretion of the milk gland becomes reduced or ended.

Influences exerted on the milk gland by oestrum or puberty, and also the impulse of pregnancy, have not yet been sufficiently explained through this theory. Pfaundler enlarges upon and explains these phenomena by stating that the withdrawal of certain nutritive substances, through the germinal gland, embryo and ovum, and not the appearance of milk producing substances alone, periodically disturb the equilibrum of physiologically acting substances in the blood, and thereby the antagonizers of those substances (the stimolines, harmones of other authors), are enabled to find specific receptors (affinities) in other organs of the genital apparatus.

After birth, continuing intermittent stimulation may retain or increase the lactation of the milk glands for a longer or shorter time. Stasis of the milk diminishes and retards the secretion.

Rievel opposes Schein's view, since in his opinion it does not explain how udders of animals in which neither pregnancy nor birth has preceded, could start secretion (lactation of milk glands of the newly born or virgins, occasionally even of male animals). According to the author's view these facts would not oppose the theory of nutritive substances. Schein, himself, aims to bring these observations into harmony with his views, and asserts that the newly born may give a secretion from their milk glands, when towards the end of pregnancy the activity of the placenta is disturbed, and as a result small quantities of the "milk producing substances" enter without changing directly into the blood of the fetus, and thence into its milk gland. Sufficient stimulation for the secretion and formation of the so-called "witches milk" results. Schein explains the formation of milk in virgin mamma, or in milk glands of individuals which have passed their climacteric. by the fact that through the stimulation produced by sucking. the secretory cells are awakened from their dormant state and then utilize the milk producing substances in the blood for the performance of their functions. Finally (1910) he concludes that the occurrence of milk secretions in nullipera and in women who have passed the climacteric, which differs from the gradually inaugurated normal lactation as a result of pregnancy, and also the observed secretion by the breasts of newly born and of male individuals, represents a continuous secretion analogous to the normal secreting process in other glands, in which the product is as a rule, however, re-absorbed by the glandular elements. In pregnancy and at birth the secretion is increased to the greatest extent, but other stimulants may under certain conditions stimulate the activity of the gland. Duval's more recent observations contain data relative to the occurrence of milk secretion by women outside of their normal lactation periods.

It is not uncommon to observe secretions in virgin animals especially when young animals which are present stimulate the udder intensively by sucking. It should be emphasized however that the udder secretion of virgin animals distinguishes itself in its appearance from the milk of mature milking animals; it represents a secretion which does not even deserve the name of milk.

The experiments which were conducted by various authors in support of their lactation theories appear of interest.

The experiments of Starling aim to show the presence of bodies in the blood during pregnancy which prevent secretion, in which claim is made that an interruption of pregnancy in rabbits at a time in which alveoli capable of secretion were not yet present, led to a retrogression of the milk gland, while in the later periods of pregnancy secretion was induced.

According to Pfaundler's view the harmone theory could be effectively supported by the fact that an existing secretion may be successfully interrupted or prevented by the introduction of serum of pregnant animals of similar species.

The author does not believe that this proof is satisfactory and mentions observations made in a case in which the secretion appeared at birth of twins which were born at long intervals, that is, the pregnancy continued after the first birth, yet the milk secretion continued unchecked. Wucherer observed a case in which a sow gave birth to nine, and seventeen days later to six other pigs. At the birth of the second lot the first born pigs were taken from the sow. These continued to thrive, but of the second lot only three remained alive. He emphasizes the opinion that a transitory action of blood serum, as used in Pfaundler's experiment, which corresponds only slightly in its composition with the normal blood serum, can never be favorably compared with natural influences in the body. This exception must hold also for the indecisive experiments of Starling, who by injections of juices from rabbit embryos, but not with injections of preparations from rabbit ovaries, placentas and mucous membrane of the uterus, produced a development of the glands, and at times a degree of milk secretion. He believes that the true cause of the secretion may be found in the chemical changes which are produced by the growing embryo and are brought to the glands through the placental circulation. According to Basch, secretion may be established in the mammary glands of virgin rabbits by injecting them with placental extract (serum of unlike origin, from man), which was so powerful that it also brought on a secretion of milk in mother animals without the intervention of pregnancy. The placental extract could induce the secretion only when the teats of these animals were stimulated to hyperplasia by the implantation of ovaries from pregnant animals.

According to the author's observations these questions can only be determined through experimentation, when by uniting two female individuals of like species a basic condition is established, by which the activity of the glands of one of the impregnated individuals as a consequence of its pregnancy may be observed upon the other, and the result of the impregnation of the latter on the lactation of the first mother may also be determined, Such experi-ments have already been made by Cristea of Vienna, by coliotomy of a virgin and a pregnant animal, and uniting both by suturing of the peritoneum, the musculature and the skin, the author establishing a double individual, united by a broad peritoneal communication. Of eighteen such pairs (rats and rabbits) six remained alive. In the experiments after parturition of the gravid animals the milk secretion also appeared in the virgin animals to which they were united. Cristea therefore believes in a slow transition of a secretion from the gravid animal into the non-impregnated animal, namely by the way of the lymphatics, since there existed no blood vessel union between the individual animals. With this result the hypothesis that the changed distribution of the blood after birth produces the milk secretion collapses, since on account of the lack of communication of the blood vessels it is not possible that an increased blood supply of the mammæ of the virgin animal would result from parturition of the attached animal. It can make no difference whether milk producing substances or substances which are not assimilable and are not consumers of energy (stimulating and inhibiting substances), stimulate the glands to activity.

Recently Basch observed an abnormal birth to one of a pair of twins (the Blazek sisters showing a condition of pygopagus, union of the pelvis and sacrum with a common introitus vaginæ, and a common rectum), in which after the birth of a child to one, lactation commenced also in the virgin sister. In this instance nervous connections may exist in the genitals of both individuals. According to the author's view this case is not an absolute proof of the stimulation of the gland by hematogenic means.

The lactation theories may be laid aside, and consideration only be given to the fact that at birth, puberty, pregnancy, at the conclusion of parturition and also in the disturbances of the genitals influences are exerted on the milk gland the character and action of which are still uncertain, although the results manifested by the production of milk may readily be observed. Especially typical and striking are the phenomena seen at puberty and during and at the end of pregnancy. Exceptionally a condition may appear outside of these normal periods of the organs in females, and in single cases even in male individuals, which permits the conclusion that the glands react to special stimulation. Abnormalities may occur in the anatomical structure of the gland, pathological manifestations in the sense of inflammatory reactions, etc., may also be observed, and exceptionally the usual functions may be present or may develop, without their being accompanied by gross anatomical changes of the gland; these however are usually present at the same time.

These influences on the gland originate partially in the gravid genital organs and the fetus; in other instances the germinal glands and the disturbances of their functions are the cause of these influences.

Such influences may be classed according to the impulses which lead to glandular activity, as follows (Halban):

1. Embryonic impulse—action very transitory—mastitis neonatorum—witches milk.

2. Puberty-lasting effect-development of the gland.

3. Oestrum—action rapidly transitory—hyperemia, interstitial hemorrhages, disturbances to physiological lactation, secretion.

4. Impulse of pregnancy-lasting between parturitions.

Lactation may be sustained for a long period of time by the regular drawing of the milk, and ceases in healthy udders only when after frequent and absolute stasis of the milk (after about eight days), the tissue becomes affected by inflammatory irritations (absorbtion and change of the condition of the epithelium), or when the animals are soon to give birth to young. If no re-impregnation takes place the lactation period may last longer, even from one to two years although not to an unlimited extent. The activity of the gland may be retained for a long time through the sucking of the young, stimulation by milking, or artifical withdrawal of milk.

Frequent periodical and complete emptying of the milk cisterns acts favorably on the amount produced. In the cow two to three milkings per day are sufficient to retain the udder in secretion. The milk formation occurs between the milking periods and during the milkings; therefore of these two periods, the first lasts for many hours, the second with more intensive production is completed in a few minutes. The first phase is the work of continued activity of the gland, the second is brought on under the stimulation of the sucking, or milking, on the secretory nerves, and as a result of the increased blood supply (stimulation of the vasodilators). The capacity of all the milk ducts of the udder represents less than half of the quantity of milk obtained in one milking.

According to Fleischmann the volume of the entire udder of a cow with the teats is 6700 c. c. Of this 3000 c. c. is represented by the cavities; the secretion obtained in one milking may nevertheless amount to 7000 c. c.

Nüesch substantiates Fleischmann's statements by an experiment; a cow gave daily before slaughter 10 liters of milk of which 5.5 liters was the amount of the morning milking.

After slaughter before milking in the morning 2.7 liters of milk could be proven in the udder (catheterization and calculation of the amount remaining in the udder), which proves secretion during the process of milking.

The two phases may be considered as though the glandular cells which tire after the milking gradually recover (increased blood supply) and recommence their secretions. The collecting secretion will increase until a certain relative pressure between the collective quantity of secretion and the tissue with the blood vessels is established, when the secretion is retarded or ceases until renewed stimulation of the glands by milking, emptying, massage, (electric irritation), or stimulation of the central nervous system from milk accumulations causes the milk to fill the cavities of the udder again.

If the usual milking time is omitted a flow of milk may result, that is the pressure under which the secretion is held finally overpowers the resistance of the sphincter muscles at the opening of the teats (directly or by reflex), whereupon formation of milk again takes place.

Nervous influences on the secretion are exerted by the spermaticus externus and by the sympathicus.

Experiments which were conducted for the study of the enervating influences on the secretion produced contradictory results.

Röhrig severed the ramus inferior of the nervus spermaticus externus (vessel branch), and observed an acceleration of the secretion, while the severing of the glandular branch (part of the median branch) resulted in inhibition. Eckhard failed to observe any influence on the quantity of milk after the severing of the nervus spermaticus externus. Heidenheim and Partsch demonstrated an increase of the quantity of milk from the cutting of the nervus spermaticus externus, but only when strychnine or curare had been administered at the same time (test by Sinety on guinea pigs). Although Basch could not establish a quantitative increase by severing the nervus spermaticus externus, he found qualitative changes (formation of colostrum).

Pfaundler concludes from these and other experiments that an action of the peripheral nerves on the development of the gland and its functions, especially from a qualitative point of view, must figure in the consideration, but that these influences have only slight importance.

Insignificant as well in their results on the secretion were the severing and re-sectioning of the spinal cord, or interference with the sympathetic system. Basch again observed the formation of colostral milk after re-sectioning of the coeliac ganglion. From this he concludes that the regulating influence of the nervous system exists through reflex action, especially from the sympathetic, but that at the same time the gland is also capable of independent secretion.

As a matter of fact far reaching influences of a nervous character are observed.

1. Psychic influences.

2. Reflexes, which are caused by local stimulations (sucking —milking—electrical stimulations, etc.).

3. Reflexes from the genital region.

These points are only briefly mentioned here, since the various conditions will be discussed in subsequent chapters, when consideration will be given to the quantitative and qualitative changes which appear under varying influences.

An active part in the emptying of the milk from the cisterns, and in the passage from the upper part of the duct and alveolar systems, is played by the sucking and pressure exerted during the milking (pressing outwards, sucking from the gland), massage of the udder (pressing out into the cistern), the contractility of the tissue (elastic fibres, involuntary musculature, filling of the blood vessels), and the vis a tergo of the newly formed secretion.

## CHAPTER III.

#### MICROSCOPY OF MILK IN GENERAL.

If milk is examined through a microscope one chiefly sees numerous small fat cells floating in the fluid or milk plasma. These will be considered later, but at first the cells and cell fragments originating from healthy and affected udders will be discussed. Between the milk globules, by which term the small fat droplets are designated, bodies may be seen which are hard to define unless stained. After special treatment, however, they may be readily recognized as cells or their fragments, or as a precipitation of soluble or suspended substances.

Since the external skin of the udder, and the lining of the milk passages and milk secretory ducts in the udder are of similar formation, we naturally are only concerned with the upper layers of pavement epithelium, cylindrical epithelium, and the deeper cubical epithelium of the terminal ducts and alveoli, and only in severe tissue changes would cells of other parenchymatous parts appear in the milk. Naturally in such an actively working organ, even in a physiological normal condition, leucocytes of the most varied kind, and even red blood corpuscles may be found. In cases of special stimulation from physiological or pathological causes, the resulting cell mixture may be of a most varied character depending upon the location of the stimulation, and its quality and duration; hence at times certain leucocytes, and again red blood cells or epithelia, may predominate in the mixture.

1. Cells from compound pavement epithelium. Following the intensive manipulation and stimulation of the teats by milking, the appearance of cells from the upper layers of the pavement epithelium of the outer skin, and the milk ducts is natural. As a matter of fact in the fresh milking periods during which irritation from the extraction of the milk is especially evident, the milk always contains fine folded platelets of round, oval, or irregularly distended and curved borders, which frequently when folded in several layers, appear as small clasped cysts without special structure.

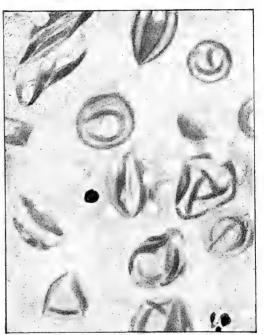
These bodies have been described by Winkler, and were considered by him as indications of pathological changes. The author took a stand against this view of Winkler, as he had observed them in the milk of entirely healthy animals, but not until the present time has he been able to offer an explanation of the nature of these bodies, designated as "skinlets" or "shell." They represent desquamated cells of the stratum mortificatum of the pavement epithelial layers singly or in groups. Although usually no particular structure is manifested yet in single instances typical flat, round nuclei can be seen.

If the teats of a slaughtered cow are taken and the cistern and milk duct are carefully cut open, and from the surface of the milk

duct a small quantity of the cellular layer is scraped off, an examination by the usual method discloses the typical "shells."

2. If cells from the cistern are prepared and examined, elongated or oval, or quadrangular cells with oval nuclei, frequently elongated at the base. will be found, singly or in groups. Single fat droplets may frequently be seen in the plasma surrounding these cells. Similar cells may also be found in normal milk. They are usually single, although sometimes united in groups arranged like flowers. In stimulation, which brings on a desquamation from the mucous membrane of the cisterns, or from the parenchyma in catarrhal conditions of the milk passages, they of course appear in masses.

Fig. 10.



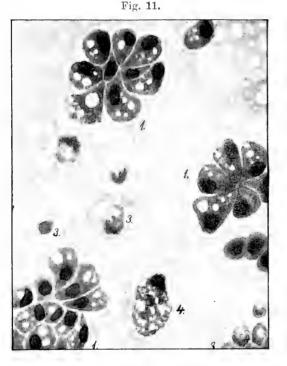
Film of sediment from milk of a fresh milking cow. Cells from the stratified layer of pavement epithelia of the teat canal. Thionin.  $1 \times 1000$ .

Such reactions occur in the cistern for instance as a result of the so-called kneading.

3. Cells from the secreting milk ducts and the alveoli, appearing large or small according to the quantity of fat globules collected in them, often become tremendously distended and bloated (foam cells). Their structure is mostly honeycombed or mulberry-shaped when they contain fat; without fat the cell is surrounded with only a narrow border of protoplasm. The nucleus is usually in good condition.

These cells are the large colostral bodies. They are in their entire structure and in their staining characteristic epithelial cells and not leucocytes; the amoeboid movements observed in them, if these observations were beyond questioning, do not prove that all colostral bodies represent leucocytes.

This point will be again taken up during the discussion of colostrum. While such cells only appear occasionally in ripe milk they are extremely numerous at the beginning and termination of secretion, and in pathological processes, in the latter especially in subacute and chronic forms, but not in peracute and acute inflammatory



Cells from the lining membrane of the wall of the cistern. Sediment in catarrh of the cistern. Thionin.  $1 \times 1000$ .

uniform condition, will the organ cease to free itself of incapable elements. In inflammation the inflammatory irritation and its consequences soon drive the cells to overproduction. At other times it paralyses or destroys them, even before the formation of milk, depending on the duration of the inflammation.

The form of the epithelium varies in accordance with the content of fat. The collection of fat is not the result of fatty degeneration, but is produced when the cell is thrown off before its time for secretion, or while still capable of taking up material and producing fat but without strength for the separation of fat. Therefore

conditions of the parenchyma. Such cells may occasionally be noted collected in groups.

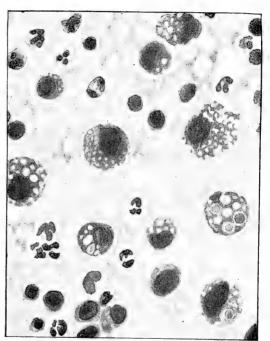
The author believes that their appearance in masses in the milk, that is, the condition increasing the expulsion of these epithelia, results from the fact that each cell, which in its singular activity precedes or follows the other cells of the union, becomes desquamated. It does not correspond functionally, with the other cells, and is therefore removed from the rows of cells which are developing for a definite purpose or are working for that purpose. Only when uniform work is performed by all of the cells working in unison, and bringing about a such cells may be found even in the epithelial groups, which is an additional proof that they with certainty represent epithelial cells.

The cells are from 5 to 25, even to 47  $\mu$  in size (Schulz). Not infrequently 2 to 3 nuclei of oval or roundish shape are present. The author has never observed more than one nucleus, and believes, with Popper and Schulz, that the appearance of more than one nucleus results from two cells lying on each other, in which case the cell thus formed may appear to possess two or more nuclei. Migrated macrophages may also simulate a polynuclear appearance.

Not infrequently epithelial cells are thrown off, with a single large fat globule in the body of the cell, known as "seal-ring cells." In such cases the fat globules have a "moon" or "cap" appearance.

4. Leucocytes of all forms are frequently met with in milk such as mononuclear basophiles, eosinophiles, polynuclear basophiles, acidophiles, or cells with neutrophilic and eosinophilic granules in the protoplasm.

If the polynuclear cells show no nuclear bridges, they may be found with 3/4 or more spherical shaped nuclear granules (spherical granule polynuclear leucocytes, Babs.) The nucleus is usually in the Fig. 12.



Sediment in milk of a cow after milk stasis. Numerous desquamated epithelia, among these an "albuminophore," and polynuclear leucocytes.

shape of a ribbon, or clover leaf, or heartshaped. The protoplasm usually contains fat globules, which in stained preparations appear as fine vacuoles.

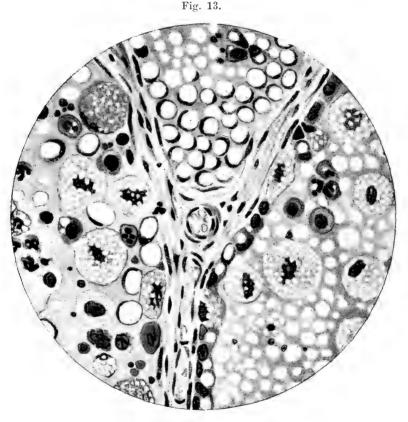
The lymphocytes are small cells with round nuclei and a very small border of protoplasm. According to Schulz they never contain fat. Large mononuclear leucocytes are also supposed to be present in the milk. If they gorge themselves with fat they are filled to their fullest extent, and can no longer be distinguished from fat-containing epithelial cells.

5. The red blood cells may be seen as small, round or thorn-

apple shaped bodies, with metachromatic staining substances. They may be readily recognized as erythrocytes.

6. Degeneration of these various kinds of cells may result in the finding of the most peculiar formations.

The protoplasm of the epithelial cells becomes shredded; the nucleus splits up and eliminates its chromatin into the plasma in the form of dust or flakes. It diffusely passes into the cell protoplasm, which appears darkly stained, and in the place of the



The formation of large colostral spheres and desquamation of "seal-ring cells."  $1 \times 800,$ 

nucleus a pale vacuole appears. If the breaking down continues there may appear a disintegration of the cell and of its nucleus into small droplets and fragments of roundish appearance, either with or without a lightly stained border around a small darkly stained center of chromatin (Heidenheim, Cohn, Popper, Schulz). These chromatin flakes are probably identical with the so-called free nuclei (Michaelis), which were also observed by Lenfers.

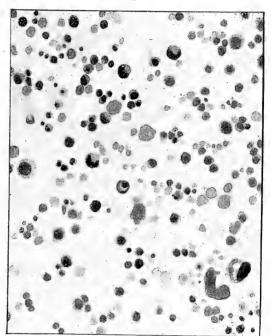
The flakes which result from chromatolysis have been des-

ignated up to the present as "Nissen's Globules." According to Ottolenghi they are derived either from leucocytes or from epithelia.

If fat-containing cells break down in this manner, fat globules in the shape of grape-like bunches, and single fat globules result, which are united by a mesh of fine protoplasm, or they are surrounded in the form of a moon by a narrow border of protoplasm, which crowded to one side rests like a cap on the fat globules. Such moon and cap formations may also result in another way.

The leucocytes (macrocytes), crowd on to the dead or dying cells, eat their way into the cell bodies and establish in the more and more distending cell actual lacunæ, in which the devouring leucocytes lie. The remains of the protoplasm and of the cell and nuclear membranes float in the shape of caps and moons in the milk protoplasm until the swelling or further breaking down converts them into spheres or globules. At the same time of course the macrocytes may themselves degenerate in the cell, and no longer present a recognizable nucleus. In such cases its respective lacuna contains homogeneous. sharply circumscribed proteid globules.

Fig. 14.

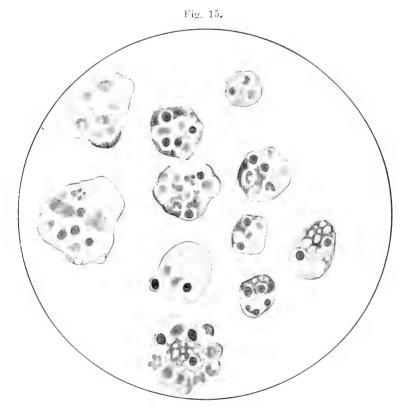


Budding globules, free nuclei, Nissen's globules, that is cell fragments, in the sediment of cow's milk,  $1 \times 1000$ .

The author considers these epithelial cells which have been destroyed by macrophages, as identical with the albuminophores of Bab and Schulz which they described as large lymphocytes,  $(15 \text{ to } 20\mu)$ , containing fat and 1 to 4 or more proteid globules.

Besides these regularly formed constituents of the milk, its sediment contains flaky constituents, small irregular shaped coagula, which readily tinge with basic anilin dyes, or with nuclear staining substances. Frequently they are without any structure. At times they appear in individual milkings, almost completely dominating the microscopical field. They are the early stages of the **corpora amylacea**, soon to be described, which appear either round, oval, bean-shaped, or nodular, ranging from very small  $(1 \text{ to } 2 \mu)$ , to an enormous size (5 to 200  $\mu$  according to Zimmermann). These bodies show no concentric formation, or radial stripes. They usually appear during abnormal activity of the gland, and are found in colostrum, in stasis of the milk, in mastitis, in the inactive glands of older animals, etc. Their varied thickness makes active turning of the micrometer screw necessary.

These corpora amylacca (according to Siegert, corp. flava in contra-distinction from corp. versicolorata, are the same as amylacca) were seen by Herz, Ottolenghi, Iwanoff, and later described by Martin, Lenfers, Winkler and Zimmermann. Wederhake



Epithelia in different stages of destruction by macrocytes, that is so-called albuminophores.  $1 \times 1000$ .

confirmed their occurrence in the colostrum of women, and compared them with the corp. amylacea of the prostate gland.

A section offers the best opportunity for the microscopical study of the nature of these bodies. In preparations of acute mastitis, their development is especially clear. Around small flakes of proteids, possibly precipitated nuclear or cell fragments, layer after layer will be formed until a concrement results, which may even fill the entire alveolus. Lime and salts of magnesium are later absorbed by this basic structure of concentric layers, and fine

30

radiated stripes appear upon its surface in consequence (Fig. 13, Fig. 16 and Table I.).

While the alveolar epithelium succumbs to the pressure of the growing concrement, and may be absorbed for some time, the concrement resists the influences of the organs, and finally is surrounded by connective tissue. Zimmermann states that the bodies may be either in the alveolus or on or under the epithelial layer, and even free in the connective tissue. These observations have been confirmed by the author.

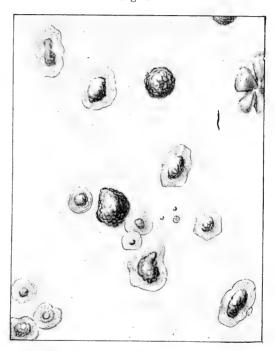
They stain with methylene blue, iodine green, and gentian violet, similar to other amyloid substances, but do not give the

starch reaction with iodine solution and sulphuric acid (Zimmermann, and author's observations). Wederhake and Winkler claim to have obtained a bluish violet coloration with iodine.

The corpora amylacea of the mammary glands resist few acids (sulphuric acid, hydrochloric acid). Ottolenghi and Zimmermann obtained a solution with pure sulphuric acid.

They are therefore pure concrements of secretion which form under peculiar conditions. Their quality varies, depending on the character of the precipitations, which combine to form them.

Fig. 16.



Lime concrement in the milk sediment of a cow.  $1 \times 1000.$ 

What remarkable significance may be attached to such conditions may be indicated by the views of Herz, who considers them as the initial formation of casein, and those by Winkler, who believes that they change into fat or that they are degenerated epithelium.

Leucocytes also crowd upon these bodies, and attempt to dissolve them just as osteoclasts attack bones. Under their influence, combined with that of the body juices, a destruction, solution and absorption of the concrements may take place, or on the other hand new layers of thickened secretion may form around the old debris, and a new concrement develops.

This describes, with the exception of the fat globules, the cell elements which may be demonstrated under the microscope, as far as they originate from the udder of the cow. The fat globules will be discussed under the heading of milk fat.

## CHAPTER IV.

# COMPOSITION OF MILK AND ITS BIOLOGICAL, CHEMICAL AND PHYSICAL CHARACTERISTICS.

There is very little known with absolute certainty relative to the development of the individual constituents of milk. The theories in this regard are almost entirely hypothetical. It is certain that milk constitutes the specific product of cell activity of the glandular parenchyma, and does not represent a simple transudation of the constituents of blood, with a mixture of broken down products of cells (nuclear masses of leucocytes and epithelia, and fatty detritus), nor the fatty breaking down of the epithelium (Reinhardt, Virchow, Skanzoni, Koelliker), nor partial epithelial degeneration of the parts lying adjacent to the lumen (Heidenhain), nor transformation of leucocytes and lymph cells (Rauber). None of these is the basic pheonomenon in the formation of milk. but it is due instead to the assimilating activity of the cells, which send their secretion into the lumen of the cell tube (Ottolenghi). A breaking down of cells of course occurs to a greater or lesser extent, in accordance with their increased activity, and therefore the milk contains cells and cell fragments in varied quantities, without this throwing off of cells or breaking down of cells having anything to do directly with the secretion proper. The throwing off of useless material, and its natural replacement by functionating elements are only signs that the organ desires to maintain itself in a condition capable of continued secretion.

Our attention has previously been directed principally to the functions and activities of the milk gland from a physiological point of view; the morphological condition of the udder and some constituents of the secretion have also been noted. In this chapter the chemical qualities of the milk will be considered, as far as this is necessary for the most ordinary conception of these properties.

The quality of the milk—in the broadest sense—adjusts itself to the requirements of the young. The milk gland offers it nutritive and protective material in a form which most favorably meets the requirements of the off-spring.

In order to give only a few examples attention should be directed to the established facts, which show that there exist absolute relations between the time required for the doubling of the weight of the young and the percentage of proteids in the milk; between the proportion of certain salts and the ash constituent, and the rapid growth of the young; between the growth of the brain and the supply of proteids and lecithin.

Milk consists of dissolved constituents, and this solution contains substances in suspension; in the entire mixture there are also undissolved substances in emulsion.

The dissolved and suspended substances are designated as milk plasma, which after coagulation separates in milk serum and coagulum. The fat is present in an emulsion; there are in addition to this several salts, coagulums, cells, etc., undissolved or in a precipitated condition. In coagulation the casein which at first is in suspension, thickens, and carries down the undissolved substances, separating more or less from the milk serum in which the soluble salts, milk sugar, certain proteids, ferments, coloring matter, etc., remain.

The principal constituents of the milk, which constitute as well the principal properties of the glandular secretions, are the parts which have received the most thorough study.

**The proteids.** Casein, milk albumen, and milk globulin (traces of lactomucins, and possibly traces of other proteid substances, which remain after acid precipitation and boiling, being known collectively as lactoproteins) are the protein constituents of milk.

The fat; the milk sugar. The milk further contains lecithin, sarcin, kreatinin, nuclein, urea and sulphocyanic acid.

Nothing is known at the present time of some of these constituents, whether they occur originally in the milk, or whether they are only split products, which result during the final production of the various principal constituents, or through bacterial action in the milk; of such substances may be mentioned peptone, ammonia, leucin, etc.

Of non-nitrogenous substances milk also contains citric acid, cholesterin and under certain conditions free lactic acid, alcohol and acetic acid.

Gases which occur free in milk are oxygen, nitrogen, and occasionally carbonic acid; the salts are combinations of the bases of sodium, potassium, magnesium, calcium, and iron, with hydrochloric acid, sulphuric acid, phosphoric acid, carbonic acid, and citric acid.

## **Principal Constituents.**

**Casein** is a proteid especially characteristic of milk, occurring almost exclusively in the milk gland secretion of mammalia, in quantities of from 2 to 4 per cent.

(It is supposed to occur also in the secretion of the sebaceous glands of mammalia and in the coccygeal gland of birds.)

The origin of case in is unknown. It was formerly supposed that it originated from an enzymic change of serum albumen produced by the action of enzyme-like bodies upon the albumen. However, since it has been found that the assertion of Kemmerich, relative to the increase of the case in at the expense of the lactalbumen, after the digestion of milk at blood temperature for several hours was incorrect (Schmidt and Tierfelder, likewise that casein is not produced by mixing blood serum and macerated milk gland structure, or milk gland juice and ovalbumin, and especially since it is known that casein represents a nuclear albumin containing phosphorus, the enzymic origin of the casein in the above sense is denied. For a time Basch's hypothesis relative to the origin of the casein was accepted, namely that the nucleic acid which is set free in the alveoli by the activity of the gland, combines with the transuded blood serum, forming the nucleo-albumin, the "casein." Investigation of the experiments of Basch by Odenius, Mendel, Levene and Lobisch proved however that Basch's hypothesis cannot stand.

At the present time it must be admitted that the cells of the milk gland break up the proteids into more simple bodies, and then build up the casein from these products.

The case is distinguished from other proteids containing phosphorus, as for instance from the nucleo-proteids, by the absence of the xanthin group, the pyrimidins, and the pentose group. The consistence of case in from various species of animals varies chemically to a considerable extent. By special reactions with case in anti-serum (precipitation, complement fixation), the case ins from different species of animals may be differentiated one from the other. In the splitting up of case in into its various constituents, quantitative differences in these split products are found which indicate the differences in the individual case ins.

The cow case in contains

according to	С.	H.	S.	Р.	N.	О.
Tangl						
Ellenberger	53.07	7.13	0.76	0.80	15.64	22.60 %
Burow	52.825	7.095	0.725	0.808	15.64	22.906%
Hammarsten	52.96	7.05	0.758	0.847	15.65	

It is insoluble in water and in alcohol, but with bases forms solutions, the so-called caseinates. Alkali-caseinates form opalescent solutions, while solutions from caseinates of earthy alkalies represent cloudy, milky fluids. Casein is slightly acid, the solution of which with the bases is accompanied by the formation of salt-like compounds.

The characteristics of casein are of especial interest, as they give to the milk its well known properties of **rennet-coagulation**, and easy **acid coagulation**, etc.

Casein is present in the milk as caseinate of lime, in suspended condition as dicalcium-caseinate, which gives an acid reaction to phenolphthalein, and a neutral reaction to litmus.

Acid abstracts calcium from the caseinate, the casein being precipitated (that is casein from the milk of cows and other ruminants) as coarse, flaky material, while the casein from the milk of solipeds and women is precipitated as a fine, flaky substance.

This difference in its properties is traceable to the physical condition which is manifested by the casein molecule of the various kinds of milk (Fuld and Wohlgemut); but it may also be the result of a variation in the quantity of salt and proteid present in the milk.

In the presence of di- and tri-phosphates the casein dissolves by combining with a part of the bases, so that the neutral and alkaline phosphates change into monophosphates (Hammarsten, Arthus).

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Case in is also soluble in other salts, but not, or only to a very slight extent in NaCl, Na<sub>2</sub> SO<sub>4</sub>, NaNO<sub>3</sub>, KCl and others.

In the presence of an excess of acid the casein which is first precipitated is again dissolved into a syrup-like mass, but may be again recovered as casein after neutralization. Neutral calcium casein suspensions do not coagulate in boiling, but they form a pellicle on the surface. (The nature of this manifestation is not entirely clear, but depends probably on the drying and transformation of the casein into a more solid form.)

Casein is precipitated even in the presence of relatively small quantities of acid while boiling under this condition changes it slowly into a body not susceptible to the action of rennet. In overheating and likewise in boiling and over-heating with small excesses of alkali, casein is split up through hydrolysis.

Even under the action of water, casein is split up into a proteid body which is coagulated by heat, passes through a filter and is probably identical with whey casein.

The latter substance is formed after the precipitation of the cheesy substance, through the action of rennet, and is a mixture of reduction products of the casein originating through the action of the rennet (Raudnitz).

One characteristic property of casein is its precipitation by rennet in the presence of earthy alkali salts. The precipitation of casein has no connection with the action of the rennet as such. This may occur even without having precipitation as a result. If for instance a casein solution is mixed with active rennet, and another solution mixed with inactive boiled rennet, then in the mixture containing active rennet, para-casein is formed without any action being noticeable. Only after the addition of soluble calcium salts will precipitation of the para-casein calcium result in the glass which contains the active rennet, but not in the glass containing rennet which has been inactivated by heating.

In the change of casein by the rennet ferment, there results in addition to the substance designated as para-casein, another proteid body free of phosphorus, with the properties of albumose, the whey-proteid (Hammarsten).

The change of the case to para-case in, and whey proteid may be a splitting up of the case in, or it may depend on a change in the grouping of the molecules, or it may correspond to a change in its physical condition.

The action of rennet in the curdling of milk is practically the same as in casein solutions; however it is influenced by the other (dissolved) substances, by the other proteids and salts, and possibly also by the physical condition of the fatty emulsion.

Curdling with calf rennet develops in accordance with definite laws. In milk that has been brought to low temperatures (refrigerator) the action of the rennet may be established by subsequent heating; the precipitation, however, will not take place until the mixture is heated to 37 deg. C. (Morgenroth.) Coagulation may not always appear if the milk is immediately heated to 37 deg., which would indicate that some of the rennet is destroyed at 37 deg.

If the same milk is utilized under the same experimental conditions, it can be seen that the amount of rennet necessary for the coagulation of the milk is nearly proportionately opposite to the length of time necessary for the coagulation to be completed; this fact is expressed by Storch and Segelke as follows: "The product from the quantity of ferment and time of coagulation is constant."

Each kind of rennet has a certain strength which of course is changeable, and relative for each sample of milk. In strong dilutions of the rennet the action does not correspond with the time rule, the time of coagulation becoming continually longer unto infinity; that is, coagulation finally no longer takes place.

The action of rennet depends on the most varied factors, which may either hasten or retard its action and influence the precipitation.

Acids for instance strengthen the rennet action, likewise earthy alkali salts, while alkalies, albumoses, neutral salts of higher concentrations, heating of the milk, talcum, caolin, and mucilaginous substances retard the rennet action. Shaking reduces the strength of the rennet if it is in solution.

The following data are taken from a work of Smeliansky in order to show the influence of various additions on the rennet coagulation of cow's milk.

It appears that:

1. Heating the milk results in retarding the action. The longer the heating lasts the softer and smaller are the flakes.

2. Addition of water likewise retards the action.

3. Mucilaginous substances retard the rennet action from taking place, and the flakes formed are soft and loose. Barley water especially influences its consistence while corn water principally alters the time of coagulation.

If boiled milk is diluted with equal parts of a mucilaginous infusion and water, the mucilaginous portions coagulate more quickly than the watery parts.

4. The addition of soda solution renders the flakes soft, and retards coagulation. Milk containing 0.5% of soda is entirely prevented from coagulating even after standing for 24 hours.

Four per cent of table salt renders the flakes softer. Potassium carbonate acts the same as soda while the other salts respond according to their alkalinity. 5. Milk of lime retards the action; chlorate of lime accelerates it. If boiled milk

5. Milk of lime retards the action; chlorate of lime accelerates it. If boiled milk for instance coagulates after  $6\frac{1}{2}$  hours, the time required for coagulation after the addition of Ca Cl<sub>2</sub> is only 8 to 15 minutes. It causes the flakes of raw milk to become loose and soft.

According to Smeliansky, the reaction indicates the character of the coagulation, and the time required for it. Sugars exert no influence.

On the other hand Reichel-Spiro have determined a slight retarding of coagulation in the presence of a high content of cane sugar.

Cooking the milk retards the process (lowering the acidity as a result of the loss of  $CO_2$  and precipitation of lime salts, Raudnitz). In overheated milk no coagulation or only poor coagulation takes place. The addition of water retards coagulation (Weitzel), likewise physiological salt solution or whey which is free of rennet (Reichel-Spiro). Hammarsten, Lorcher, Peters, Weitzel, Gerber and Raudnitz conducted experiments relative to the action of salts on coagulation, the results of which according to Raudnitz may be interpreted as follows: Rennet.

1. The chemical reaction of rennet is hastened by the distribution of the rennet and its quantitative relation to the casein, possibly also by elevated temperatures up to an unknown limit. Alkaline earths and acids probably act in a similar manner by activating the rennet.

2. The chemical reaction is retarded: (a) By the destruction of the rennet: temperatures over  $41^{\circ}$  C., free hydroxylions; (b) by inactivation of the same: anti-rennet; (c) by changes of the casein: temperatures over 80 deg.; formalin.

3. The physical reaction is hastened by higher temperatures, free hydrogenions, and the neutral salts up to a certain concentration, especially the salts of alkaline earths.

4. The physical reaction is retarded by reducing the concentration of the mentioned salts below a certain point, especially of the alkaline earths; therefore heating the milk and the salts which precipitate lime, and calciumions will produce this result. Higher concentrations of neutral salts have the same effect. It may also be possible that some of the alkaline action should be considered here.

It is known that by the injection of rennet into an animal an anti-rennet may be produced. The rennet acting as antigen induces in the body of the rabbit the formation of a specifically acting anti-body, which works against the action of the antigen in the re-agent glass, very likely through fixation. Normal serum also contains rennet-inhibiting substances.

The action of the rennet may be inhibited or entirely prevented by the addition of horse blood as has been proved by Hammarsten, and later by Roden. The same inhibition is exerted on the action of trypsin and pepsin and is referred to as an anti-ferment action of the blood serum. Blood of cattle added to cow's milk also shows this characteristic (Schern). Inhibition action is traced back to the anti-ferment substances of a specific nature contained in the blood, and the presence of an anti-rennet is considered probable. It should however be noted that Raudnitz and Jakoby prevented inhibition by neutralizing the serum with acid.

The strength of the rennet may be tested in various ways. That quantity of milk is measured which is coagulated by one part of rennet in 40 minutes at 35 deg. Market rennet has a strength of 1:10,000 to 1:100,000 (fluid rennet and solid rennet).

Meunier ascertains the quantity of milk which is coagulated by one c. c. of undiluted gastric juice in ten minutes. Schern employs solutions of rennet (standard rennet prepared according to Morgenroth) of varying density (1:100:200:300, etc). One part of these rennet dilutions is mixed with nine parts of milk, so that milk-rennet dilutions of 1:1000:2000:3000, etc. are obtained. After an action of two hours the samples are placed in the incubator. The dilutions in which coagulation may now be demonstrated give the relative value of the rennet for the respective milk, and if a mixed milk of healthy animals had been used it establishes the "rennet-titer."

It is to be regretted that the standard rennet solutions are not constant, and that they weaken by storing, etc. For this reason it is necessary to establish the rennet-titer before each test on the milk of healthy animals, or on casein solutions.

In addition to the rennet of calves, extracts and ferments from other organs of these animals act on milk in a similar manner, such as extracts of spleen, kidney, liver, lung, thymus, intestine, ovaries, testicles and muscles. Rennet from the stomach of a calf is known as chymosin; rennet from the stomach of a hog, and from the gastric juice of man as parachymosin (Bang). Rennet enzymes may also be demonstrated in the bodies of other animals, fish, birds and snails. Enzymes with the action of rennet have been found in various plants and parts of plants, such as the artichoke, branches of fig trees, candytuft (Iberis pinnata), yellow mustard (Isatis tinctoria), etc., also in bacteria (proteolytic) and in yeast.

The individual kinds of rennets vary considerably in their sensitiveness to various influences.

Whereas the rennet of calves is very susceptible to heat, and exerts its action readily in alkaline solutions, the parachymosin is less influenced by the harmful action of heat, but is greatly affected in its action by the presence of alkalies.

The rennet enzymes obtained from plants act in an optimal way at high temperatures (sykochymas at 65-70 deg. C. for raw, at 85 deg. C. for sterilized milk).

Aside from casein, milk contains proteids which are coagulable by heat.

(1) **Lactalbumin** which is related to the serum albumin but is not identical with it (it has a slight optical polarization:—36.4 to —38 against—60.1 to —62.6, Sebelien).

(2) Lacto-globulin may be precipitated with the aid of magnesium sulphate. It is contained in milk in quantities of about 0.1 per cent. of the total proteids. The lacto-albumin is obtained from the residual solution after saturation with magnesium sulphate and acidifying it, or by almost complete saturation with ammonium sulphate.

3. Lacto-mucin has been also demonstrated in milk by Storch, Siegfeld, Voltz and Rosengren, whereas other proteid substances such as albumose, peptone, albuminose, lacto-protein, gelatin, galactozymase and opalisin, are considered more recently as products of the preparation of other proteid bodies, at least so far as their appearance in ripe milk is concerned.

The proteids which remain in the fluid after precipitation with acid and boiling are collected under the term "lacto-protein."

The milk fat consists of a mixture of triglycerides, cholesterin, lecithin, and a coloring substance, and distinguishes itself considerably from the fat of the body and from the nutritive fat by its chemical and physical characteristics. Although the milk fats manifest considerable dependence upon the nutritive fat, as will be seen from the later chapters, nevertheless a transition of the nutritive fat into milk fat cannot be asserted. The same statement would also apply to the transition of body fat, although in this instance a closer relationship between the substances must be admitted.

It is possible that transitory relations exist, by means of which split up body fat may be converted in the milk gland into milk fat, and thus the nutritive fat takes part indirectly in the formation of milk fat after first having been deposited as body fat.

It should be considered however, that the specific activity of the cell builds up the fat from the constituents at hand, and utilizes whatever material is placed at its disposition, such as nutritive fat, when such is present, or body fat in emergencies. The product will approach in its properties the material which has been utilized, but will always remain peculiar to the species of animal producing it.

A formation of fat from proteid is possible, as may be seen when cows are fed with substances free of fat, and after the body fat deposits have been used up. It is probable that the carbohydrates of the food here take part in the formation of fat. The fat which is contained in milk in the form of very fine globules, causes in part the white color of the milk through the reflection of light. The size of the fat globules varies in the milk of the same cow and depends upon the individual, length of the period of lactation, the race, feeding, and upon whether the first, middle or the last part of the milking is examined. According to Woll, D'Hunt, Schellenberger and Gutzeit the diameter varies between 0.8 and 22  $\mu$  with an average of 2.2:2.5:2.9:3.6  $\mu$ .

Variations in the percentage of fat are caused by change of food, etc. These changes also have an influence on the size of the fat globules, and according to Woll the fat globules become larger with dry feeding, a statement which could not however be confirmed by Schellenberger and Pankowsky. According to the investigations of these authors the feeding of green forage, especially clover, produces large-sized fat globules.

The length of the period of lactation should be considered since the variations of size at the beginning of lactation are more considerable than in ripe milk, in which the milk globules appear more uniform and mostly of medium size.

In colostrum they vary from the sizes of dust to  $20\mu$  and over. Donne and Schulz found that colostrum contains large, broad oil drops in addition to the small and minute fat globules, which show a less uniform appearance and contour, when compared with the usually spherical fat globules of ripe milk.

In interrupted milking the size of the milk globules bears a certain relation to the fat content. With the increased quantity of fat which obtain in the milk toward the end of a single milking, the size of the fat globules also become larger (Schellenberger, Woll).

With the extension of the lactation period the size of the fat globules decreases, but their number increases.

According to Gutzeit and Schellenberger the following values were obtained in milk from different breeds:

	Size in 1/1000	mm. No	o. per cc. in millions.	
	Gutzeit:	Schellenberger:		
Voigtlander		2.73	1944 to 4476.9	
Jersey	3.5	2.95	2064.1 to 4643.3	
East Friesian		2.30	2521.0 to 5911.0	
Angus <sup>-</sup>	2.95	2.20	2886.0 to 6200.0	
Simmenthal		2.56	2995.0 to 5210.3	
Dessau			3070.0 to 6308.6	
Swiss		2.33	4008.0 to 5326.7	
Shorthorn				
Montavoner	2.62			
Holstein	2.58			
Breitenburger	2.46			

According to Grimmer the number of milk globules fluctuated in 21 tests on three herds of blackish-brown lowland cattle in Pomerania, from 1,330,000 to 3,073,000 per cubic millimeter, having an average diameter of  $2.6 \cdot 3.7 \mu$ .

The milk globules retain their form through their surface tension and are not surrounded by special capsules which could be considered as membranes, as has been thought by former authors. Although the milk globules cannot be entirely freed from proteids by washing (covering the milk with water and allowing the separation of fat), the demonstration of the remains of proteids cannot be considered as proof of an actual "haptogen membrane" which must be broken down during the butter-making process, in order to make possible the flowing together of the milk fat, but it does constitute a proof that remnants of proteids, even after the most careful washing of the cream, remain around the fat globules. At least it has never been possible to demonstrate membranes of the fat globules, neither in boiled milk, in which during continuous heating larger fat clumps develop, nor in fat extractions (Soxhlet, Quineke, Morres).

Milk sugar is also a specific substance of milk. It is formed in the gland and is found only in its secretion. If sucking is interrupted, it may be present in the urine, from which it immediately disappears upon amputation of the lactating gland, or it may not appear at all when the gland is amputated before the appearance of lactation (Sinet, Magnus-Levy, Zuntz). After the complete removal of the gland in goats and cows, however, a temporary hyperglycosemia and glycosuria appear. If parts of the gland remain, lactosuria results.

After the injection of glucose, lactose appears in the urine (Porcher), likewise after the ingestion of large quantities of dextrose. Since the blood in the mammary vein before parturition and during lactation contains considerably less glucose than the blood of the jugular vein (Kaufman and Lagne), it may be accepted that glucose has been utilized in the gland, and further that glucose is the material from the constituents of which the lactose is formed in the gland.

Of the various **salts** milk contains compounds of potassium, calcium, magnesium, iron, traces of manganese, aluminum, phosphoric acid, hydrochloric acid, carbonic acid, sulphuric acid, citric acid, fluorine and iodine.

Carbonic acid, oxygen and nitrogen have been demonstrated as gases in the milk.

Besides these substances, lecithin, cholesterine and coloring matter are present in the milk, besides ferments and substances which are collected as residual substances; these have been previously mentioned.

Raudnitz and Grimmer have recently published compiled articles relative to the individual constituents and chemical properties of milk which contain the collected material of many experimental results, and at the same time show how much is still unsettled in regard to the composition of milk and the characteristics of the substances which it contains.

Certain **physical characteristics** of milk correspond to its chemical condition. These adjust themselves according to the proportion of the various constituents, and to the conditions attending the mixing of the different component parts.

The **appearance** of the milk is influenced by the suspended casein and the proportion of fat. Skimmed milk, which is almost free from fat constitutes a non-transparent, somewhat bluish fluid, as compared with the whitish yellow color of whole milk. The ad-

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dition of alkalies to milk free of fat renders it transparent. Hammarsten furnished the proof that a calcium caseinate solution which corresponds to the composition of milk is almost as non-transparent as milk. The milk becomes less transparent the smaller the fat globules are. This is most strikingly apparent when the fat globules are broken up to dust-sized bodies (for instance through homogenization). The appearance of fresh milk is also influenced by the coloring matter present in the milk plasma and in the fat. It is known that the skimmed milk of certain cows varies considerably in color; at times it is bluish white, sometimes more yellowish green, again transparent, other times of a non-transparent whitish color, and also the fat has a more yellow color during the pasturing of the animals than at the time of stable feeding.

The non-transparency as mentioned above is no proof of the presence of fat in the milk; therefore all methods which are destined to establish the quantity of fat or addition of water by the establishment of the whiteness, are of no use, as for instance, Heeren's pioscope, Feser's lactoscope, etc.

If milk is allowed to stand for a time, **cream** forms on the surface; the fat globules rise and collect usually as a distinct layer of cream above the milk. The rapidity of the separation depends on the temperature, the size of the fat globules, and the density of the milk plasma. The quantity of the cream is not in parallel relation to the quantity of fat; it depends on the size of the fat globules.

The separation of cream may be hastened and increased by centrifugalization. During separation while allowing to stand, about 85% of the fat rises to the surface, while by a perfectly operating centrifuge the separation of cream may be accomplished up to 0.01% of its fat.

The **specific gravity** of the milk depends on the solid substances, the relation of the mixture and the condition of the suspended, dissolved, and emulsified constituents of the solid substances. Corresponding to the variable composition of cow's milk it is natural that the specific gravity of the milk should vary. It fluctuates considerably, varying from 1.027 to 1.034 at a temperature of 15 deg. Similar to the impossibility of speaking of milk of normal composition, one cannot speak of milk of normal specific gravity, and even to give average figures would be of very problematical value; but to take such average figures or even smallest values as a basis for the calculation of falsification would be a gross error. Milk from many cows would under ordinary conditions have a specific gravity of 1,029 to 1,033.

The specific gravity is measured, or is calculated from the values of fat contents and solids, according to formulas, which, depending on the milk from certain breeds, or certain localities, show slight variations. This formula made on the basis of the value of the specific gravity of the milk fat (about 0.93), and the solids or dry substances (1.6001), which is quite constant, is according to Fleischmann:

$$1000-3.75$$
 (d-1.2 f)

In these equations s stands for specific gravity, d for dry substances or solids, and f for fat.

The following values may also be calculated from the fat contents of the milk and its specific gravity.

1. Total solids:

$$d=1.2 \text{ f}+2.665 \times \frac{100 \text{ s}-100}{\text{s}}$$

2. The fat-free solids are shown by deducing the percentage of fat from the percentage of the total solids.

3. The specific gravity of the solids

$$\frac{s \times d}{s \times d}$$

 $= \frac{1}{s \times d - (100 \text{ s} - 100)}$ 

4. Finally the fat contents when the solids and specific gravity are known:

$$f=0.833 d=2.22 \frac{100 s=100}{s}$$

The values obtained from formulas are of course not absolutely correct, but represent the results only approximately with the analytical methods of weights, the fat-free dry substance of the milk is not of absolute constant composition, but varies, so that its specific gravity which is based upon the sugars, proteids and salts, varies more or less from the number which has been accepted by Fleischmann as the average value (1.60).

The equations hold only for cow's milk.

If milk is freshly drawn, and immediately tested it shows a considerably lower specific gravity (0.0008-0.0015), than after cooling. The milk "contracts" and becomes constant in its specific gravity only after standing for several hours. The cause of this manifestation is not yet entirely clear. Toyonaga aims to explain it by the fixing of previously uncooled and fluid fat globules, which is the most plausible explanation; other authors believe that the contraction is the result of a cessation of the expansion of the casein.

The density of the milk varies in accordance with the temperature. The maximum (for water at 4 deg.) lies almost near its freezing point, namely at 0.3 deg. C.

The freezing point of milk is somewhat lower, namely -0.54 to -0.57 deg. This is especially influenced by the presence of salt, less by the sugar contents of the individual samples of milk, and it is induced by the relatively constant amount of soluble salts in the salt contents, which is subject to only slight fluctuations in the milk of healthy animals.

For the sake of completeness the electrical **conductibility** of the milk should also be mentioned. This varies according to the resistance which is offered by the fluid to the current. It fluctuates within wider borders than the freezing point, and is influenced according to Zanger by general diseases, through local affections of the udder, by estrum, pregnancy, feeding, etc. The conductivity is diminished by the fat globules; therefore skim milk conducts better than whole milk or cream. The conductivity of the different quarters is inversely proportional to the quantity of milk, in milk from different quarters of one cow (Schnorf).

The **viscosity** of milk is a factor which principally depends on the condition and on the quantity of the casein and the fat. Higher temperatures reduce the viscosity, likewise shaking; quiet standing increases it.

The surface tension of milk is lower than that of water (0.053 against 0.075).

Of the physical properties the specific gravity of milk and its serum, and the polarization of milk serum, are of special importance for the practical testing of milk (see technique). For practical results, however, the determination of the fat contents is also necessary.

As it has been shown the total solids may be determined by the aid of the fat contents and the specific gravity and the fat-free solids may be established by deducting the percentage of fat, these four factors are generally sufficient for the preliminary tests. For more accurate study these preliminary tests are completed by the establishment of the specific gravity of the milk serum, or still better by the refractoscope to determine the chlorids of calcium serum, which renders more rapid work possible. This is a method whose satisfactory use in practice has been proven by the numerous works of Mai and Rothenfusser.

Publications relative to the **polarization of milk** were issued by Valentin in 1879, and later continued by Villiers and Bertault, Braun, Utz, Lam, Radulesku, Ripper, Schnorf and others, on rennet serum, acetic acid serum and milk serum, which had been prepared by voluntary coagulation.

The given values of the authors varied in accordance with the method of preparation of the serum; nevertheless it could be established that comparatively uniform figures were obtained whenever the work was carried out under similar experimental conditions. In 1908 Cornalba showed that contrary to the variance in the amount of colloidal substances dissolved or suspended in milk, the sum of the dissolved constituents of milk is very constant. Whereas in samples of mixed milk the sum of the first substances varied between 5 and 8.585 per cent. the differences for the total dissolved substances were only 6.05 to 6.25 per cent.

Milk serum which contains the dissolved substances, offers therefore constant results in the examinations, the same as the examinations which lead to the establishment of the fat-free solids, which still include the casein. Examinations of serum are therefore of the highest practical value for the demonstration or establishment of the addition of water, provided that the serum is always prepared in the same way. Ackermann, Mai and Rothenfusser have in their fundamental works, determined the practical importance of the polarization of the proteid-free serum, and have proved that with the polarization of the chloride of calcium serum we possess means which are better adapted than any other to the detection of the adulteration of milk by water. Refraction is the most valuable accessory to the various methods of tests of recent times.

Ackermann found in 2,800 samples of normal milk, variations in the scale division of Zeiss's immersion refractometer, from 38.5 to 40.5.

Even slight additions of water reduce the refraction considerably; the addition of 5% of water results in a 1.3 lowering of the scale division, while 10% lowers it 2.3.

According to Mai and Rothenfusser the original refraction of 39 scale divisions is lowered to a refraction of :

37.9	with	about	4%	addition	of	water
37.7	66	" "	5%	66	66	66
37.5	"	6 6	6%	6 6	"	"
37.3	"	66	7%	6 6	"	"
37.1	"	66	8%	"	"	66
36.9	"	٤ ٤	9%	"	"	"
36.7	"	" "	10%	"	66	" "
36.5	"	. 6. 6	11%	" "	"	"
36.3	"	"	12%	" "	"	"
36.1	"	66	13%	6 6	"	66
35.9	"	"	14%	6 6	66	"
35.7	"	66	15%	<i>4 4</i>	"	66
35.5	"	66	16%	6 6	"	66
35.3	" "	"	17%	66	6.6	66
35.1	" "	"	18%	66	"	66
35.0	"	"	19%	66	"	66
34.8	" "	66	20%	6 6	"	"
34.0	" "	"	25%	66	66	66
33.3	"	6.6	30%	" "	"	"
32.6	"	66	35%	66	"	"
32.	"	66	40%	66	"	66
30.9	" "	"	50%	6 6	"	"

In the establishment of the refraction index of the chloride of calcium serum it was also discovered that it is impossible to establish normal values for the chloride of calcium serum, as well as for other constituents of milk. Mai and Rothenfusser also established the general rule for milk, that only in the presence of rigorous controls of the same origin can the addition of water be satisfactorily determined, and the extent of the adulteration established. The experiments of Weigner and Yakuwa are of interest since they demonstrate that the refraction and specific gravity of the chloride of calcium serum are theoretically of equal value. Mai and Rothenfusser, on the other hand, emphasize the fact that of two theoretical methods of equal value the man in practice has to prefer the method which offers, with the same certainty of the results, greater advantages in regard to rapidity, convenience, and saving of material, advantages which the method of refraction possesses.

The investigations of Mai and Rothenfusser prove that the variations in the results of continued tests, from day to day may reach in mixed milk of one stable 0.1 to 0.55, and in longer periods (22 days), up to 1.0. Changes of feeding have no marked influence. The milk of individual cows failed to show any important fluctuation during the time in which the tests of the entire stable were made (0.2 to 0.6).

More considerable may be the fluctuation between the findings of normal milk and the secretion from cows with an affected udder, and the variation between the findings of milk from the same animal while healthy, and within 24 hours after the udder becomes diseased.

The milk of individual animals with affected udders shows, not infrequently, values which are considerably below the values of normal milk. This has been proved by the work of Metzger, Fuchs, Jesser and Henkel, and from the experience of the official milk control station.

These abnormal values, however, do not affect the worth of this method, if the results are compared through the use of satisfactory control tests, and confirmed by other methods.

# Ferments in Milk. Immune Bodies. Milk as Antigen.

For the testing of milk special characteristics which it possesses, which may be collected under the name of reaction manifestation of ferment action, and for which at present there is still no satisfactory explanation, are of importance.

Under the term ferments (enzymes) those substances are included which hasten chemical changes with an explosion-like rapidity (Uexkuell), and without using themselves up they act in relatively minimal quantities. Their activity is inhibited by the products of the reaction. Higher degress of heat and certain toxins (ferment toxins, as for instance hydrocyanic acid) inhibit their activity, the ferments being thermolabile. The author designates as ferments all of those bodies with ferment-like action, without consideration as to whether the nature of the ferment is known or not.

A careful distinction must be made between original fermen-

tative action and ferment-like bacterial activity sometimes taking place in milk.

The original "ferments," the nature of which is disputed, originate from the blood, or are formed from the cells of the blood and the parenchyma of the udder. They are either eliminated the same as products of metabolism into the surrounding parts, or they are anchored to the cell and are only set free in the breaking up of the cell (ecto- and endo-ferments). The ferments in their action are destined to certain substances to which they fit, "as the key fits the lock" (Fischer). They act either through hydrolytic splitting, through oxidation, or through reduction.

Those ferments are of importance to the milk inspector, where diminished or increased presence or complete absence offers certain conclusions as to various conditions in the udder or in the milk. These are the amylase (diastase), the indirect oxydase (peroxydase), the superoxydase (catalase) and the indirect reductase (aldehydreductase, ''aldehydcatalase'').

Besides those mentioned, milk also contains other bodies which are included among the ferments; for practical milk examinations, however, they have little or no bearing. Mention need only be made here of the proteolytic ferment, "Galactase," found by Babcock and Russell and bodies acting like pepsin or trypsin (Jensen, Freudenreich, Spolverini and others). These are only present in very small amounts. Kinase and fibrin ferment have also been demonstrated in milk.

The proteolysis could be explained through the presence of leucocytes in the milk. Similar to the proteolytic ferments which cannot be utilized for diagnostic purposes, the lipase and the salol-splitting salolase (the existence of which as a ferment is disputed by Desmouliere, Miele and Willen; the alkaline reaction of various kinds of milk is sufficient to split up the salol) can not be likewise utilized for the purpose of diagnosis. Rullmann in 1910 proved by the examination of aseptically drawn milk, that salolase is not an original ferment; the author considers the splitting of the salol to be the result of bacterial action.

Of the ferments in milk which split up the carbo-hydrates, the amylase (diastase, galactoenzyme), whose action is similar to the ptyalin of saliva splitting up the polysaccharid starch into dextrose and maltose, is of the greatest interest (Moro). This ferment was first found by Bechamp in the milk of women, later by Zaitscheck, Koning, Seligmann and others in cow's milk. One hundred c. c. of mixed milk can be split up by 0.015 to 0.020 gm. of amylase. Amylase is destroyed by heating for 30 minutes at 68 deg. C. (Koning); the optimum of its activity lies at 45 deg. C.

The substances designated as oxydase and peroxydase exert a special action. They transmit the oxidation either by "activation of the oxygen of the air," (direct oxidation) or by abstracting the active oxygen, for instance from peroxide of hydrogen (peroxydase). Substances acting as reagents indicate their oxidation by the formation of coloring matter.

The occurrence of direct oxydase in milk, the action of which appears even without peroxide of hydrogen, is uncertain. Rullman has found traces of direct oxidation in milk drawn under sterile conditions; the quantity however is almost nil for practical purposes.

The indirect oxydase acts only after the addition of hydrogen peroxide or other oxygen carriers (for instance super-borates),

### Catalase.

by abstracting active oxygen after the formula  $H_2O_2=H_2O+O$ (Jensen). The active oxygen oxidizes the added "chromogenic" substances, as guaiacol, ursol, paraphenylendiamin, etc., to coloring matter. The peroxydase is injured by long heating, even at the relatively lower temperature (50–60–70 deg. C.), and is destroyed at about 75 deg., so that boiled or pasteurized milk may be distinguished from raw milk by the non-appearance of the color reaction.

The action of the superoxydase (Raudnitz) or **catalase** (Loew) develops in a different way. It splits the  $H_2O_2$  according to the formula  $2 H_2O_2 = 2 H_2O + 2O$ , which join to a molecule of  $O_2$ . Other authors include the superoxydase with the oxidizing ferments, as the freed oxygen is utilized in the body for the oxidation (Seligmann). According to others it is included with reductase, as the action of the ferments on  $H_2O_2$  equals a reduction of  $2 H_2O$ , and molecular oxygen  $O_2$  which passes out without being utilized for oxidation, whereas the oxygen freed by peroxydase is immediately utilized for further oxidation changes; therefore the peroxydase is an oxidyzing, while the catalase is a reducing ferment (Grimmer).

Original catalase has been demonstrated in the milk of all animals; it originates in the cells of the milk gland, especially from the leucocytes. It is secreted, but may be set free in the breaking down of cells or may appear bound to the cell. That catalase is derived from the cells (especially leucocytes) is not contradicted by the fact that cream is richer in catalase than skim milk since leucocytes and other cells are also included in the separation of the cream. These conditions were indicated by Friedjung, Hecht and Pallazzi, and later confirmed by Koning. This also explains the reason for the centrifuge foam, rich in leucocytes, giving such a strong reaction. Since the formed elements (cells) are precipitated with the casein, and probably a part of the free ferment is also drawn down with it, milk serum is always poorer in catalase than the original milk.

Catalase passes through infusorial earth filters, but considerable quantities are retained. Light, storage, etc., affect catalase, even if it is relatively resistant. A leucocytic extract, which was kept exposed to the light in the laboratory of the author, showed even after months, an unweakened action to  $H_2O_2$ , while hydrogen sulphide, hydrocyanic acid, potassium cyanide, mercuric cyanide, barium nitrate, hydrochloric acid, sulphuric acid, acetic acid, oxalic acid, and potassium nitrate affected its action (Faitelowitz). It appears noteworthy that  $H_2O_2$  inhibits the ferment in its action. In the presence of excessive amounts of peroxide of hydrogen the ferment splits up less  $H_2O_2$  than if the diluted peroxide of hydrogen is gradually added.

Heating to 62-70° C. destroys the original catalase in a short time. The optimum temperature appears to be about 37 deg. C. An original "ferment," the nature of which is by no means definite, is Schardinger's formalin methylene blue reductase, which according to Trommsdorff, will be designated as Schardinger's ferment (synonyms are indirect reductase, aldehydcatalase, aldehydreductase). Fresh milk in a mixture of formalin and aqueous methylene blue solution (Schardinger's re-agent), is decolorized inside of a few minutes. Smidt explains the action of Schardinger's ferment by the fact that the formalin changes into formic acid and thereby reduces the methylene blue. The character of its action however is not yet solved. The Schardinger ferment exerts its best action, at 65 to 70 deg. C., it is destroyed above 70 deg. As has already been indicated by Smidt and confirmed by Trommsdorff, Schardinger's ferment is very sensitive. It is injured by small excesses of formalin, and by relatively larger quantities it is destroyed.

Römer and Sames established more recently, the interesting fact that boiled milk with 0.3 c. c. of a 1% of ferrosulphate solution also gives the reaction, and this disappears again when the mixture is boiled for a half hour. The authors point to the care which must be taken in judging the so-called enzyme reaction, since it is possible, with the aid of simple chemical reagents, to produce similar effects to those obtained in the supposed enzymatic reaction.

Very little is known relative to the origin of the formalin reductase in milk. This ferment is not in every sample of milk, being frequently absent in milk from an animal whose off-spring is still sucking, and in animals which are just fresh in milk (Schern). It is absent when the time of milking is over-extended, and in stasis of the milk (Römer and Sames), and it does not decolorize, or only incompletely so, in the first part of the milking, better in the middle of the milking, and rapidly in the last portion of the milking. This also corresponds to the relative frequency of fat in milk but no one however has been able to establish a complete parallelism. The authors conclude from this that the same conditions under which the gland excretes especially large amounts of fat, cause the quantity of Schardinger's ferment to be likewise increased.

# Milk as Antigen and Carrier of Anti-Bodies.

Since the fundamental experiments of Ehrlich relative to the formation of **immune substances** in the animal body, we possess an explanation for manifold manifestations between the inter-action of the disease-producing agent and the animal's power of protection, known as Ehrlich's theory of immunity.

The substances which are formed in the body in the combat against certain invaders are the anti-bodies; the harmful substances which are capable of stimulating the body to the formation of anti-bodies are the antigens.

Antigens may be substances of the most varied kinds; animal proteid, animal cells, plant cells, plant proteid, living and dead bacteria, bacterial substances, toxins, etc. The antigens are distinguished by groups, which make possible their combining with certain groups of the cell substances of the body. The "haptophore" groups of antigens under certain conditions fit as a key fits the lock, into the haptophore group of the "receptors," thus making possible the binding of the antigens to the cell. These terms were applied to these bodies by Ehrlich.

The simplest way of explaining the mechanism of the antigen action and the anti-body formation is by using toxin as an example.

A toxin is an antigen with a haptophore binding group, and a poison-producing group, the toxophores. If the toxin enters the body of an animal it may find groups on the cell to which it fits, the so-called receptors, which bind its haptophore group. If this has been the case the toxophore group exerts its action, the effect of the toxin becomes noticeable and the animal suffers as a result of the toxin. If there are no receptors present for the specific toxin it is impossible for the toxin group to exert its action, and the animal is therefore resistant against this respective toxin.

It is possible that as a result of the receptors of the cell combining with the toxin, the cell molecule is destroyed. But if the damage is not too serious, the protoplasm is stimulated to produce numerous receptors,—an over-production in fact. As not all of these are necessary for the performance of the cell function, the superfluous ones are rapidly thrown off into the body fluids. If such free receptors combine with the haptophore groups of the toxin, the latter is no longer able to combine with the protoplasm of the cell. These free receptors therefore protect the body against renewed action of the toxin, that is they act as antitoxins, and constitute the antitoxic part of the serum.

Besides the antitoxins, the action of which lies principally in the neutralization of the binding group of the toxin (anti-bodies of the first order), there are still more complicated receptors, for instance those which possess an active or ferment-producing group; they are anti-bodies of the second order. Finally there are anti-bodies of the third order, which are unable to act by themselves, but must utilize a third body in order to exert an action on the antigen.

Immune bodies of the third order become complete in their action only through the utilization of the complement. These immune bodies of the third order possesss therefore a binding group for anchoring the antigen, and a binding group for the complement. They are amboceptors, in contradistinction to the uniceptors of the first and second order.

Some anti-bodies resist heating for a half hour at 56 deg. C.; they are thermostabile, as for instance the antitoxins, the agglutinins, the amboceptors, while others, as for instance the complement, are destroyed at this temperature, as they are thermo-labile.

If, for instance, hemolytic anti-bodies are produced in a rabbit by treating the rabbit with red-blood corpuscles of another animal, then the hemolytic rabbit serum loses its action by heating to 56 deg. C.

The red blood corpuseles however are again dissolved when to the heated, "inactivated" rabbit serum, guinea-pig serum containing complement is added. Therefore, whereas neither the amboceptor in itself, nor the complement in itself can dissolve blood corpuseles, the combination of the two is capable of doing it.

The action of the anti-body is specific for the substance which induced its formation, on homologous antigen. Diphtheria antitoxin acts only on the toxins of the diphtheria bacillus, and not on the toxins of the tetanus bacillus. The specificity is a very high one, nevertheless it is only relative; that is, a similar, although somewhat weaker action is exerted on related antigen, as compared with the specific antigen.

The chemical structure of the anti-bodies is unknown, and they are generally designated according to the action which the g exert in the animal body.

Anti-toxins neutralize toxins, agglutinins agglutinate (stick together) animal cells and bacteria, and drag them to the bottom, precipitins and coagulins produce precipitation in antigen solutions, hemolysins dissolve erythrocytes, bacteriolysins dissolve bacteria, cytolysins dissolve animal cells, etc.

If anti-bodies are produced by injecting antigens into an animal, then the animal is actively immunized against the antigen. On the other hand, if another animal is injected with the produced anti-bodies it is given a passive immunity. The active immunity lasts for a long time, the passive immunity does not last beyond several weeks.

If anti-bodies are present in the blood in certain quantities they are excreted by the milk gland, and may be demonstrated in the milk.

Ehrlich succeeded in proving the passing of anti-toxins into the milk of anti-toxic immune mothers, by showing that young mice from non-immune mothers acquired a high degree of resistance against the toxin when they were allowed to suck activelyimmunized mothers.

The passing of anti-bodies into the milk even in the presence of passive immunity was proven by Ehrlich, Schmidt and Pflanz, although the passage was only slight. The action of such milk was 15 to 20 times weaker than that of the blood.

Similar to the action of the anti-bodies of the first order are those of the uniceptors of the second order; for instance, bacterial agglutinins and precipitins. The passage of agglutinins from the blood into the milk has been demonstrated by Kraus, in goats which had been immunized against colon-bacilli, typhoid and cholera. The later works of Bensaude, Bertarelli, Bamberg and Brügsch, de Blasi, Courmont, Cade, Figari, Maragliano, Rodella, Stäubli and others confirm the findings of Kraus. The agglutination value of milk, as compared with blood, may be lower or identical, or it may even be greater than that of the blood. As it has been found that bacterial agglutinins may pass into the milk, so it also has been proved that agglutinins against animal cells may do likewise. That under certain conditions amboceptors, as immune bodies of the third order, may pass into the milk, is proved by Bertarelli's experiment on a sheep treated with the red blood corpuscles of a chicken. The specific hemolytic amboceptor which resulted could be demonstrated in the milk. Therefore although amboceptors may pass into the milk and although normal milk contains nonspecific hemolytic amboceptors in small amounts, nevertheless the passing of hemolysins into the milk is very uncertain. According to Kraus, Kopf and others hemolysins do not occur in milk; likewise bacteriolysins are absent, or their presence is very doubtful, according to the investigations of Bab. Of course one of the hemolytic factors, the amboceptors, might be present in the blood, while the complement under the special conditions present in milk, may be inactive.

While Pfaundler and Moro state that hemolytic and bactericidal complement may be found in cows' milk, Bauer and Kopf, and Bauer and Sassenhagen, on the other hand showed that in normal, ripe milk complements are not present; that is, even by special examinations only traces could be established.

On the contrary in samples of colostral milk, and milk from udders affected with mastitis, both amboceptor and complement may be demonstrated.

The complement content of milk drops with the duration of time which has elapsed between parturition and the taking of the sample, until from the sixth to the twenty-seventh day after calving the amount of the complement disappears.

This observation may possibly be of great practical value in ascertaining whether or not a cow is fresh in milk. Mastitis milk, which bears a close relation to colostral milk, showed a relative richness in amboceptor and in complement, thereby making it possible to establish the affection of the udder by the demonstration of the complement. Of course it is not certain that the complement occurs early enough to enable this method to be utilized more readily than for instance the Trommsdorff test, the catalase test, or microscopic examination of the centrifuged sediment, and others.

Sassenhagen found in one case that the presence of mastitis could be determined by complement-fixation 18 days before the first clinical appearance of the disease, even when the quantity of sediment, after the Trommsdorff reaction was insufficient to afford a basis for a diagnosis of mastitis.

Bauer further proved that complement inhibiting substances are present in milk; Hausmann and Pascucci traced this inhibition of hemolysis to the presence of lecithin or cholesterin in the milk.

According to Kopf the complement passes from the colostral milk into the blood of the calf; it may be demonstrated in the serum of the calf from the third to the fourth day, before which time the blood cells of guinea pigs were not dissolved. As proved by Kraus bacteriolytic immune bodies also pass into the milk, in artificially immunized animals, and into the body of the suckling consuming the milk, provided the mother possesses active immunity (de Blasi).

Of other immune bodies which are present in the blood and have been demonstrated also in the milk of the same animal, should be mentioned the opsonins (Wright), which influence the bacterins in such a way that they may be readily assimilated by the phagocytes (Turton and Appleton, Eisler and Sohma).

Other substances which induce the so-called hypersensitiveness (anaphylaxis), have also been demonstrated (Otto). At least it has been proven in the study of hypersensitiveness, that the off-spring of hypersensitized guinea pigs possess an increased sensitiveness for homologous antigens, and this may not only be the result of the intra-uterine transmission of the anaphylaxis from the mother to the young, but also of the transmission of the immune bodies, through the milk of the mother.

To the subject of immune substances belong possibly the observations made by Tage, Duhat and Dobrowits, during the treatment of nursing syphilitic mothers with salvarsan which shows its effect upon the untreated syphilitic children. Syphilitic children thrive splendidly after the treatment of their mother. Tt was impossible to demonstrate arsenic in the milk, either in organic or non-organic combination. Ehrlich explains the action by the fact that a rapid breaking down of the syphilitic spirochaetes in the mother is produced through the action of the new syphilitic remedy, and thereby an elimination of the endo-toxins is induced. The antitoxins which develop in the mothers pass into the milk, and cause a passive immunization of the child, through the gastro-intestinal tract; Jesionek, on the other hand claims the passage of the arsenic from the blood of the treated mother to the milk, and explains thereby the remarkable results in untreated children which are nursed by the treated mothers.

Very little is known with certainty relative to the quantitative relation which exists between the immune bodies appearing in the circluating blood, and those in the milk. The views expressed are too widely divergent. It is known of the anti-toxins in which this relation has been mostly studied, that of 15 to 30 parts of the anti-bodies which are demonstrable in the blood a certain amount appears in the milk.

These relations are still somewhat vague, since the passing of the anti-bodies which are bound to the albumins and globulins depends on the quantitative relation of these proteids in the milk, and the experimental results therefore must vary in accordance with the species of animal used, the stage of lactation of the respective individual, diseases of the udder, etc.

In infections of the udder, for instance with colon bacilli, anti-

#### Agressins.

bodies accumulate in the glands so that as a result the milk serum agglutinates more readily than the blood serum.

Not only anti-toxins and other protective immune bodies pass into the milk, but substances also which inhibit the protective power of the body, for instance aggressins, at least so long as the body has not formed anti-aggressins. The aggressins for instance act against the dissolving of bacteria. Schenk demonstrated antistaphylolysins and anti-vibriolysins in the milk of goats, cows and women.

Otherwise the passage of toxic substances of the character of antigen, which are closely allied to proteids, could be just as plausible as the passage of the constituents of the blood which are indispensable in the composition of the milk. The passage of toxins into the milk has not yet been satisfactorily proven for all toxins.

A large number of known substances from animal and plant life are known as toxins, that is, bodies which do not act like chemical poisons, but exert their toxic action only after a period of incubation, in which time fixation takes place.

These toxins do not affect all animals in a similar degree, but only those which are susceptible. Certain species of animals are not susceptible to certain toxins; they are immune. This immunity may also be artifically established in susceptible animals. The toxin is an antigen, and under certain conditions it produces an anti-toxin contrary to the toxins which act purely chemically.

Among toxins acting in this manner may be mentioned the products of metabolism of the *Bacillus diphtheriæ*, the *Bacillus tetani*, the bacillus of certain forms of meat poisoning—the *Bacillus botulinus* and the *Bacillus pyocyaneus*, the bacillus of blackleg, and the body substances of certain bacteria (endo-toxins). They may be of animal origin: snake toxins, spider toxins, scorpion toxins, turtle toxins, toxin of the blood of eels, salamander toxins, wasp toxins, or of plant origin, such as the abrin, robin, krotin, ricin, etc.

If it is considered that the gastro-intestinal tract of very young individuals is readily penetrable for proteids, although proteids of unlike origin pass with greater difficulty than those of like origin, the question as to whether the milk of the mother may contain toxins when toxins are circulating in her blood, assumes practical importance. This becomes, however, unimportant when it is considered that even in severely affected individuals only very small quantities of toxins are circulating free in the blood. Should a part of these minute amounts be secreted in the milk, this quantity itself is of only little practical importance even when the great susceptibility of the intestines of the suckling is considered.

It is true that Miessner succeeded in proving that mice die from tetanus when they are fed with raw milk from a cow affected with tetanus, whereas the feeding of meat has no influence on the health of the animal; this proves the passage of the tetanus toxin into the milk.

Older animals do not become affected even after the administration *per os* of large doses of toxins, at least not from diphtheria or tetanus toxins, and the *Bacillus botulinus*, the toxins of which are absorbed by the stomach but the bacillus does not thrive in the body; therefore the possibility of secreting these toxins through the milk gland is from the first of small importance, especially since in severely affected animals the secretion ceases.

From a practical consideration of the question of toxin elimination, the plant toxins come principally into consideration, especially ricin, as food adulterations to a great extent take place with ricinus seed and its flower. An elimination of ricin with the milk, however, has not been observed up to the present time. Ehrlich was unable to observe an elimination of ricin in mice which were under the action of ricin; the offspring of these mice were not actively immunized against ricin but acquired only a passive immunity of short duration.

Of more importance however are the bacterial toxins, and products of decomposition acting like toxins, which subsequently develop in the milk after certain fermentation processes.

The above-mentioned immune substances are probably of great importance for the nourishment of the young and the sucklings. The passage of genuine proteids in very young individuals with injured mucous membranes, is an established fact, and with the globulins anti-bodies also pass into the blood of the young, while in older individuals the relatively labile anti-bodies are changed or destroyed by the splitting up of the proteids.

The absorption of anti-toxins through the intestines of the young has been proved by the classical experiments on sucklings by Ehrlich.

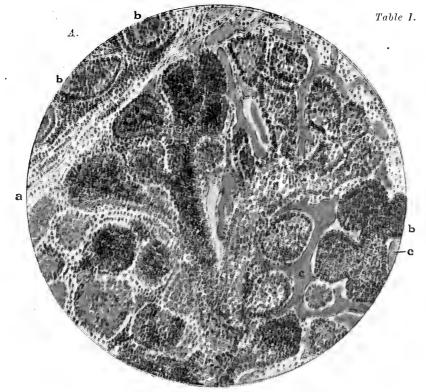
Other works by Brieger, Ehrlich, Salge and Römer prove that certain immune substances of milk of like origin pass through the intestines, while in feeding sera or anti-substances of like origin contained in milk of unlike origin the quantity passed was only very slight. Thus Römer succeeded in demonstrating passive immunity in foals after feeding them with anti-toxin milk of like origin, but was unsuccessful after feeding anti-toxic sera of like origin.

In calves of course the results were positive even when the anti-toxin was mixed with the milk as a serum of unlike origin, but the quantity of immune bodies of unlike origin absorbed was smaller than that of like origin. The absorption diminishes with the increase of the age of the animal.

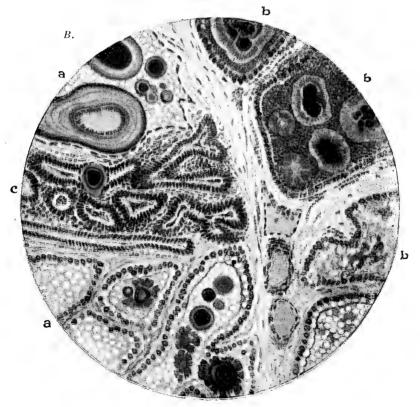
Relative to the passage of other immune substances from the milk into the blood of the suckling, the same experiences hold as a rule as in the case of milk containing anti-toxin.

Milk is not only a carrier of anti-bodies, and possibly of anti-

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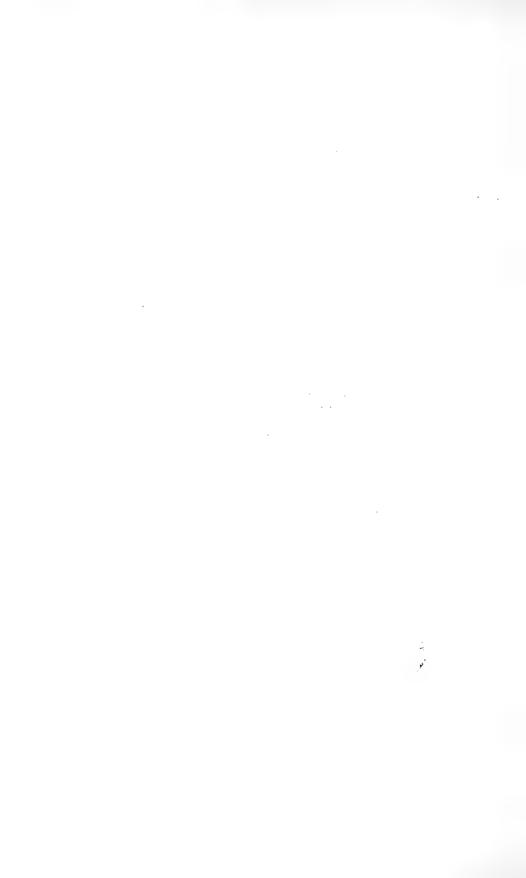


Acute purulent fibrinous mastitis. Dilation of the blood vessels (c); with exudate of numerous cells into the alveoli and excretory duct. Hematoxylin—Sudan III.



Concrement formation in milk stasis (a), and in mastitis (b); inactive portion of the gland (c).

Ernst, Milk Hygiene.



### Precipitin.

gen, but as a proteid-containing material it is an **antigen** in itself, or rather a collection of antigens, which may again produce antibodies in the body of an animal. These anti-bodies against milk not only develop in artificial administration by injections, but also under certain conditions during the natural ingestion of the milk *per os* as a food. Although usually such anti-bodies against nutritive proteids of unlike origin appear only in intensive over-feeding of proteids (Ascoli, Michaelis and Oppenheimer, Uhlenhuth, and others), nevertheless in the presence of an injured intestinal mucous membrane the absorption of proteids of unlike origin may, under natural conditions, take place, and thereby induce the formation of anti-substances.

Moro succeeded in finding cow milk precipitin in two instances, and milk proteid in one instance in an examination of 22 anemic bottle-fed children.

Bauer found precipitating substances of cow's milk in the blood of an emaciated man.

Kentzler, with the aid of the precipitation test, demonstrated milk proteids in the blood of six human subjects in which the gastric secretion was disturbed, out of 61 cases that he examined two to three hours after feeding.

Although milk is absorbed through the intestines of older individuals only after the splitting up of the proteids, nevertheless in case of an injured mucous membrane, or in greatly emaciated and in very young individuals the direct absorption of unchanged proteids is possible. Ganghofner and Langer succeeded in proving this on very young rabbits, on pigs and on newly born cats, and they succeeded also in demonstrating a precipitin formation in the blood. Schkarin describes similar results after the feeding of cow's milk to young rabbits.

## Lactoserum.

It will be advisable and appropriate to include at this place a subject which as a matter of fact belongs to the chapter dealing with the characteristics of the milk of various species of animals. Milk is an antigen and contains various antigens. After injecting the milk of species A into an individual of species B, the formation of various anti-bodies, precipitins, amboceptors, etc., may be observed in the blood serum of the treated individual, which gives to the blood serum the specific characteristics of lactoserum. This specific characteristic is shown by the fact that the cow lactoserum of rabbits produces a **precipitation** only when cow's milk is used for the precipitation, but not with milk of women or goats. Works of Bordet, Fish, Morgenroth, Wassermann and Schütze show the specific action of lactosera. With the aid of such sera the possibility is afforded of differentiating the milk from various species of animals. This however does not end the degree of the specificity, as it is possible with the aid of the precipitation method to differentiate various kinds of proteids of one and the same milk. If the soluble proteid bodies are separated from the undissolved easein by filtration (Schlossmann), then the rabbits which are treated with soluble proteid bodies furnish sera which react only to milk albumin and globulin (Hamburger).

It is of further interest that cow-casein sera gave precipitation with cattle blood (Hamburger), the same as is the case with lactosera (Landsteiner, Halban, Dungern, F. Meyer, L. Aschoff).

Moreover lactosera immobilizes spermatozoa of bulls, and dissolves red blood corpuscles of cattle. However no reaction results from the addition of cattle blood serum containing antibodies to cow milk (Meyer). The anti-serum sensitized against cattle blood only gives slight precipitation when it possesses especially high value (Uhlenhuth and Schütze). The same conditions were found in preparations of human blood, and women's milk, by Halban and Landsteiner.

Uhlenhuth and Schütze proved that the differentiation of various kinds of milk shows that the biological method succeeds even when the milk is heated to a high temperature (114 deg. C. in an autoclave); if the milk antigen was heated 20 minutes at 120 deg. C., lactosera resulted, which only contained coagulins, but no hemolysins. Sion and Laptes showed that the most varied splitting and decomposing changes of cheese-making and cheese-ripening do not influence the antigen to such an extent that the kind of milk used in making the cheese could not be determined by the biological method. This specificity is of course manifested even in the use of lactosera, but is not absolute, only relative. Lactosera also gave a reaction with the milk of closely related animals, the same as has been established for blood sera, meat sera, etc. Thus for instance it is impossible to differentiate sheep's milk from goat's milk (Uhlenhuth, Moro, Gengou), although it is possible to draw conclusions from the comparison of the intensity of the reaction in the homologous milk.

Other authors, as for instance Bauer, succeeded in demonstrating by the so-called complement-fixation method, the presence of cow's milk in woman's milk, even when only 1 c. c. of the former had been added to 1000 c. c. of the latter.

If specific serum which has been heated for a half hour at 57 deg. C. is mixed with milk and as much complement is added as is necessary for the dissolving of the subsequently added blood corpuscle suspension, with the aid of certain quantities of hemolytic amboceptors, then the amboceptors of the lactoserum bind the complement, provided they find in the milk the specific antigen (cow lactoserum-cow milk), and the subsequently added hemolytic system, free of complement, no longer finds complement, so hemolysis does not occur, but instead fixation of complement results. If there is no specific antigen present (if the milk to be examined contains no cow milk), the complement remains free to be utilized later by the hemolytic amboceptors and the blood cells, for the

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functionating hemolytic system, and a solution of the blood—hemolysis—results.

The appearance of "anaphylaxia" may also be produced experimentally with milk (Arthus and Besredka). It may be brought on by raw as well as by boiled milk.

The phenomenon of hypersensitiveness as is known, results when a proteid of unlike origin is injected into an animal and later after a period of time the same proteid is re-injected. At the second injection (or only after later ones, depending on the experimental animal and the quantity of proteid), the experimental animal reacts violently with indications of extreme sickness (Von Behring, Richet and Arthus), which may even result in death from convulsions and pulmonary edema.

Miessner succeeded in producing a hypersensitiveness against homologous kinds of milk in guinea pigs, sometimes after one injection, but more markedly after repeated subcutaneous injections, and with the greatest certainty after intra-abdominal injections of small quantities of raw milk. The best reaction was obtained in the animals after three intra-abdominal injections of .5 c. c. of milk, on three successive days. After the preparation of the animal 40 to 50 days should elapse before the test which is made by intra-cardial injections.

After repeated injections it is possible to demonstrate an anaphylaxis in most cases even with boiled milk.

In order to utilize the biological test for milk differentiation several rabbits should be prepared. This is carried out by intravenous injections of small quantities (5 or more c. c.) of milk heated for a long time to 65 deg. C. The injection is repeated 5 to 8 times at intervals of 1 to 4 days. In from 14 to 20 days after the last injection the lactoserum may be tested for its effectiveness and if found suitable, may then be drawn. For this purpose the animal is kept without food for one-half day, (in order not to obtain a cloudy serum), a venous hyperemia of the ear is produced by intensive lighting of the ear with the aid of an electric globe, or by rubbing it with xylol, and the vein is then punctured with a fine hypodermic needle. Several cubic centimeters of blood are drawn, which is allowed to coagulate; the blood clot is separated and allowed to stand for 24 hours in an ice chest. The test is made as follows:

1. Establish the dilutions of the serum which are capable of producing a visible precipitation in 3 c. c. of milk dilution, with 1:60 physiological salt solution, or

2. Establish the dilution of milk with 1:10 physiological salt solution, in which when mixed in the relation of 1:6, the lactoserum still produces a precipitation.

The most active lactoserum is the best adapted for use.

The rabbit is bled to death (slight anesthesia-opening of the thorax-puncturing of the heart), the blood for the collection of the serum is allowed to stand, the serum is drawn off in quantities of 2 to 5 c. c. into small vials, and placed in an ice box for safekeeping.

# CHAPTER V.

## PROCUREMENT OF COW'S MILK.

As has already been mentioned milk secretion may be retained for a long time by proper emptying of the gland and by the stimulation exerted on the gland in the process of emptying. In the presence of incomplete milking, in over-extending the time of milking, and in stasis of the milk, a condition of the gland results, which finally passes into a state of inactivity, when the stimulating condition which is exerted by the retention of the secretion on the secreting epithelia does not again appear with the act of a complete milking. The milk secretion therefore is largely dependent on the activity which is exerted on the gland from the outside, such as the sucking act of the calf, or artificial milking.

Artificial emptying is carried out in various ways:

1. By closing the upper portion of the teat with the aid of the thumb and index finger, and pressing out the contents of the cistern by gradual closing of the hand to a fist in such a way that first the middle finger, then the ring finger, and finally the small finger presses the milk downward and from the opening of the teat. The open hand is passed up again, forcing the milk into the cistern from the upper part of the quarter, the thumb and index finger again squeeze the cistern at its base, and the procedure ends as before. This manipulation is known as "fisting" or full handed milking.

2. By stroking with the closed thumb and index finger from the base of the teat to its point the milk may also be pressed out ("stripping" or "tipping"). This method of milking requires much less strength than the full handed milking, but causes a lengthening of the teats, and is a painful operation for the animal, as it is frequently accompanied by injuries to the tissue, and tearing of the mucous membrane.

The full handed milking may be carried out by dry milking, while "stripping" succeeds only when the teat and hand are moistened (moist milking), since the necessary smoothness and slipperiness of the skin result only from moistening.

If the teat is not pressed with the extended thumb (a brace for the index finger), but the thumb is crooked and the teat is pressed and stringed with the bent index finger against the nail surface of the thumb and the knuckle of the joint, this is spoken of as "streak milking" or stripping with bent thumb. The "streak milking" may be completed by stripping or by full handed milking. Full handed milking and this method combined with "streak milking" are according to Henkel permissible; the other kind of milking should be prohibited since the teats are too much extended.

The udder should be milked by the dry method since this method of milking is more cleanly than moist milking, in which the fingers become moistened by the milk, and although they slip easily, at the same time they wash off the dirt from the entire teat.

It is to be regretted that moist milking and stripping because of their labor saving advantages, are preferred by many milkers on account of their convenience, and even if they are urged to carry out the ordinary dry milking, as soon as they are left without supervision they will at once fall into the same fault.

The order of milking the various teats differs. Milking from the same side is supposed to induce the development of the side first milked, since the half of the udder first milked is worked with fresh strength while the subsequently milked quarters are not emptied as well on account of the beginning weariness of the milker and therefore they develop less perfectly. The hind quarters are either not emptied entirely when the milkers have completed the milking of the fore quarters, or else one hand of the milker rests while he finishes milking the hind quarter with the other. The same applies in milking the teats crosswise, when the hind quarter of one side of the udder is milked at the same time as the fore quarters together and the hind quarters together, and the milking should be undertaken first on those quarters which appear to be most distended.

With the drawing of the milk from the udder through milking the teats, the complete act of milking is not concluded, as the udder has not yet been sufficiently exhausted in its production. The cause of this may lie in the fact that the milk cannot be emptied by the simple sucking action from the smallest milk ducts and alveoli, or that after the apparent entire emptying, the milk production still goes on if the gland cells are properly stimulated in their functions.

As the flowing in of milk may be accomplished through the so-called "preparation," that is stroking or massage of the bases of the teats and quarters, the same result is possible through the so-called "clean milking," or "after milking," to obtain an additional quantity, which is especially rich in fat.

These methods vary and are practiced in different ways in different localities.

The best known method of "clean milking," and one which has been mostly studied, is that practiced by Hegelund, a Danish veterinarian. This method is divided into the following phases: 1. Milking through simultaneous full handed milking, first of the fore and then of the hind teats, until the milk flows no longer.

2. This milking is followed by the "clean milking," which consists in massaging the udder, beginning at the teat up to the base of the teat, and as high as possible extending on to the parenchyma. While the first act corresponds with the usual full handed milking, the second act massages with a milking motion, the base of the cistern, and the third is carried out by surrounding between the thumb and the hand, and stroking down the lower part of the quarter, that is, through simultaneous pressing against each other of both quarters of opposing sides.

3. The first manipulation of the after milking is carried out by pressing the right quarters of the udder against each other, the left hand being placed on the hind quarter and the right hand on the fore quarter. In case of a large udder, only one quarter is grasped at one time. The hands are then pressed upwards with a rubbing motion on the gland which exerts a massage on the parenchyma of the udder, this being repeated three times, followed by milking out the cistern. This manipulation is repeated until no more milk is obtained, when the left quarters are treated in a similar manner.

In the second manipulation the fore quarters are milked by placing one hand on the outside of the quarter and the other in the division between the two fore quarters. The hands are pressed against each other followed by milking of the teats. Then the hind quarters are milked by placing a hand on the outside of each quarter in such a way that the fingers are turned upwards and the thumb placed in front of the hind quarter. The hands grasp the quarter and are pressed upward; then they are lowered and the milking follows. This is also repeated until no more milk is obtained.

In the third manipulation the milker imitates the butting motions of a calf during sucking. The hands loosely surround the teats and the quarters are lifted and pushed against the abdominal wall so that the gland tissue is shaken. This lifting and pushing motion is repeated three times and the teats are then milked out. Following this procedure on the fore quarters the hind quarters are treated in a like manner, until no more milk is obtained.

The works of Aashamar, Alfonsus, Woll, van der Zande and Henkel, and Wenk, speak of the excellence of the method of Hegelund.

According to Henkel the increase of the milk yield in 37 Simmenthal cows was 217.4 gm. (3.4%) per milking on an average. Wenk succeeded in obtaining from 24 cows 4.5 kg. of milk per day more than with the ordinary method of milking.

Of course against the increase of yield must be placed the

additional work and time, which is an additional expense and considerably diminishes the profit derived from the increased yield of milk, and may even nullify it, since it involves the employment of additional help. The principal advantages of Hegelund's method lie in the fact that the milkers are held down to thorough work, and the milk glands are subjected to more correct and appropriate handling.

A modified form of Hegelund's method is the so-called "New Algäuer milking method," which combines the acts of the Algäuer method with those of Hegelund. The massage of each quarter is carried out with both hands. The method of Sondergaard aims to simplify the time-consuming work of "clean milking" inasmuch as the residual milk is obtained by a wide extensive hold of the halves of the udder at their bases, and pressing at the same time and stroking downwards. The principal factor in each method of milking is that the udder should be thoroughly emptied, and this can only take place when each part of the milk gland is stimulated by massage to the limit of its production.

#### CHAPTER VI.

### INTERNAL INFLUENCES ON THE CHARACTER OF MILK.

The influences which must be considered in the formation of milk may be separated into internal influences which lie in the individual characteristics and in the immediate condition of health of the animal, and in outside influences, such as stabling, feeding, etc., which again act only in that they influence the internal condition.

As internal influences may be considered the characteristics of the breeds, strains, family, individual, age, influences of the lactation period, pregnancy, and the general and local conditions of health. The outside influences may be considered under care and attendance, feed, medical treatment, climatic influences, methods of milking, etc.

Following this outline the internal influences on the formation of milk will be considered first.

#### Breed, Family, Heredity, Individual Characteristics.

# Age, Lactation and Other Special Conditions of the Individuals.

The influence of the breed on milk formation is generally known. There are beef breeds which fatten especially well, milk breeds in which milk production is especially preminent, and breeds which possess the ability to produce both milk and meat.

Breeds of low lands and their crosses produce more milk with lower percentage of fat than breeds from the highlands. According to the quantity of milk produced, the breeds are headed by the Holsteins, Angler, Oldenburger, East Friesian, Breitenburger, Wilstermarscher, Dithmarscher with 20 to 25 liters of milk per day per animal at the height of production, with percentages of fat from 2.5 to 3 to 3.4. Smaller quantities of milk are given by the grayish-brown mountain cattle, the Swiss and Algäuer, with 3.6 to 3.7% of fat, and the spotted mountain cattle, for instance Simmenthal, Misbacher and Pinzgauer, with 3.5 to 4% of fat. The fat content of the Westerwalder and of the Schlesian red cattle varies between 3.5 to 3.6%, while the English Shorthorns and Ayrshires give 3.7 and 3.8%; the richest in fat is the milk of the Vogelsberger and Harz cattle (3.9 to 4.2%) and that of the Jersey with 5 to 5.4% (Ramm). Reference should be made here to the following results of tests of production made with Simmenthal, Vogelsberger, Westerwaldern and Lahn breeds of cows.

Average weight of the	Simmenthal	Lahn	Vogelsberg	Westerwal	d
tested animals Average production Fat contents	2495 4 001%	$502 \\ 2650 \\ 4.001\%$	$427 \\ 1919 \\ 3.74\%$	395 1878	kg
Individual maximum production For 100 kg. body weight *The cow with maximal produc duced over 3000 kg. each.	$4562 \\ 431.1$	3955 528	3800 450	3234	kg

The fluctuation is not in fat content alone, but also in the other solid substances. Milk which is rich in fat as a rule contains more of the other solid constituents as well. The proportion of individual factors of the dry substances is variable; in cattle from the highlands for instance, the casein was 76.24% of the dry substance, in cattle of the lowlands it averaged 73.78% (Fischer).

Fat from the milk of the mountain breeds is generally under otherwise similar conditions, the richest in fatty acid of molecular weight; the fat globules of the breeds of the lowlands are smaller than the fat globules of the cattle from the highlands. Babcock mentions that Jersey and Guernsey cows produce larger and more uniform fat globules than the Holsteins, while Ayrshires have small, irregular fat globules in their milk. Milk with large fat globules is preferable for butter making, since these produce butter of good consistence and good taste with a low melting point.

The ash content of milk from highly bred animals is sometimes somewhat lower in CaO and  $P_2O_5$  than that of the common breeds, but the fluctuation is such that definite deductions cannot be established. In highly improved breeds Pages found: CaO in 0.143 to 0.227% and  $P_2O_5$  in 0.18 to 0.273%; in common breeds the same elements amount to 0.15 to 0.204, and 0.153 to 0.296, respectively.

These characteristics of breeds are general, but they are not so constant that individual **strains**, individual **families**, and especially particular animals may not present exceptions. This fact forms the basis of breeding for increased **milk production** within individual breeds.

Good milk cows should be bred to bulls, whose mothers and sisters were or are known to be good milkers, and then it may be expected that the good characteristics of the family will be inherited. In experiments extending over eight years Hogstrom tried to discover whether the characteristics of producing milk of certain fat contents could be transmitted by bulls to future generations. The large majority of female progeny produced a higher fat content than the milk of their mothers, which points to a positive influence from the male animal. In cases in which the fat content of the mother was 3.08 to 3.77% the fat content of the milk of the daughter increased materially; but as soon as the fat reached or exceeded 3.77%, the influence of the bull was no longer sufficient to further increase the percentage of fat, and the percentage remained lower than in the mother. (The experiences of Hogstrom's were confirmed by the rule laid down by Galton.) The great variation which occurs in the milk production of individuals of the same breeds depends on hereditary qualities. According to the statistics of the dairy control station at Algäu, animals of the Algäuer breed produce:

	Quantity of Milk	Fat Percentage	Quantity of Fat
Maximal production Minimal production			181.93 kg. 45.31 kg.
Difference	3946 kg.	2.11	136.62 kg.
Among 50 of the Jeverland bree Maximal production Minimal production	8699 kg.	$\begin{array}{c} 3.713 \\ 2.482 \end{array}$	286.76 kg. 75.21 kg.
Difference	6250 kg.	1.231	211.55 kg.

The production of single individuals during the lactation period is sometimes remarkably large. Some of the following data relative to production is taken from Kirchner's handbook.

Author	Cow	Quantity of	Milk	Percentage of Fat	Quanti of Fa	
Kirsten	Wesermarsch	11291	kg.	2.78 .	324	kg.
Kirsten	5th calving East Friesian	9047.75	kg.	3.07	277.77	kg.
Woll	6th calving Guernsey ''Yeksa Sunbean		kg.	5.745	388:8	kg.

Kirchner also quoted a case in which a farmer observed that a seven-year old cow in the second month after calving still produced 50 liters of milk per day.

Just as the quantity of milk and percentage of fat may vary, so also the fat-free solid substances may vary in the individuals, although only within narrow limits. The rule also holds good here that an individual with milk rich in fat will at the same time produce more fat-free solids.

Normally fed animals which are not individually large producers cannot be brought up to a remarkable increase of production through any agency. The elimination of the poor assimilators of food in favor of good producers, which is a matter of economic necessity, should be based upon the capacity of the individual cow to properly utilize her food. Only through a systematic test of milkings and production records can the profits of the dairy be increased.

In judging individuals as milk producers by their external conformation, the following rules of the German Society for Breeds and Breeding may serve as a basis:

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(a) Heavy milk production is usually associated:

1. With low body weight,

2. With low measurement at the shoulder,

3. With a straight back, although slight deviations should not be considered as signs of small milk productiveness.

4. With more or less prominent hips and rump according to the characteristics of the breed.

5. With the more pronounced depth of thorax; heavy milkers are often narrow and flat chested;

6. With long shoulders,

7. With long rumps,

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8. With long, narrow head,

9. Generally with fineness of horn,

10. With fine bony structure;

11. The most important is the udder. The best cows have large udders of spongy-granular consistence, with large tortuous mammary veins, large milk wells, and easily movable skin.

The skin should lay together over the perineal surface of the udder in 4 to 6 or more large, well developed folds. The udder should collapse thoroughly after milking, and the animals should be easy milkers. Relatively early calving seems to have a good influence. The possibility of estimating the qualitative production of milk from external conformations is only very slight. As a rule, as shown by investigations, the smaller and shorter animals with fine long bones produce milk of higher quantity, and above all milk with a large yield of fat.

The productiveness of one and the same individual varies, especially with **age** and the lactation period. Cows with the first calf, provided normal conditions prevail, do not produce as much milk as after subsequent calvings; as a rule when cows reach the age of 7 to 9 years with the fifth and sixth calf, the maximum production is obtained. With the advance of age the production again gradually recedes. The proportion of solids is higher in cows with the first calf than in those which have calved several times; the quantity of fat on the other hand, as compared with that of older cows, is smaller (Teichert, Hittcher, Hogstrom, Vieth and others).

The variations which are manifested in the production of milk during **single lactation periods** are considerable, and depend entirely upon the individual, as does the length of the milking period.

For a few days after parturition a product is secreted which has very little in common with milk, and which may be considered as a product of glandular inflammation as a result of physiological irritation. It corresponds strikingly in its appearance and composition, as well as in the microscopical appearance of its cream and sediment with the inflammatory product of the milk gland.

This product called *colostrum* is a yellowish or even yellowish-red, slimy fluid, with an acid reaction. Corresponding to the increased content of albumen, globulin and

fat in colostrum, as compared with ripe milk, the amount of dry substances in colostral milk is very high, and its specific gravity is increased. The amount of urea, creatinin, cholesterin, and lecithin in colostrum is increased. The milk at this stage is rich in fat-containing glandular epithelium in the form of foam cells, and seal-ring shaped cells with so-called caps and moons, and in albuminophores. Numerous leucocytes are to be found, and during the first days red blood corpuscles are also present in great numbers. According to Emmerling, cow colostrum, on the morning after the birth of the calf, consists of 76.14% of water, and 23.86% of dry substance, of which 4.705% is casein, 0.58 albumen, 8.320 globulin. Compared with normal milk, the fat content is increased or diminished, the milk sugar diminished, and the ash contents increased.

Engling found the following values for colostrum:

Immed	iately after calving	• After 10 hours	After 24 hours	After 48 hours	After 72 hours
Specific gravity	1.068	1.046	1.043	1.042	1.035
Solids	26,83	21.23	19.37	14.19	13.36
Casein	2.65	4.28	4.5	3.25	3.33
Albumin and globulin	16.56	9.32	6.25	2.31	1.03
Fat	3.53	4.66	4.75	4.21	4.8
Milk sugar	3.0	1.42	2.85	3.46	4.1
Ash	1.18	1.55	1.02	0.96	0.82

The composition of the ash differs from that of ripe milk, as may be observed from the findings of Schrodt and Hansen:

		Ripe Milk (10 days
	Colostrum	after calving)
K <sub>9</sub> O	17.4	24.12
NaoO	10.10	8.72
CãO	22.99	22.69
MgO	6.88	2.92
$Fe_2O_3$	0.42	Traces
$SO_3$	2.82	4.10
$P_2O_5$	34.30	30.73
Cl	6.85	8.30

The ferments in colostrum also deserve special consideration. The amylase content is considerably increased, also the amount of catalase. During the colostral period the milk further contains hemolytic amboceptors and increased complement. Formalin methylene blue is not decolorized. The reaction of the colostrum is acid.

The colostral period lasts from 3 to 5 days after calving. In heifers the transition period results more slowly than in old cows (Deisman, Hittcher). Up to the end of this period there is a constantly increasing approach to the properties of ripe milk, together with an increase in the yield, which continues to increase until the first or second month, and then gradually recedes and finally rapidly diminishes towards the end of the lactation period. The reduction of the milk yield corresponds with an increase in the percentage of fat. The fat globules become smaller and more numerous. At the end of the lactation period the milk again assumes the character of colostrum, becoming especially rich in chlorine, and sodium oxide, while the phosphoric acid and the potassium contents appear diminished. The milk becomes salty, bitter, and its reaction alkaline. The entire time of lactation or one lactation period, usually lies between the birth of two calves, and is divided into the lactation period and the dry period. Good milk cows give milk on an average for 300 days. Cows which are not bred again, or which cannot be impregnated, may have a considerably longer lactation period.

During estrum a considerable diminution of milk in quantity and quality may be observed in cows. Sucking calves may at this time become affected with digestive disturbances. Hittcher and Neumann state that the quantity and the proportion of fat diminish, while the casein contents and the specific gravity of the milk are increased. There are however no set influences in one and the same animal, and still less so in different individuals. Sometimes the quantity of milk even increases, and not infrequently the milk becomes abnormally rich in fat (Martiny). Fascetti and Bertozzi found diminished quantity, increased specific gravity, and increased dry substance, which they supposed resulted from the increased proteid contents, especially from the increase of fat. The volatile fatty acids in the fat according to Nilsen are diminished, and the degree of acidity of the milk is frequently increased (Mezger). As a whole, however, the milk is not materially changed (Weber).

No observations have been made on the influence of **coition** and the beginning of another pregnancy. However the milk of cows far advanced in pregnancy frequently has a lower value. It coagulates sometimes as early as in the sixth, seventh, or eighth month of the gestation period. Finally it becomes slimy, yellow, and shortly before the cow goes dry it shows a similarity to colostrum. The amount of phosphoric acid and lime, contrary to that in colostrum immediately after calving, is diminished, and the taste is bitter and rancid (Backhaus).

If cows are **spayed** 5 to 6 weeks after calving the milk is supposed to be richer in fat, casein, and ash. The lactation period of such cows is considerably lengthened, according to Gouin extending to 6 years. Lajoux on the other hand states that in healthy animals the quality of the milk remains the same, but during the course of lactation the cows do not dry off so rapidly and the yield is therefore greater.

Milk which is produced after **abortion** is supposed to be similar to that of ripe milk (Schaffer and Hess). The lactation period however is short, and the milk yield small.

If the cow remains farrow for a long time a greater yield is obtained. This however is only slight, and does not compensate for the shrinkage during the latter part of the milking period.

#### Influence of Diseases.

Relatively little is known of the chemical changes which milk undergoes from the influence of general affections of animals. We are in possession of better information relative to the occurrence of specific disease agents in milk, and it is known that these pass into the milk either directly from the blood being then eliminated with the milk, or else they reach the milk through subsequent contamination of the milk with excretions.

A rapid diminution of the milk yield is characteristic in all acute diseases associated with great pain and fever, and in some cases a sudden cessation of the secretion may be observed.

Whenever the yield of milk of a cow suddenly shows a considerable diminution, all of her milk should be excluded from market, even though the animal shows no visible affection and before the disease can be recognized as a general or specific affection. In the sense of the pure food law the milk of every severely affected cow should be considered unfit for food without any further consideration.

Sometimes this unfitness of the milk is manifested by strong objective perceptible changes, as compared with the secretion of healthy animals.

The milk may become bitter, salty, have an increase of ash and albumin, and coagulate more rapidly than healthy milk (Jensen). The fat content of the milk is at the same time diminished or increased, while the sugar and ash contents may show fluctuation. The amount of catalase present, according to Spindler, may increase considerably, especially in cases of peritonitis and tuberculosis. The reaction of the milk remains acid or becomes slightly According to Schnorf, most of the internal affections, alkaline. even when the udder is not involved, produce a diminution of sugar and proteid contents as a result of increased metabolism. The electrical conductivity of the milk of animals with general affections is subject to great fluctuations. After tuberculin injections with subsequent fever, the milk shows a slight increase in its electrical conductivity. The index of refraction in pathological milk is normal, and not diminished; the temperature at which freezing occurs is not infrequently higher.

During the course of individual diseases the following should be considered:

An elimination of toxins and toxic products of metabolism with the milk is to be feared in all septic and pyemic diseases. If with this there is a possibility of contamination with pathological excretions, as for instance in **septic metritis**, hemorrhagic or ichorous enteritis, or in the retention of putrid afterbirth, the milk should be considered harmful. In septic metritis the infective agents pass from the uterus into the meat and into the udder, from which they may be eliminated. Basenau demonstrated the *Bacillus* morbificans bovis, a meat poisoning organism of the colon typhoid group, in the meat during the existence of septic metritis. The stapylococci and streptococci which are frequent participants in mixed infections of the uterus, are also eliminated with the milk, provided the udder has not already ceased its secretion. Milk from cows affected with acute and sub-acute intestinal inflammations should be judged in the same way as milk from animals affected with septic metritis.

The ingestion of milk from cows affected with bloody or fetid diarrheas should be especially guarded against. The appearance of sickness in man after the ingestion of such milk has been satisfactorily proved by Gaffky and Follenius.

Two assistants and a helper of the Hygienic Institute of Giessen drank milk of this character and became sick with dullness, headaches and chills. After two days diarrhea, vomiting and high fever appeared. The clinical manifestations in the two assistants simulated those of typhoid fever, while in the helper they were similar to those of Asiatic cholera. The milk originated from a cow affected with hemorrhagic enteritis. Gaffky demonstrated rapidly growing and strongly virulent colon bacilli both in the bloody excrements of the cow and in the stools of the affected patients.

In the presence of infectious diseases the milk of the entire stable should be withdrawn from use, or should be rendered safe by suitable treatment, as for instance by pasteurization. Such milk should never be sold as certified or infants' milk. Jensen extends this prohibition even to milk from stables in which white scour of calves, and other calf affections of an infectious nature have occurred.

Dangerous properties of the milk should also be considered in the appearance of other diseases, as for instance malignant catarrhal fever, purulent broncho-pneumonia, traumatic pericarditis, rinderpest, etc. (Bongert). In all cases of hemorrhagic, purulent, acute or chronic inflammations of the kidneys the milk should be judged similarly to milk from animals with intestinal inflammations. In such affections the freezing point of the milk approaches zero, and the refraction index is lower. At the same time these values in animals affected with inflammations of the kidneys vary extensively.

#### Special Infectious Diseases.

Tuberculosis of animals, especially its hygienic importance, is considered here in connection with tuberculosis of the udder. It should be mentioned at this point that some investigators believe that the toxins of the tubercle bacillus pass into the milk. A change of the quality of the milk will occur only in cases in which the advanced chronic affection of the animal results in lasting emaciation, or when an acute attack of the disease, associated with fever, appears during the chronic course of the disease. In tuberculosis the milk may become bluish, and poor in fat, the sugar and proteid substances may be diminished, or the latter may be even increased (Storch). Several tables, which indicate the experimental results of Monvoisin, are taken from Grimmer's "Chemistry and Physiology of Milk."

(D) 1 -

#### 1000 gm. of milk contained

1000 gm. of mink contained		Tuperculous	cows without
	Healthy cows	tuberculosis	of the udder.
Acidity as lactic acid	1.543	0.664	1.292
Total nitrogen	5.87	8.67	4.21
Fat	46.5	29.6	59.7
Sugar	43.5	29.8	43.9
Solids	142.3	126.05	147.5
Ash	7.3	8.2	6.7
Chlorin (sodium chloride)	1.4	4.13	1.05
Freezing Point	0.55		
Refraction at 15 deg	1.3434	1.3416	1.3442

In rinderpest, according to Busson, the amount of fat and sugar diminished rapidly, whereas the casein, albumin and salt increased. The passage of the contagion of rinderpest into the milk in a direct way from the blood is probable; the milk, however, can be contaminated with certainty through infectious secretions and excretions. Rinderpest is of no practical importance from the standpoint of milk hygiene, to most of the European countries (with the exception of Turkey), since it has been eradicated with the aid of veterinary police measures and even in the event of any possible introduction, it will be immediately suppressed.

Milk from cows affected with contagious pleuro-pneumonia is supposed to have caused the death of children (Randou, Lecujer and Wiedemann). Secretion of milk is immediately reduced at the onset of this disease, it becomes poor in fat and sugar, richer in albumin and ash, its appearance resembles that of colostrum, and its taste is peculiar. The contagion of pleuro-pneumonia appears to pass into the blood but rarely, and therefore its elimination in the milk can occur only exceptionally, if at all. Contagious pleuropneumonia is also subject to the most stringent veterinary police measures, and therefore has but little practical importance for milk hygiene.

Similar conditions prevail with **pox** of cattle. This disease however demands our interest for the reason that the infectious agent of cow pox must be considered as a mild form of smallpox of man. Cattle usually become affected through transmission of the disease from naturally infected men, or from those vaccinated with cow pox. The infection occurs if during milking the contagion of pox is rubbed into visible or invisible wounds of the skin of the udder. The infected teats manifest roundish or oval, hard papules of the size of a pea, which after 1 to 2 days change into yellowishwhite vesicles of a mother-of-pearl luster. After ripening into pustules which requires from 8 to 10 days, the lesions show a characteristic depression in their center, the so-called navel of the pox. They either rupture and suppurate, or dry and heal, leaving a superficial scar.

The udder becomes sensitive to pain, the milk is thinner, and

of lower specific gravity, but richer in albumin (Jensen). The injection of the contagion of pox into the ducts of the udder results in the development of pox vesicles on the walls of the milk ducts. After 2 to 3 days a swelling of the udder, with increased sensitiveness, develops and the secretion is changed. It becomes purulent and bloody on the eighth to the tenth day (Lienaux and Hebrant).

Transmission from animal to animal may be brought about by milking, and the entire herd in a stable may rapidly become affected. The course of cow pox is usually benign. According to Herz the milk becomes rich in cells, contains colostral bodies, and it has an unpleasant taste. Careful examination showed the following results:

	Beginning of observations	After 13 days	After 40 days
Specific gravity of the milk	1.0265	1.0270	1.0215
Specific gravity of the whey		1.0235	1.0209
Acidity according to Soxhlet-Henkel.	5.3	6.6	4.1
Fat	5.36%	4.02%	5.54%
Solids	13.31	11.82	12.25
Fat-free solids	7.95	8.81	6.72
Ash	0.72	0.72	0.8

Transmission of pox from cattle to man is of course very readily possible, and is not at all uncommon as a result of milking affected animals. After the ingestion of infected raw milk the pox exanthema may develop on the face (Jensen).

The so-called false or **gangrenous variola** which may be frequently observed on the teats of fine-skinned, fresh milking animals should not be mistaken for true pox. These eruptions are produced by the ordinary pus-producing organisms, which have been rubbed into the skin during milking or have penetrated the skin by means of various injuries. Small furuncles and skin abscesses result, which heal without influencing the formation or secretion of the milk. Healing is of course retarded through the act of milking, and during the presence of the pus cells, blood and pyogenic organisms may pass into the milk in small quantities. These false pox lesions are not very important.

Of much greater importance than cow pox is **foot-and-mouth disease** which sometimes appears extensively. This is a highly acute febrile disease which is transmitted to cloven-footed animals with remarkable ease. The most striking symptom which occurs in association with the disease, the vesicular eruptions, may also affect the udder, and especially the teats.

The udder swells, becomes painful, and red-bordered vesicles develop in sizes up to that of a walnut, which burst during milking or spontaneously, leaving painful ulcers. During the beginning of foot-and-mouth disease the yield of milk is considerably diminished, sometimes one-quarter less than the usual yield, as a result

of the febrile affection and on account of the inappetence due to the pain caused by the vesicles in the mouth and on the feet. The effect of the disease upon milk secretion varies according to the individuals. the age and the lactation. Siedamgrotzky, Weber and Born have published the effects of the disease on milk secretion during outbreaks in certain herds. In 43 cows the quantity of milk at the height of the disease dropped from 745 to 364 liters, and again rose after the eradication of the outbreak to 522 liters. Thirty cows of another herd gave only 30 liters instead of 300 liters of milk during a period of eight days. In a third herd the quantity of milk dropped from 510 to 260 liters, later rising to only 350 liters. Other figures showed a decrease from 750 to 280 liters, with a subsequent rise to 400. The diminished yield per cow per day was from 5 to 6 liters and even more. In cows that have been milking for a long time the loss in milk reaches as high as 75%. in animals in the middle of the lactation period up to 43%, while in fresh milkers it may amount to 55% (Hutvra and Marek). Sugar and fat contents diminish, but at times the amount of fat may become considerably higher. The volatile fatty acids are diminished, but the milk contains more albumin and salts, an increased amount of thrown-off epithelium, colostral cells, pus cells, and also red blood corpuscles (Lavena, Kalantar, Herberger, Kreis, Vogler and others). The catalase content is increased even if the udder manifests no changes (Bertin-Sans and Gaujoux).

Honigmund examined five cows affected with foot-and-mouth disease, one of which was not visibly affected on the day of the examination although already infected. The individual data inside of nine days were as follows:

Quantity of Milk	Tempera- ture	Specific Gravity	Fat Con- tents	Nitrogen- ous Subs.		Solids	$\operatorname{Ash}$
15 L	. 38.7	1.032	3.05	2.99	4.24	11.62	0.74
6 — 7 "	39.6	1.031	5.4	2.97	3.63	13.00	0.63
6 — 7 "	38.9	1.030	4.3	2.99	3.80	12.81	0.89
about 8 "	39.0	1.030	3.43	3.04	3.91	11.33	0.65
8 - 10 "	38.6	1.031	3.06	3.04	4.15	11.01	0.70
8 - 10 "	38.4	1.029	2.9	3.1	4.49	12.03	0.69
about 10 ''	38.6	1.030	2.84	3.19	4.57	11.21	0.71
10 - 11 "	38.5	1.032	3.45	3.24	4.41	11.77	0.67
about 12 ''	38.4	1.031	3.25	3.33	4.38	12.30	0.70

It appears also from the other investigations of Honigmund, in which the animals showed symptoms of the disease as early as on the first day of the examination, that the fat and ash content is greater in the first day than in normal conditions. The total solids and also the fat-free solids fluctuate considerably.

When catarrh of the milk ducts becomes associated with footand-mouth disease, the milk becomes yellowish, of a rancid, bitter taste, colostrum-like, and similar to the secretion during other inflammatory conditions of the udder, that is, slimy, watery, and intermixed with coagulum.

It is an important fact that milk from animals which are affected with foot-and-mouth disease will contain the virus of footand-mouth disease, if it has been contaminated by the vesicular contents. Nocard succeeded in proving, however, by careful sterile drawing of the milk from cows affected with foot-and-mouth disease, that the milk does not contain the virus of foot-and-mouth disease as it leaves the udder.

Nevertheless it is not satisfactorily proved that a direct elimination of the virus may not take place at the beginning of the febrile state, as at this time the virus is present in the blood. If the udder itself is affected by the eruptions of foot-and-mouth disease it is hardly possible to avoid contamination of the milk with the vesicular contents. Considering the ease with which the virus of the disease is spread, it may be assumed that the entire milk of a herd affected by the disease, under ordinary conditions of milk production, contains the contagion of foot-and-mouth disease. Strict veterinary police measures must be inaugurated to prevent the spread of the disease. Sale of the milk should be permitted only after sufficient heating. The maintenance of a temperature of 70 deg. C. for one-half hour will make the milk perfectly safe.

Milk containing the living virus of foot-and-mouth disease must be considered deleterious to human health, since it has been established by experiments and observations that the disease is transmissible to human beings. Vesicular and ulcerated inflammatory changes of the buccal mucous membrane with fever and general symptoms develop with possible vesicles and ulcers on the hands, arms, breast, lips, ears, and in the throat. Vomiting and diarrhea may be associated with symptoms of a gastro-intestinal inflammation, and the affection may even terminate in death. (Bussenius and Siegel, Jensen, annual reports of the Imperial Board of Health, Hertwig, Stickler, Schreyer, Krajewski, Walkowski, and others). Bongert suggests the separation in dairy stables of the non-affected, slightly and severely affected animals into isolated groups, and in order to reduce the economic losses as low as possible, the milk of these groups should be treated in different ways. Heated milk from the non-affected animals for instance, could be utilized as infant's milk. The milk from slightly affected animals could be marketed as ordinary milk [after pasteurization], while the milk from the severely affected cows or milk changed in its consistence, should be excluded from consumption even in a heated condition. Even with this separation the losses will necessarily be high as a result of the enforcement of stringent sanitary regulations.

According to Ebert sour milk 3 to 4 days old is no longer capable of transmitting the infection. The transmission is possi-

ble through cheese and butter (Fröhner, Ebstein, Thiele, Schneider, Frick, Fröhlich).

The general rules which have been indicated above obtain also in changes of the milk in malignant œdema, blackleg, or parturient blackleg of cattle. Transmission of these diseases through the consumption of milk from affected cattle, or through the diseased products of contaminated milk, is not to be feared; besides milk production ceases very rapidly in the affected animals.

The same rules should apply in judging milk from animals affected with **hemorrhagic septicemia**, a disease which is produced by a bi-polar bacterium. This disease is transmissible to calves, through sucking or feeding milk from affected animals.

Anthrax of cattle should also be mentioned. This runs in an acute or sub-acute form, and as a rule is associated with a sudden cessation of the milk secretion, which occurs even as early as at the beginning of the fever. The anthrax bacilli only multiply towards the end of the disease sufficiently to cause a direct passage from the blood into the milk. If the secretion has continued to some extent this direct passage is possible even if no hemorrhages, such as are typical during the course of anthrax, have developed in the parenchyma of the udder. The demonstration of anthrax bacilli in milk has been accomplished microscopically, and by inoculation and cultural experiments, but not in all the cases which have been examined (Bollinger, Chambrellent and Moussou, Feser, Monatzkow).

In severe cases the milk becomes yellowish, bloody and slimy. At the appearance of the fever the fat and sugar contents are increased, while the proteid contents are diminished.

The danger of infection through the ingestion of raw milk containing bacilli is slight, since the anthrax bacilli are digested by the gastric juice. More dangerous than the bacilli which may pass into the milk from the blood are the anthrax spores which may reach the milk through contamination with manure of affected animals, or through straw and stable dust, since the resistant spores are not destroyed by the gastric digestion. The virus may also be present at times in normally healthy animals after they ingest food containing anthrax spores. The milk may become infective through contamination with feces from such bacilli carriers. In spite of the fact that there are remarkably frequent opportunities to obtain milk with bacilli and spores from localities in which anthrax persists epizootically as a disease of the soil, yet only one anthrax infection of man is known to have occurred through the ingestion of milk. This resulted in a patient with typhoid fever, who after drinking 11/2 liters of milk became affected with intestinal anthrax. The milk was derived from a cow with a malignant pustule on the udder, which had died in the meantime from anthrax.

Lehnert states that the calf of a cow affected with anthrax remains well, although it may suck the mother through the entire course of the disease.

Even though milk offers a splendid nutritive medium for the anthrax bacillus, an increase of bacilli only occurs during the first three hours. Keeping the milk at room temperature for 18 to 24 hours, is followed by the death of the bacilli (Caro). At the beginning of souring the vegetative forms of the virus are quickly destroyed; the spores however remain active (Inghilleri). If anthrax bacilli are cultivated in milk, coagulation occurs under the rennet action of the peptonizing bacterial ferments. The coagulum again slowly dissolves, and the milk separates into fat and whey.

Less important than anthrax is rabies, as this disease occurs much more rarely in cows. According to Nocard and Bardach the milk of animals affected with rabies contains the virus. Neverthe less the danger to man from the ingestion of such milk is hardly probable, since it is impossible to affect experiment animals by feeding fresh milk (exceptions are rats and mice). A nursing infant of a woman affected with rabies remained well, although it was fed with the milk of the patient until one day before her death (Bardach). The uninjured mucous membrane of the mouth, pharynx, and the intestinal tract does not offer opportunity for infection. This opportunity is afforded only when destruction of tissue and small wounds permit the entrance of the contagion. Thus for instance Galtier succeeded in producing rabies through rubbing brain material of rabid animals into the mucous membrane of rabbits. According to the observations of Virschikowsky the rabid virus is destroyed by the gastric juice.

Very little, or nothing at all is known relative to the special relationship of other infectious diseases to milk, as for instance malignant catarrhal fever, croup of cattle, the blood diseases of cattle caused by spirochætes, trypanosomes and piroplasma, or infectious vaginal catarrh and infectious abortion. In the presence of infectious vaginal catarrh and contagious abortion the milk secretion is supposed to be diminished.

It should be remembered that in such affections the passing of the disease agents from the blood into the milk is possible. [That the bacillus of infectious abortion is eliminated by the milk has been definitely established. See Bureau of Animal Industry Circular No. 216].

In a case of icterus in a woman Mayer observed the passage of bile acids, especially taurocholic acid into her milk.

Finally two other diseases should be mentioned which may be transmitted from animal to man:

1. Milk Sickness. A rather peculiar disease, called "milk sickness," is found in the central part of the United States, where it at times occurs as an epidemic among cattle and people. In cattle, the first indication of disease is dullness, followed by violent

trembling and great weakness, which increases during the succeeding day until the animal becomes paralyzed and dies. Through the ingestion of flesh, milk, or dairy products of an affected animal the disease is transmitted to man or to another animal, and attacks produced in this way most frequently prove fatal. In man the disease develops with marked weariness, vomiting, retching, and insatiable thirst. Respirations become labored, peristalsis ceases, the temperature is subnormal, and the patient becomes apathetic. Paralysis gradually follows and death takes place quietly without rigor mortis.

Many efforts have been made to elucidate the question regarding the nature and cause of this disease, but although many theories have been discussed none of them has so far been generally accepted. Some investigators hold that the disease is of microorganismal origin, some that it is due to auto-intoxication, while others think it is caused by vegetable or mineral poisons All seem to agree, however, that the disease is limited to low, swampy, uncultivated land, and that the area of the places where it occurs is often restricted to one or a few acres. Furthermore, when such land or pastures have been cultivated and drained the disease disappears completely.

The discovery of a new focus of this disease in the Pecos Vallev of New Mexico in November, 1907, gave Jordan and Harris the opportunity of studying this peculiar affection by modern bacteriological methods. As a result they have succeeded in isolating in pure cultures from the blood and organs of animals dead of this disease a spore-forming bacillus which they name "Bacillus lactimorbi." With this bacillus they have reproduced in experiment animals the symptoms and lesions peculiar to milk sickness or trembles, and from these animals the same organism has been recovered in purity. It therefore appears to have been demonstrated that the bacillus in question is the probable cause of the disease. As Jordan and Harris have already indicated, more comprehensive studies, based on a larger supply of material, are desirable in order that the many obscure and mystifying features connected with the etiology of this rapidly disappearing disease may be elucidated.

From the above facts it seems evident that milk sickness is an infectious disease communicable to man, and the cattle owners should therefore not be permitted to make use of the meat or milk of affected animals for human consumption. Trans.]

2. Malta Fever. On the coast of the Mediterranean, in South Africa, India, China, Philippines, America, and especially on the Island of Malta, there occurs in goats a disease which exists in the animals without producing any or at most only very slight symptoms. Cows may also possibly be affected. The infected animals eliminate for months, frequently at intermittent periods, the virus of the disease (*Micrococcus melitensis*, *Bruce*). Follow-

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ing the ingestion of such milk "Malta Fever" develops in man. It has a protracted course with recurrences, and is accompanied by anemia, headaches, rheumatic pains, constipation and swelling of the joints. Malta fever terminates fatally in about 3% of the cases. The goats show on postmortem, swelling of the spleen and lymph glands, frequently also inflammations of the kidneys and lobular pneumonia. The virus is relatively resistant against souring of the milk, but at 70 deg. C. it dies in 10 minutes.

According to Zammit about 10% of all the goats on the Island of Malta eliminate the virus, while 50% of the animals show by the agglutination test that they are or have been under the influence of the *Micrococcus melitensis*.

We are in possession of better information concerning the changes which milk undergoes in inflammations of the udder than we have regarding the effect on the milk secretion as a result of general diseases, or regarding the importance of milk from affected animals from a hygienic standpoint.

## Changes in Appearance, Consistence, Contents, Etc., During an Attack of Mastitis.

Relatively very little is known as to the influence of the diseases of the udder on the chemical and physical character of the milk, although it is well known that with the changes in function and condition of the organ the product is also changed, as compared with the product of the normal gland. Even in the same disease the product varies in accordance with the intensity, duration and the extension of the disease, the same as it naturally varies in accordance with the nature of the injury to which the parenchyma is subjected. As a result of these conditions the results of the data of different authors vary considerably.

It may be said in general that in affections of the udder the proportion of the proteids, sugar, salt, fat, and enzymes in the milk becomes altered and that the relation of the individual proteids, the salts and the enzymes, also undergoes fluctuations. In acute and greatly extended chronic inflammations, both fluid and cellular constituents of the blood may pass into the milk, cells of the parenchyma are thrown off, coagulation sets in, and briefly, the milk changes more or less rapidly in appearance, taste and contents, so that it deviates considerably from the milk of healthy cows.

At times none of these characteristics appears, especially in the early stages of chronic inflammations of the udder, or after the subsidence of the acute symptoms, and it is then only possible with the aid of certain methods of examination to differentiate such affected milk from normal.

Therefore of special importance to milk hygiene are the chronic inflammations, and inflammatory stages in which the changes of the secretion appear slowly, and relatively late, while inflammations of an acute character very quickly produce a tremendous change in the secretion, the mixing of which with market milk would be the grossest negligence. It is to be regretted that such cases occur.

Appearance of affected milk: In forms of inflammation which are associated with rapid development, painful swelling and increased temperature of the udder, the milk usually has a bloody discoloration, later becoming yellow (colostrum-like), and finally changes into a custard, or honey-like secretion, in which thick, yellow and yellowish-brown flakes are suspended in a more or less clear serum or plasma.

Such changes are observed in samples of milk in acute forms of mastitis, through infection of bacteria of the colon group, in mixed infections, in acute attacks or in great extension of streptococcic mastitis, and in infections with the *Bacillus pyogenes*, etc.

In chronic affections the milk changes only slightly or not at all during the beginning of the disease, or it may appear normal long before the disease as such is considered cured. If such normal appearing milk from affected quarters is allowed to stand for several hours a white, yellowish-white or yellowish sediment settles to the bottom. At the same time the quantity of cream is increased and changed, appearing yellowish, tenacious, and when shaken it assumes a cloudy or wavy appearance. If the migration of the pus corpuscles from the blood vessels becomes more intensive the milk appears thick, yellowish, cream-like, and after standing separates into a yellowish-white to ocher colored sediment, which may amount to two-thirds or more of the entire mass, and into a dark, transparent, yellowish-grey to greenish-yellow skim The sediment layer is at times increased, at other times milk. decreased. The cream becomes granular, shredded, and tenacious. If red blood corpuscles are eliminated in great numbers they collect in the form of a red disc on the yellow to yellowish-brown base, which is composed of leucocytes and coagulation masses. In hemorrhagic stages of the inflammation the milk is pinkish or brownish-red; by sedimentation it separates into a Bordeaux-red or rust-colored precipitate, and a pinkish-red layer of cream over the reddish-gray skim milk.

In other cases the milk becomes grayish and watery, and only a few thin conglomerates and fat globules indicate the layer of cream.

Cream and sediment are especially rich in cells in all forms of inflammation. Epithelial cells are desquamated into such milk in the form of colostral cells, or entire epithelial bands, and numerous polynuclear leucocytes, besides single epithelial cells, into which macrocytes penetrate (albuminophores), erythrocytes, cell debris, fragments of nuclei, as well as Nissen's globules are found.

Besides concrements of the most varied quality, casein and

#### Mastitis.

fibrinous flakes appear (Zschokke, de Bruin, Kitt, Sven Wall, Doane, Russell and Hoffman, Ruhm, Ernst, Bähr and others).

The taste of milk from affected quarters of the udder is also affected markedly, the milk becoming salty, bitter, and pungent. According to Craandijk in 67% of cases the taste of the milk changes in streptococcic mastitis.

From the appearance which the affected quarter manifests. as compared with healthy quarters, from the change in the behaviour of the animal, from the varying quantity of the secretion against the quantity from healthy quarters or the previous yield of the same quarter, the milker becomes suspicious of the existence of an abnormal condition in the suspected quarter, and the tasting test reveals a salty, bitter taste which assures him of the appearance of a change in the activity of the gland. If the udder secretion could be examined on the hollow of the hand before being milked into the pail, in order to determine the possible presence of flakes, etc., as should be the duty of the milkers, then the mixing of such mill: from affected quarters would not occur to the extent that it does at present, as has been proved on numerous occasions. A great deal would be gained if the milk from those quarters which produce a milk so changed that its abnormalities can be recognized by its appearance or taste could be totally destroyed. As a matter of fact milkers can much more readily recognize developing inflammations of the udder (as for instance streptococcic mastitis) from the varying conditions of the udder, or quarter, the quantity of milk, and the behavior of the animal, than the veterinarian can by a single clinical examination. Therefore the method applied in practice consisting of a single clinical examination of the cows producing infant milk at the time of purchase, or every 3 to 4 weeks is not sufficient to determine the presence of udder affections. Periodical examinations of all cows producing certified milk, supplemented by tests of the milk obtained at the time of the examination, are necessary when the inspection is to serve its purpose.

At the examination in the stable a comprehensive history should be taken from the milkers relative to the general condition of the cows, their action during milking, the condition of the teats and the gland tissue, the inflammatory changes noted, in fact all points which may offer valuable supplements to physical examination. One may learn from questioning that the cow milks very hard from one quarter, that she sometimes refuses to "give down" her milk, or that she "draws up" the milk or that recently the cow has shown a tendency to kick during milking. At other times one may hear that the parenchyma contains knobs or lumps or that the teats contain beads or warts, or are "fleshy," the quantity of milk is diminished, the milk is sometimes hot, "heated," or that the cow has the "cold garget" without any inflammatory indications of streptococcic infections. The milk is ropy, the quarter is "blind," the milk contains stringy clots and other things.

The keeping of milk records and the taking of milk samples at least every four weeks, should be required of all owners of animals which produce milk for city consumption and those furnishing it to wholesale milk dealers.

Together with the visible changes in the milk, changes of the value of the chemical and physical properties occur which have been especially studied by Guillebeau and Hess, Schaffer and Bondzynski, E. Seel, Mezger, Fuchs and Jesser and Mai and Rothenfusser.

These changes in the contents and properties are therefore especially important since frequently values are obtained which suggest adulteration with water. Irreproachable comparative tests of milk obtained directly from the stable may indicate however that in the specific cases the investigations were being made with abnormal milk.

According to Schaffer and Bondzynski's examinations the milk from cows affected with mastitis showed the following values:

Water	Solids.	Fat	Pro- teid	Milk Sugar	Total Ash	$\overset{\%}{\mathrm{P}_{2}\mathrm{O}_{4}}$	% Cl.
In non-infectious		0.00	1.07	0 70			
	7.17	0.82	4.01	0.53	0.79	7.35	35.76
In yellow galt	10.66	1.99	6.00	1.84	0.83		
In parenchymatous							
mastitis 90.26	9.74	2.16	4.21	1.01	0.97	19.21	27.79
In comparison with healthy							
milk	12.25	3.4	3.5	4.6	0.75	20.0	14.0

The milk sugar content was also considerably diminished; the amount of mineral substances on the other hand was increased. Guillebeau and Hess give the following values in milk from cows with affected udders:

Duration of the Disease and Origin of the Milk	Specific Gravity	Solids.	Fat	Nitrog- enous subst.	Milk Sugar	Ash
1½ days		7.45	0.52	6.17		0.85
$2^{\prime}$		5.15	0.22	4.26	0.	0.82
5 "udder recovering		9.80	1.95	2.98	4.06	0.81
7 "	1.0314	11.28	2.72	3.50	4.35	0.70
2 "		7.69	1.09	5.74	0.	0.87
2 "		23.58	9.30	8.53	4.68	1.07
$1\frac{1}{2}$ '' from two affected quarters		15.88	4.50	5.37	5.14	0.87
$1\frac{1}{2}$ "from two affected quarters		9.66	2.09	6.74	2.09	0.85
1			0.53	5.13		
8 '' from two affected quarters		15.88	4.50	5.37	5.14	0.87
2 '' from two affected quarters	1.0430	20.94	0.97	16.65	2.61	0.71
21 "'	1.0379	18.18	2.80	7.93	2.04	0.91

In most cases the specific gravity is lower (Seel, Mezger, Fuchs and Jesser) and approaches the normal only towards the end of the disease. In mixed milk from all four quarters the specific gravity is less influenced. The quantity of fat, according to Seel, and in some cases of Mezger, Fuchs and

The quantity of fat, according to Seel, and in some cases of Mezger, Fuchs and Jesser, is very much reduced. The latter authors emphasize the fluctuation of the fat in sudden jumps. The experience of the official milk control station in Munich also gives similar results, at the beginning of the affection frequently finding abnormally high or again abnormally low fat contents of the milk.

The amount of milk sugar as a rule is reduced, and rises only with the appearance of recovery.

The solids are likewise usually diminished; the fat-free substance is also, and only becomes increased after signs of recovery have been noticed.

The amount of proteid coagulable by heat frequently increases enormously, as compared with the contents of casein which diminishes.

The ash content on the average is increased. Relative to the composition of the ash the data appear to be contradictory. Although Seel found in 15 cases out of 18 a diminution of chlorides against an increased quantity of  $P_2O_5$  Mezger, Fuchs and Jesser observed an increase of the chlorine content and a diminution of the phosphoric acid, while Steinegger and Allemann found that the amount of  $P_2O_5$ , CaO,  $K_2O$  and MgO diminishes, in general, while the quantity of Cl., No<sub>2</sub>O and SO<sub>3</sub> increases. According to Hashimoto the ash of abnormal milk closely approaches the ash of blood serum (0.78%), consisting of S.9863  $K_2O$ ; 36.544 Na<sub>2</sub>O; 7.44 CaO; 1.738 MgO; 17.380  $P_2O_5$  and 33.627% Cl.

The reaction of affected milk is mostly alkaline (Seel, Mezger, Fuchs and Jesser, Hoyberg, Auzinger, Ernst), or more rarely acid (Zschokke, Henkel, Wyssmann and Peter, Ernst). The determination of acidity is recommended by Plaut as a means for the diagnosis of udder affections.

Independently from the degree of acidity, the coagulability on the addition of alcohol is frequently considerably increased but not always, and in some cases not constantly. (Henkel, Rullmann and Trommsdorff, Auzinger).

The refractability of the calcium chloride serum not infrequently suffers considerable changes upward and downward. Ripper, Ertel, Mairhofer, Schnorf, Mai and Rothenfusster, Henkel, Mezger, Fuchs and Jesser found considerable changes in this respect and proved that the daily variations in the refraction may be very great in milk of individual quarters, and even in the full milk of an animal. Frequently however the refraction of the calcium chloride serum shows no change when compared with the milk of healthy animals.

The same variation obtains in the lowering of the freezing point of milk from affected quarters; the values may be considerably higher than that of healthy milk, or on the other hand they may be lower. More frequently a high value is observed (Schorf, Quiraud and Laserre, Crispo, Bertozzi, Pins). According to Schnorf the electrical conductivity is always increased, never normal or lower. According to Bonnema the increase of chlorides results in an increase of the electrical conductivity.

A change in the contents of original ferments appears very early during the affection, together with an increase of cellular elements, especially leucocytes (Zschokke, Bergey, Trommsdorff and Rullmann), and fibrinous flakes (Doane, Russell and Hoffmann).

According to Koning the increase of the catalase content in freshly obtained milk is an indication of the affection of the udder, provided the colostral period has passed. The publications of Spindlers and Rullmann (who were enabled to obtain aseptically milked samples with which to work) and the author's observations confirm Koning's findings. The author observed that in slight, local affections of a chronic nature, without febrile manifestations, the content of catalase usually runs parallel with the cell content, and it rises when there is an especially marked throwing off of epithelia (presence of typical colostral cells).

The faculty of splitting up added starch solution likewise increases in milk from affected udders as compared with that from healthy udders. There are no observations relative to the quantitative effects of peroxydase. According to Weichel the peroxydase content of affected milk from an artifically affected goat disappeared, whereas the healthy milk gave the guaiac reaction. The reaction again appeared when the secretion became of a milklike consistency.

Affected milk behaves in various ways on the application of formalin-methylene blue solution; frequently a very rapid decoloration of Schardinger's reagent may be observed (Rullmann, Sassenhagen, Rievel). Sometimes in typically changed samples the reduction does not take place (author's observation).

As the above-mentioned enzymes (not anylase), at least in part may appear to be brought on by bacterial action, their abnormal presence in milk has a diagnostic importance only in freshly milked samples. The case is different with the complement content. As indicated in the chapter on antigens, blood constituents pass directly into the milk during periods of physiological and pathological irritations. Therefore in mastitis, as proved by Bauer and Sassenhagen, complements are demonstrable in the milk. This, according to Sassenhagen, is possible even in affections in which the Trommsdorff value of the centrifugalized cells per 1000 parts of milk is still remarkably slight.

The alkaline reaction of affected milk, the altered proportions of mineral salts, at times the passing into the milk of bloody particles, and the diminution of casein, reduce the coagulability of the milk towards added rennet. Affected milk therefore generally utilizes a considerably larger quantity of rennet than normal milk before it becomes coagulated (Schern).

The spontaneous coagulation of affected milk also appears to be considerably delayed.

#### Infectious Agents of Mastitis.

Nocard and Mollereau, Kitt, Lucet, Bang, Guillebeau and Hess, Zschokke, Jensen, Streit, Glage and Sven Wall have offered sufficient information regarding the infectious agents of the different forms of mastitis.

Most cases of mastitis are produced by **streptococci**; they consist of chronic inflammations of one or more quarters of the udder. The disease is of relatively small influence on the general condition of the animal.

Bacilli of the **coli-aerogenes-paratyphus-paracolon** groups produce highly acute, parenchymatous lesions. The general condition is severely influenced through infections by bacteria of the paratyphus-B group. Locally a gangrenous, septic mastitis develops with this infection, and the milk is markedly ichorous, while in colon infections the secretion is of a serum-like character (Weichel).

A third form of inflammation of the udder, also of a chronic nature, is produced by a representative of the group of the *Bacillus pyogenes*, Sven Wall's **pyobacillosis** of the udder. The *Bacillus pyogenes* colonizes with a special predilection in the presence of streptococci or staphylococci, and in these mixed infections causes severe necrotic inflammations of the udder, and may continue to produce chronic mastitis in the affected udder tissue after the disappearance of the other bacteria.

Other forms of mastitis are produced by **tuberculosis** and actinomycosis, and they usually result through emboli of the infective agents. They may be of a traumatic origin (actinomycosis) induced by irritation with particles of straw, or barley beards.

Furthermore all possible infective agents, as for instance the *Bacillus necrophorus*, may be found in inflammations of the udder, either independently or as mixed infections.

Only the more important infections of the parenchyma will be described here.

## Streptococcic Mastitis.

By far the most widely spread type is the streptococcic mastisis, described by Sven Wall as streptomycosis of the udder.

The works of Bergey, Craandijk, Trommsdorff and Rullmann, Kunze, Russell and Hoffmann, Savage, Rühm, and Ernst give general information on this condition. The disease is either sporadic or epizootic among the animals of a stable according to the stable conditions. The disease may attain an especially wide distribution when the secretion of the affected quarter is milked upon the floor or into the bedding, and the milkers fail to wash their hands, both bad practices which, it is to be regretted, are quite common.

Zschokke, Jensen, Bang, and Sven Wall proved experimentally that bacteria injected into the cistern penetrate even into the farthest alveoli in from 2 to 24 hours.

By inoculating with strains of streptococci of different origin varying reactions may be produced in the udder (Bang: Streptococci equi and Streptococci agalactiæ; Gminder: streptococci of the stable air and of infectious vaginal catarrh). The manifestations also vary after the injection of individual strains into the same animals, and from injections of the same strain into various animals. In other words the **course** of the disease varies in accordance with the virulence of the organism, the resistance of the body, and the extent of the local invasion which again is influenced by the lactation period. According to de Bruin fresh milking animals more frequently become affected with the acute form having inflammatory manifestations, while in old milking animals the disease confines itself mostly to the altered appearance of the secretion.

The result of the disease is that sooner or later the affected part of the gland becomes destroyed.

Sometimes the streptococci remain for weeks in the folds of the mucous membrane of the cistern without infecting the parenchyma; in other cases again the entire quarter quickly becomes affected. Unfortunately the disease does not often subside even through the physiologically dry period, and the affection re-appears immediately after parturition.

The destruction of all streptococci involves a difficult task for the entire body. The dissolution of the streptococci progresses only very slowly even in actively or passively immunized animals. Living streptococci may be demonstrated in the abdominal cavity of test animals, many hours after an intra-peritoneal injection. Not infrequently a delayed death appears in apparently recovered animals (v. Lingelsheim). The long streptococci appear to represent specially adapted forms which have great tenacity. Nevertheless at times recovery takes place. According to Zschokke the relation between recovered and unrecovered cases is as 7:5.

According to the experience of the author in practice, infectious mastitis is not curable, or only with the greatest difficulty, and if so, always with a loss of productiveness, which even remains after the physiologically dry period. The chronic irritation causes a change in the connective tissue structure of the parenchyma of the udder so that the usual development of the gland during pregnancy cannot take place. The principal aim in treatment therefore should be prompt drying of the suspected udder, in order to make possible the most rapid and most complete recovery, which, according to Zschokke may be expected only when the quarter has been allowed to remain dry for a long time.

This is also necessary in order to prevent a spread of the disease, which is to be feared since the hands of the milkers and milking upon the straw may transmit the infective agent to other quarters. Care should be taken therefore to keep the milk from the healthy quarters of the udder separate from the secretion of the affected quarter.

As long as the most primitive requirements of clean milk production on the part of the milkers are so carelessly neglected, which unfortunately has been the case up to the present, the immediate drying of the affected quarter offers the only means of preventing the further spread of the disease.

If, however, there is assurance that the affected animal or the affected quarter, respectively, is individually milked, and the milkers follow instructions, an attempt may be made by special frequent milking (into a jar) to produce a hyperemia of the udder. With this method success can only be expected in the early stages.

The extent of the **spread** of the disease may become obvious by the findings after examination of individual herds. In such cases it is necessary to milk each cow, or still better each quarter, separately. The results vary, depending on the technique of the examination. The lowest number of affections is obtained when only a clinical examination is made. This therefore does not suffice in order to eradicate the disease effectively, or to single out the affected animals. The data of the different authors vary relative to its occurrence. The following figures are given which were obtained by systematic examinations of entire herds. Out of 260 animals Trommsdorff found 15.6% affected, Rühm 31.25% out of 16 animals, Russell and Hoffmann found in 188 samples 50% with "streptococci." Savage found similar values (55%).

The author examined from April 1, 1907, to November, 1908, 1697 samples of milk from individual cows, and found in 348 samples the typical signs of streptococcic mastitis.

In 1908 and in the following years he has demonstrated:

1908.	No. of animals, 1695.	Streptococcic cases353
1909.	No. of animals, 738.	Streptococcic cases301
1910.	No. of animals, 597.	Streptococcic cases203
1911.	No. of animals, 876.	Streptococcic cases279

Therefore 20.6; 20.9; 40.6; 34 and 31.8%, respectively, of the animals were found to be affected with streptococcic infections of the udder.

If the milk of the individual quarters of the affected udder is examined various stages of the affection in the different parts of the udder may frequently be found. Out of 528 quarters of animals with affected udders 276, or 52.2%, showed lesions in individual quarters. 39.2% of the cows had the disease in one quarter, 25.9% showed it in two quarters, 18.5% in three quarters, and 16.2% in all four quarters. According to Zschokke out of 662 affected quarters 193 occurred in one quarter of the animal, 211 in two, 109 in three, and 149 in all four quarters.

The contamination of market milk with the secretions from animals with udder affections is relatively high.

In spite of the fact that proof of the mixing of milk from affected udders with market milk is possible only in very pronounced cases (typical streptococcic chains with characteristics of animal origin), nevertheless the following results, obtained in examinations, demonstrated conclusively that the secretion from quarters affected with streptococcic mastitis had been added to the whole milk:

> 1908, in 352 out of 1578 samples=22% 1909, in 501 out of 1629 samples=40.5% 1910, in 243 out of 1211 samples=20% 1911, in 432 out of 1273 samples=33.9%

The **hygienic importance** of the affection to the consumers of milk may be illustrated from the following data.

I. Holst, in 1894, had the opportunity of examining in Christiania four series of affections of acute gastro-intestinal catarrh.

I. Four grown persons and four children out of three families in the same street became affected four hours after the drinking of milk which originated upon one farm. Those persons who drank no milk or only that which had been boiled were spared with the exception of a child who became affected, although only slightly, after drinking boiled milk.

The appearance of the milk showed nothing abnormal, but it coagulated on boiling and showed a tremendous number of bacteria, especially streptococci, which could not be distinguished from the Streptococcus pyogenes.

The veterinary examination confirmed the suspicion that a pus-containing secretion was being yielded by one cow.

The milk from the cow with mastitis on the day in question was added to the whole milk through the neglect of a newly hired attendant.

2. Several hours after the drinking of raw milk five persons, and as found later other cases also became affected with acute gastro-intestinal catarrh. In this case a milk dealer was implicated, and it was found on inquiry that the milk contained secretion from a cow affected with streptococcic mastitis.

3. According to the observations of Johannesen two persons (mother and child) became sick after the drinking of milk. The milk was thin, flaky, and contained pus-like lumps. In the herd from which this milk originated two cows were found to be affected with streptococcic mastitis.

4. After the drinking of freshly drawn raw milk four children of the same family became affected with acute gastro-intestinal catarrh. The milk appeared apparently normal, but contained large quantities of streptococci. It originated in a stable from which on the day in question a cow was sold on account of mastitis. The milk from this cow appeared to have been mixed with the whole milk due to the neglect of a new milker (the regular one being sick).

The affections which occurred in Stockholm with symptoms of fever, dullness, attacks of fainting, nausea, vomiting, diarrhea and cramps in the calf of the leg (nine families being involved), cannot according to the obtainable reports, be declared to be streptococcic infections. The milk, through the drinking of which the cases could be traced, originated from a dairy of 14 cows, among which one cow had mas-titis. It is possible that in this case an infection with bacteria of the paratyphus group, which plays an important part in the development of acute mastitis, was concerned.

Further contributions to the casuistics of "milk poisoning" were published by Jakobsen and Weigmann and Gruber.

II. In 1905, Jakobsen observed symptoms in several persons which he traced to fit. In 1505, Jakobsen observed symptoms in several persons which he fraced to the drinking of milk from one stable. The symptoms were diarrhea, vomiting and fever. Out of 17 persons, 10 who drank the milk became affected, while 7 who did not take any remained well. On May 30, 1905, other persons became affected. The examination of the 32 cows of the dairy showed a streptococcic mastitis

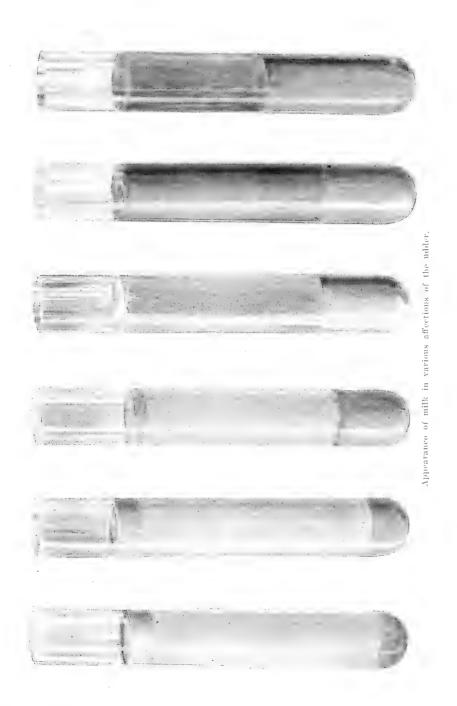
in one animal. The cow was slaughtered and no further cases were reported.

III. Edwards and Severn described an epidemic of follicular tonsilitis which developed from the drinking of milk. They found in the exudate of the throat, and in the milk, in addition to other bacteria pyogenic streptococci which as shown by the investigation were also contained in the secretion of a cow affected with mastitis.

[In various cities of the United States epidemics of sore throat with swelling of the cervical lymph glands, colic, diarrhea and fever lasting several days have occurred which were traced to the use of milk from cows affected with streptococcic mastitis. Such milk when examined was found to contain pus and streptococci in great abundance.—Trans.]

IV. Lameris and Harreveld observed an outbreak of diarrhea among the inmates of a hospital after the drinking of boiled milk, which in part was obtained from cows affected from streptococcie mastitis.

Whether the authors of the last cases are correct in their view that very likely a heat-resisting toxin brought on the disease, or whether the streptococci might remain alive in the milk foam or in the formed membrane, etc., as suspected by Jensen, it becomes evident that boiling does not carry with it an assurance that the danger from streptococcic milk is eliminated. If Jensen's suspicion is correct a proof would be offered that even the smallest



Ernst, Milk Hygiene.



quantities of mastitis streptococci are sufficient for the production of severe intestinal affections.

V. On December 17, 1907, a sample of boiled milk was brought to the official milk control station of Munich; about a half hour after the drinking of this milk the man who delivered the milk, and his family, as well as a neighboring family using milk of the same origin, became sick. The milk contained 1.5:1000 streptococcic pus. It originated from a large dairy. Three producers and one distributor were suspected. In tracing down the cause of the trouble two producers were found whose herds contained six animals with affected udders, their milk being mixed with the whole milk.

tracing down the cause of the trouble two producers were found whose herds contained six animals with affected udders, their milk being mixed with the whole milk. The affection was marked by chills, diarrhea, headaches, and lasted not quite an entire day. The milk constituted the only common food partaken by all, and therefore could be considered, although not with absolute certainty, as the probable cause.

From the examples cited it may be seen that the drinking of milk which contains the secretion of streptococcic infected udders is capable, under certain conditions, of producing injurious effects upon the health of human beings. Considering the frequency of the disease, and the numerous cases where the prohibition of milking affected udders into the whole milk is disregarded, it is to be wondered at that affections which could be traced to the drinking of such milk are not observed with greater frequency.

This may be due to the fact that the secretion of affected quarters is usually very greatly diluted with the milk of healthy quarters, showing that the harmful actions are not necessarily induced by the predomination of the injurious material, and further it may also be due to the fact that the milk is mostly used after being boiled (Trommsdorff, Jensen). That the boiling of the milk is not always sufficient to destroy the injurious properties may be seen from the cases of Holst and Lameris and van Harreveld; the milk of course is marketed in a raw state, and must therefore be judged in the condition in which it is sold.

The factors which induce the harmfulness of the milk from streptococcic animals are not known. Whether the injurious factors are due to the toxin produced by the streptococci of mastitis, or to the products of the disease, as for instance pus (Jensen), or to streptococci which are pathogenic to man as such, cannot at the present time be definitely determined. This, however, is of little importance from a practical standpoint. Some authors, such as Petruschky and Kriebel, consider that infected cows are the sources of milk streptococci, and that these are the principal cause of the summer mortality of children. Seiffert considers the streptococci originating from affected udders as more dangerous than the saprophytic streptococci which contaminate the milk as a result of unclean milking. This view was also expressed by the author in May, 1908, and was confirmed by Trommsdorff.

Neither studies nor animal experiments have succeeded up to the present time in proving the harmfulness of the streptococci by themselves, or the relationship of the mastitis streptococci to human pathogenic strains of streptococci, the animal experiments offering only a relative conclusion on the susceptibility of the respective species of animals.

The differentiation of the mastitis streptococci from other milk streptococci is however, absolutely necessary for milk control since only in the presence of typical mastitis streptococci can the milk dealers be held responsible, and be obliged to prevent the contamination.

Escherich and Holst found streptococci in almost every sample of milk, and Hellens repeatedly isolated them from milk. In 1840 in samples of market milk from Munich and vicinity the author succeeded in isolating streptococci either by cultivation or recognizing them by bacteriological examination in 100% of the cases. Other investigators confirmed a positive finding in a strikingly high percentage.

Beck-Market milk of Berlin
Savage—17 samples of market milk100 %
10 samples of market milk 100 %
Kaiser-Market milk of Graz
Brüning—28 samples of Leipsie market milk
Easten—186 samples $\dots 57^{-57}$
Eastles-185 samples from all parts of England

The simplest proof of the constant occurrence of streptococci in market milk is the usual acid fermentation of cow milk induced by streptococci.

A method for distinguishing these frequently observed streptococci from mastitis streptococci has not yet been discovered, either through the fermentation of various kinds of sugars by the streptococci, or through the investigations of creatinin formations, hemolytic action, acid formation or their actions in the presence of various temperatures of cultivation. It should be borne in mind that the behavior of the various strains of mastitis streptococci has been described in such a variety of ways, that either the presence of remarkably numerous strains or a strong instability of characteristics, or confusion with saprophytic forms, must be accepted.

The formation of acid by the streptococci is sometimes described as strong (Zschokke, Nenski, Groning, Kaiser, Heinemann, Müller, Koning), at other times it appears insignificant (Sven Wall, Rullmann).

Lohnic classes the streptococci of mastitis with the group of lactic acid streptococci, especially with the group of *Streptococci* guntheri, with close relationship to the group of *Streptococci* rosenbach, having the following characteristics:

"Form of the cells variable; capsule formation is frequent and appears to be associated in certain forms with the presence of sugar in the nutritive media. Spores are not formed; the bacteria are Gram-positive; the intensity of the growth has no significance. Coagulation of the milk results, in these varieties, either through acid formation or through a rennet-like ferment; gas formation is rare; the pathogenicity varies remarkably."

Müller in his work on comparative examinations of lactic acid bacteria (Typ. güntheri, etc.) presents the following:

1. "The strains studied manifest marked differences either in their cultural or morphological characteristics with the exception of the strain causing "sour brood" among honey bees."

2. "The action on carbohydrates is practically uniform."

3. "Influencing individual strains relative to their acid formation in the sense of increasing or decreasing it, is possible. The characteristics which the freshly isolated strains possess are more or less permanent."

4. "There exists a relation between the group of Streptococcus güntheri and the Streptococcus agalactiae since their capability of forming acid is about the same." 5. "The off recurring confusion of the two may be explained by certain similar

5. "The off recurring confusion of the two may be explained by certain similar forms of growth which both possess."
6. "The supposition that the pathogenic streptococci represents lactic acid bac-

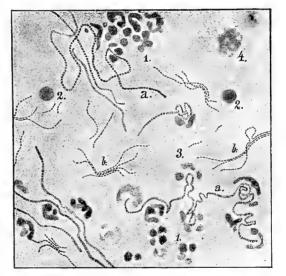
6. "The supposition that the pathogenic streptococci represents lactic acid bacteria of the Typ. güntheri which have adapted themselves to parasitic conditions, is substantiated by the findings, since it was possible in the various strains of streptococci to produce transition forms, which correspond to the Typ. güntheri."

Therefore from these few examples it may be seen that it is impossible to separate the streptococcus of infectious mastitis from the group of the lactic acid streptococci. Nevertheless it would be

a great error to identify the ordinary lactic acid streptococci with pathogenic streptococci of man and animals.

If the fact is taken into consideration that some streptococci, as for instance that of Kefir, the streptococci of sour milk, and others, have a favorable influence on the nutrition of man, the necessity of their strict identification for controlling the milk supply is apparent.

Although it is not possible to **absolutely** differentiate one strain by cultural and biological characteristics, from a culture strain of different origin, nevertheless Fig. 17.



Sediment of milk, one day old, from an udder affected with streptococcic masilits; (a) streptococci of infectious mastitis, (b) subsequently developed streptococci; 1, 2, 3 and 4, cells from the udder.

there are certain morphological characteristics of the streptococci in the smears made from sediments, which are sufficiently constant to absolutely warrant the definite assertion that the streptococci in certain positive cases originated in an infected organ, and were not incidentally leading a saprophytic existence in the milk.

It has been known for a long time that parasitic bacteria in the animal body, under the influence of the animal's protective strength, attain certain peculiarities of form which they lose under ordinary cultural conditions (under certain conditions it is possible to cultivate capsulated anthrax bacilli). Reference is made to the capsule of anthrax bacilli and to the formation of botryomycotic clumps by streptococcic forms. Consideration of the question whether such changes of form in bacteria are developed as protective agents against the immunizing powers of the body, would require too lengthy a discussion. The fact should suffice that streptococci originating from affected udders almost invariably show signs of such transformation. It is not intended to assert that a steptococcus in milk which does not possess these form peculiarities is not a streptococcus of mastitis, or that it does not originate from the udder, and that under abnormal conditions (for instance cultivation at 37 deg. in raw milk or in serum) the streptococci which are present could not undergo changes of form which under certain conditions simulate the forms of "animal" streptococci; but for normal conditions of milk inspection the morphological characteristics of animal streptococci offer certain definite appear-

Fig. 18.

Sediment of market milk in which the typical animal forms of streptococci (a, b, c) make possible a diagnosis that the milk contains the secretion of an animal affected with streptococci mastitis in spite of the occurrence of other forms of streptococci (d and e).

ances of recognition which have always been proved by control tests made in the respective stables.

These characteristics are the following:

The streptococcus takes on a diplococcuslike separation, the cocci apparently press each other, become discshaped, and in profile appear like a dash. Thev stand at right angles to the length of the chain (compare with equine distemper streptococci according to Rabe.) A fine capsule is formed "animal" the around milk streptococci, which is sometimes more, at other times less pro-

nounced. This sometimes swells to a broad mucin capsule (compare Lingelsheim on streptococci, Wassermann-Kolle's Handbook of pathogenic micro-organisms III, pp. 309 and 310, and Sven Wall, p. 29). The endococcus, especially in short chains is spherical or swollen to a club shape.

With slight practice one almost invariably succeeds in distinguishing, by one or the other given characteristics, the "animal" mastitis streptococci from streptococci which have gained access to the milk accidentally (even though they may also possibly be descended from "animal" mastitis cocci).

In this way the author succeeded, from April, 1907, to November, 1908, in demonstrating by the aid of smears that secretions from cows with streptococcic mastitis were mixed with market milk. Out of 1840 microscopically examined samples 336, or 18.26% showed the presence of such an infection.

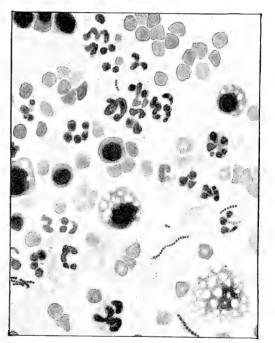
In 91 cases, or 4.945% the changes were not very pronounced; later control however proved that milk from cows affected with yellow garget had been mixed with these shipments.

18.26% + 4.945% = 23.205%, proved contaminated with streptococcic pus out of 1840 milk samples.

Müller intended in his work to distinguish milk streptococci, especially the streptococci of mastitis, from strains of streptococci pathogenic to man. In confirmation of the work of Nieber, Fischer and Borgor, Müller come

and Berger, Müller came to the conclusion that the recognition of milk streptococci pathogenic to man is impossible. Although milk streptococci as a rule coagulate milk somewhat more quickly, there are also strains which coagulate milk somewhat more slowly, and strains which dissolve the blood cells in Schottmüller's blood agar, and these in their agglutination value stand very close to the pathogenic streptococci of man, that is, they agglutinate even in dilutions of the serum of 1:400—800. At the same time several of the absolutely pathogenic strains fail to give any agglutination, and other apparently saprophytic varieties give a higher agglutination value. Bau-

Fig. 19.



Sediment of red milk. Many red blood corpuscles, several polynuclear leucocytes and colostral cells. Streptococcus brevis with capsules.  $1 \times 1000$ .

mann proved that there is no uniform agglutination value of the individual kinds of streptococci, and that spontaneous agglutination frequently appears in tests of their cultures.

Together with Horauf, the author found that mastitis strains show similar characteristics on Schottmüller's blood agar to the less pathogenic strains of man, a fact which has recently been confirmed by Gminder. Lingelsheim makes the statement that streptococci producing toxins are always obtained from subacute and chronic processes.

Acid formation and milk coagulation are common to the entire

group of pathogenic streptococci. Of the pyogenic strains of man, according to Andrewes, the *Streptococcus pyogenes* and the *Streptococcus mitis* produce acidity without coagulation. Sven Wall proved these characteristics from the mastitis strains isolated by him. According to Adametz, the mastitis cocci sometimes coagulate very intensely, the same as is the case with the streptococci of enteritis of sucklings (Petruschky). The fermentation in various sugars using Gordon's bouillon mixtures, varies greatly with the different pathogenic streptococci and milk streptococci, so that the possibility of differentiation by this means is quite impossible, which is likewise the case by testing their virulence on small test



Sediment of milk from an udder with acute inflammation. Short forms of streptococci.  $1 \times 1000$ .

animals. Pathogenic strains may at times show great variations of virulence, while according to Heinemann strains of the Streptococcus lacticus may become virulent by passage through rabbits, until they will produce changes in rabbits which correspond in their appearance, extension and character, to those caused by pyogenic strains recovered from man. Through their action on animal bile, or on sodium taurocholate, Mandelbaum differentiates the mucosus Streptococcus and *Pneumococcus* from Streptococcus pyogenes and other streptococci (Neufeld, v. Levy). The author used the mastitis strains he had on hand on cattle, hog, horse and

chicken bile, but failed to observe either a clearing of the culture media or an influence upon the form and appearance as shown by the microscope.

The establishment of the "virulence number" through phagocytic experiments also fails to yield the desired result.

In short up to the present time the absolute separation of culture strains of varied origin, the differentiation of saprophytic streptocccci from mastitis streptocccci, and these from pathogenic streptocccci of man has not been successfully accomplished. We have, however, in certain morphologic indications, for instance

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the cross-position of the segments, the capsule-like covering and other characters, a way of distinguishing streptococci originating in the udder of an animal from such as have subsequently gotten into the milk. If these distinguishing signs are present then smears from the sediment of market milk permit the deduction that secretion from an affected udder has been included in the milk. If, on the other hand, these signs are not present in the streptococci of the milk, it cannot be asserted that the milk is not contaminated with the secretion of affected udders.

Although it is not known what factors (streptococci, toxins, inflammatory products) convey the unwholesomeness to the milk, and although in spite of the frequent occurrence of mastitis injurious effects result with relative rarity. nevertheless the secretion from udders affected with streptococcic mastitis. and mixed milk which is contaminated with such secretion should be considered capable of impairing the human health, since

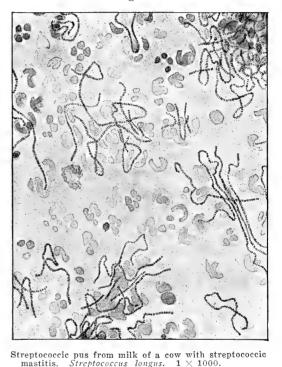
1. There are known cases in which severe disturbances of health resulted from the ingestion of such milk.

2. The investigations of streptococci scientifically justify the

suspicion of harm arising from their ingestion.

What are the conditions of the mastitis streptococci among themselves? Formerly a *Streptococcus brevis* and a *Streptococcus longus* were distinguished, but it was shown that these distinguishing features were not absolute (Staeheli). The differences of the individual strains and the forms of their growth in culture are as inconstant as their pathogenicity, acid formation, and other biological characteristics, so that, as expressed by Kitt, it would be necessary to distinguish as many varieties as there are mastitis cases if it was desired to accept the differences of the individual mastitis strains as indicative of different varieties. All the smaller and greater differences should be considered as indications of adapta-

Fig. 21.



tion to the various energies of reaction of the various animals and organs, and as the investigations of the author showed, to energy reaction of the same milk gland at different times. For instance it appears that certain changes in form bear a definite relation to the number of leucocytes in the milk. Thus the author obtained the following results in the same quarter of a cow examined at different times:

# L.—Leucocytes.

Str.—Streptococci.

Cow No. 29 1 right fore 2 left fore 3 right hind 4 left hind	Dec. 17, L. 0.3 0.2 500.0 500.0	1908. Str. 0 longus longus	Jan. L. .1 0.9 2.0 20.0	4, 1909. Str. 0 Dipl. brevis longus	Jan. 20 L. 0.5 0.4 1.9 0.5	, 1909. Str. 0 brevis brevis brevis	Jan. L. 0.2 2.0 3.0 10.0	25, 1909. Str 0 brevis brevis longus
Cow No. 34 1 right fore 2 left fore 3 right hind 4 left hind	Dec. 1 0.1 0.2 0.4 0.3	.7, 1908. 0 Dipl. Dipl. Dipl.	Jan. 0.2 Drops 0.3 0.5		Jan. 0.1 0.3 3.0 4.0	16, 1909 0 longus longus	Jan. 2 0.2 0.5 50.9 60.0	29. 1909. 0 0 brevis brevis
Cow No. 31 1 right fore . 2 left fore 3 right hind . 4 left hind			$\begin{array}{c} \mathrm{L.} \\ 0.5 \end{array}$	18, 1908. Str. Dipl. 0 Dipl. brevis	2		mediu bi	
Cow No. 33 1 right fore . 2 left fore 3 right hind . 4 left hind			$2.0 \\ 0.5 \\ 1.3 \\ 0.9$	brevis 0 brevis brevis		$\begin{array}{c} 0.3 \\ 0.2 \\ 20.0 \\ 10.0 \end{array}$	lo	Dipl. 0 ngus ngus
Cow No. 58 1 right fore . 2 left fore 3 right hind . 4 left hind			0.1	19, 1908. 0 brevis 0 0		Jan. 5 0.5 0.3 0.1 0.1	Ι	Dipl. Dipl. 0 0

These differences in the forms of streptococci may be seen during one milking on the same animal, if they are compared at the beginning, the middle and at the conclusion of the milking. These differences are only slight, so that no definite conclusions should be drawn from them.

If however the results from various animals are compared it may be seen, as already indicated above, that certain relations exist between the number of leucocytes and the forms of the streptococci since the streptococci become longer as the number of leucocytes increases. The experiments extended from December 11, 1908, to February 8, 1909, and include three stables with a total of 149 cows.

Of these 149 cows 59, or 39.6% were more or less affected. In most animals (140), all quarters were separately examined and

showed that out of 560 quarters 112, or 20% were affected. Fortytwo of these gave at times a greater, at other times a smaller secretion with a distinctly changed consistency. The other 70 manifested the infection only after sedimentation, or only through microscopical examination. Some of these 12 quarters were successively (see above) examined, so that the material used for smears from affected quarters, and which had been microscopically examined, amounted during the period mentioned, to 134.

Fifty-five affected quarters showed the *Streptococcus brevis*, 32 the *Streptococcus longus*; in 47 the infective agent was recognized in the form of a diplococcus. The 47 quarters with diplococci had as a rule a very small leucocytic number. In values of over 2.0, longer coccus-chains were always observed.

32 quarters out of the 47 had 0.5:1000 leucocytes

12 quarters out of the 47 had 1.0:1000 leucocytes

3 quarters out of the 47 had 2.0:1000 leucocytes

In the 55 cases with *Streptococcus brevis* the change in the leucocytic number varied to a greater extent.

In 18 it represented 0.5:1000 or 32.73%.

In 14 it represented 1.0:1000 or 25.47%.

In 8 it represented 2.0:1000 or 14.55%.

In 3 it represented over 2.0:1000 or 5.45%.

In 12 it represented 5-20 and more :1000 or 21.82%.

The 32 longus-cases were divided as follows:

Leucocytic Quantity.	Number of Cases.	Percentage.
Under 0.5:1000	1	3.125
up to 1.0:1000	1	3.125
up to 2.0:1000	3	9.375
up to 5.0:1000	. 3	9.375
up to 20.0:1000	3	9.375
to 100 and more:1000	21	65.625
· /1 · ·		

In other words:

up

## In leucocytic quantities

Up to	0.5, 63.00%	showed			brevis.	2.00%	Dinl.	longus
υρτο	1.0, 44.40%	showed			brevis	370%	Dinl	longue
υρτο	2.0, 17.05%	showed		64 7 22	hrovis	17 640%	Dinl	longung
Up to	<b>5.0</b> , <b>0.00%</b>	showed			brevis	50 00%	Dinl	longue
Up to :	20.0, and mo	re 0.00%	showed.	33.0%	brevis,	66.60%	Dipl.	longus

The leucocytic values will be taken up again later in the discussion of the "Trommsdorff" test.

From this tabulation it may be seen that the length of the chains actually grows with the increase of leucocytes, or with the amount of sediment. In high leucocytic values and short forms of the infective agents, the latter are frequently present in exceedingly large quantities.

The opinion that the form of the streptococcus represents an adaptation to the energy reaction of the respective animal and organ is thereby substantiated especially when the streptococci are found in the secretion of one and the same part of the udder of a cow at different times.

In the same way it is impossible to establish rules for definite differentiation of the streptococci of the yellow garget among themselves through the study of their morphological relations, by comparison of their biochemic characteristics or the pathogenic virulence of individual strains, since the acid production and acid susceptibility which are present in mastitis streptococci at first may be easily changed by artificial means, and individual strains have proven the possession of stronger, others a weaker pathogenic action for test animals (Gröning, Sven Wall).

The author does not desire by any means to establish a theory of unity for mastitis streptococci. To be sure there are marked differences in the various strains, especially in regard to the production of clinical symptoms, which cannot be attributed alone to the variation of virulence, and to unequal resisting powers.

It is possible that with the aid of newer methods of differentiation (blood media, etc.) it will be possible to establish a fundamental type of mastitis streptococci in strains freshly cultivated from animals. Even if with the continuance of cultivation new characteristics, as for instance hemolytic properties, may be acquired by the cultures, and the earlier characteristics become lost, the characteristics acquired by the respective strains of streptococci in their former growth may remain constant for a sufficient length of time to permit the establishment of the type of varieties, as has already been the case with the streptococci of man (Petruschky, Schottmüller, Baumann, Schulze and others). Enrst, Gminder and others have demonstrated that the mastitis streptococci mostly correspond to the mitior seu viridans or mucosus hom. group, respectively.

Based on the grounds previously described, milk hygienists, bacteriologists, children specialists and veterinarians sometimes more and at other times less imperatively have demanded the exclusion of cows with streptococcic mastitis from the production of milk (Jensen, Weigmann, Rievel, Sven Wall, Ruhm, Trommsdorff, Seiffert, Ernst and others).

This requirement is natural from the hygienic standpoint, but its practical execution is rendered very difficult by the remarkable prevalence of the disease, and as a matter of fact as long as the general control of production and the examination of milk of individual cows are not required a thorough enforcement cannot be hoped for.

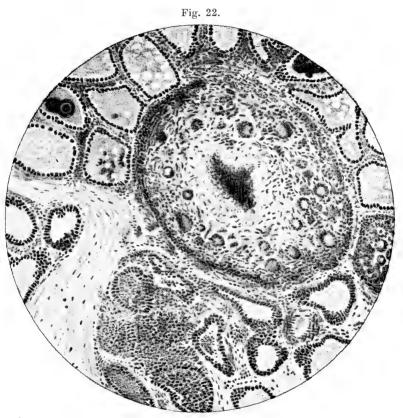
For the present the exclusion from the market of all milk which shows changes in a recognizable way, as for instance through a collection of yellow sediment, should be considered satisfactory. At the same time from an economic standpoint only milk from affected quarters should be excluded, while the sale of milk from healthy quarters should be allowed.

## TUBERCULOSIS.

# Occurrence of Tuberculosis in Cattle.

Tuberculosis of the udder in cows appears with relative frequency, corresponding to the frequent occurrence of tuberculosis in cattle.

Tuberculosis in the **udder** is manifested in different forms, the circumscribed, lobular, focal tuberculosis and the diffuse tuber-



Tuberculosis of the udder. Progressive miliary form.

culosis extending over and infiltrating the entire udder. Two of the different forms may be present at the same time in the organ, and from one form through accentuation of the infection the other forms may result.

Clinically tuberculosis is manifested by nodular swelling or hard enlargement of the affected quarter, and with enlargement and painless nodular swelling of the supramammary lymph glands. These changes, however develop very slowly and the milk from such infected quarters for weeks and months may contain millions of

### Tuberculosis.

tubercle bacilli without the udder indicating any special lesions, and without the milk showing any noteworthy changes.

The quantity of the secretion from a tuberculous quarter is at first uninfluenced, or only slightly so; later it is considerably diminished. For a long time it is of a normal appearance; later it generally becomes thick, transparent, watery, intermixed with small flakes, or again it may become thick, yellow, pus-like, depending on the intensity and extension of the lesions in the udder and the influence of the general health of the cow.

Tuberculosis of the udder as a rule is of an embolic character, and rarely represents the result of a galactiferous infection. Nocard, Meyer, Calmette and Guerin, and Zwick succeeded in producing tuberculosis of the udder of various characters, by injections of bovine and human tubercle bacilli into the milk duct of the teats. The pathologico-anatomical appearance in these instances was the same.

The extent of the spread of tuberculosis in cattle in general is best indicated by the statistics of abattoirs in the different states.

In Germany in 1904, 17.89%, in 1905, 19.16%, in 1906, 20.66%, and in 1907, 21.21% of cattle were retained on account of tuberculosis, and 0.26%, 0.30%, and 0.35%, respectively, on account of tuberculosis of the udder. In Bavaria in 1898, 5.7%; 1900, 6.0%; 1902, 6.8%; 1904, 9.2%; 1906, 10.31%, were tuberculous.

The spread of tuberculosis is especially assisted by exposure in stabling (in 1907, 7.28% of young stock, 18.54% bulls, 22.55% steers, and 29.62% of cows were found to be tuberculous on slaughter, against 5.3%, 13.9%, 18.3% and 25.3%, respectively, in 1904), and forced feeding while the percentage in pasture animals diminishes considerably, and in range cattle tuberculosis is practically unknown. In Prussia the total infection from 1898 to 1906 is estimated at 16.09 to 23.4%, in Saxony from 30.46 to 37.58%, from 1898 to 1908. In France in certain localities the infection exists in 30 to 40% of the stock, an average of 10%. In other countries similar conditions obtain.

If the abattoir findings are not considered as indicative of real conditions, and delicate biological methods are employed which prove that an animal is infected with the tubercle bacillus (without however manifesting anatomically demonstrable changes) the increase of the numbers is considerable.

In Saxony from 1891 to 1897, in round numbers two-thirds of the cattle were found to be infected through the aid of tuberculin. Ostertag accepts 25% of the cattle as infected in northern Germany and around Stuttgart, basing his estimate on sample testing in the various localities. In France from 50-80% of the animals reacted, in Great Britain 27%, in Austria 14-60%, in Hungary up to 35.18%, Belguim 48.8%, Norway 22.8%, Sweden about 31%, Finland 13.7%. In Denmark during the first years of the tuberculosis eradication 38.5 to 40% of the animals gave tuberculin reactions, while later only 8.5% reacted. [The extent of tuberculous infection among cattle in the United States is indicated by the statistical reports of the Federal Bureau of Animal Industry. According to these figures out of 400,000 cattle tested, there were 37,000 reactors or 9.25%. The majority of these animals were dairy cattle, from which fact the conclusion has been drawn that approximately 10% of the dairy cattle in this country are affected with tuberculosis. On the other hand the meat inspection statistics show that 6,978,293 cattle were slaughtered in official establishments during 1913, of which 75,870 were found tuberculous, suggesting that probably 1% of beef cattle are affected with tuberculosis to some degree.—Trans.]

The relative frequency of tuberculosis of the udder corresponds to the numerous occurrences of bovine tuberculosis.

According to Bergmann 3.5% of the tuberculous cows slaughtered at Malmo were also affected with tuberculosis of the udder. Ostertag estimates the appearance of tuberculosis of the udder in 0.1 to 0.3% for Germany. These figures of course increase in localities in which the other forms of tuberculosis occur with greater frequency. Thus Meyer-Stendal report that out of 818 cows 4%showed udder tuberculosis. Meissner found in 1910 from all cows examined in Posen 0.32% affected with udder tuberculosis.

Bugge mentions out of 16,050 cows included in the eradication work at Schleswig-Holstein in 1906, the presence of tubercle bacilli in 30 out of 318 examinations of mixed milk, and in 27 out of 562 individual milk tests of suspected cows.

In 1907 the tests for tuberculosis of 258 samples of mixed milk, revealed tubercle bacilli in 35 while out of 597 individual milk samples, tubercle bacilli were found in 32, which corresponds to 0.14% of open udder tuberculosis in the 23,278 examined animals (pulmonary tuberculosis 5.1%).

In 1908 these numbers amounted to 0.124 to 2.644% respectively out of 38,454 animals.

In the presence of such an extension it should be not at all surprising that **market milk** contains tubercle bacilli with extraordinary frequency. Examinations for this purpose were undertaken as early as 1893 by Montefusko in Naples, in 1894 by Friis in Copenhagen, in 1895 by Obermuller and Fiorentini, in 1898 by Petri, 1900 by Beck in Berlin, in 1895 by Zacharbeko in Petersburg, 1897 by Massone in Genoa, Buege in Halle, Boyce and Delepine in Liverpool, Klein in London, Nonewitsch in Wilna, Stepanow in Kasan, Bujwid in Krakau, and in 1905 to 1906 by Eber in Leipzig, with varying results.

The numbers of tubercular infection of market milk obtained (other investigators for instance Brusaferro, Roth, Schuchardt, Gröning, Petri, Rabinowitsch, Hermann and Morgenroth, Ascher, Coggi, Bonhoff, Herbert, Markl, Herr and Beninde, Eber and others experimented with butter and cheese) fluctuated between 0 and 100%. It is to be expected that all market milk, no matter of what origin, may occasionally be infected with tubercle bacilli; all milk in the production of which no special care is taken in the selection of the milking animals and no clinical examination or tuberculinization of the animals has been undertaken, should be suspected of containing tubercle bacilli, and the larger the herd which produces the milk, the greater the danger.

German investigators established the following figures for the presence of tubercle bacilli in market milk:

Author.	Samples,	Place. Tubered	ılar %.
Obermuller	13	Berlin	61
Buege	6	Halle	33.3
Petri	64	Berlin	14
Beck	56	Berlin	30.3
Proskauer	9	Market milk Berlin	55.5
Seeligmann	5	Dairies under veterinary control	0.
Croner	13	Danish milk	38.5
Eber	210	Leipzig	10.5

[The percentage of tubercle bacilli found in the milk supply of large cities in this country has been accurately determined in only a few instances. In 1907 Anderson proved that in Washington, D. C., 10.7% of the dairies supplied milk containing virulent tubercle bacilli, Schroeder found 7.7% of the 26 dairies examined were distributing infected milk to Washington, D. C., while still later Mohler showed that about 3% of the 73 samples of milk examined contained tubercle bacilli. The apparent discrepancy in these results may be readily explained by the fact that during the last 6 years strenuous efforts have been carried on by the Bureau of Animal Industry to eradicate tuberculosis from among these herds, with the result that in the District of Columbia the number of tuberculous animals has been reduced from 18.8% to 1.2% in 1914. Hess has found that 17, or 16%, of 107 samples of milk distributed in New York City contained virulent tubercle bacilli. while Campbell made extensive investigations of the occurrence of tubercle bacilli in the market milk of Philadelphia, and found 18 or 13.8% of the 130 samples examined to contain living bacilli of tuberculosis.-Trans.]

# Under What Conditions Do Tubercle Bacilli Enter the Milk?

The infection with tubercle bacilli is natural when the animal is affected with tuberculosis of the udder, or may occur indirectly when through contamination of the udder with feces in pulmonary or intestinal tuberculosis, urine or vaginal secretion in kidney or uterine tuberculosis, or with infected straw, tubercle bacilli are brushed off from the soiled udder into the drawn milk, or when in open tuberculosis of the lungs the bacteria get into the milk through the air or straw.

As early as 1869, prior to the discovery of the tubercle bacillus by Koch in 1882, Gerlach proved the infectiousness of milk from highly tuberculous animals through feeding and inoculation experiments. This was also emphasized by Zürn, Klebs, Sommer, and in 1880 by Bollinger, who first pointed to the fact that the milk of a tuberculous cow in which the udder is not noticeably affected may contain tubercle bacilli. The same results are shown by the works of Stein, Bang, Hirschberger, Ernst, Schroeder and Fiorenti, who succeeded in producing tuberculosis in test animals with milk of tuberculous cattle, even though there was no udder tuberculosis present. Milk from tuberculous udders always has been proved to be especially dangerous (May, Bang).

If special care were exercised in milking, it not infrequently happens even in extensive, generalized tuberculosis that the inoculated animals remain well; thus Nocard from injecting milk of 54 cows affected with generalized tuberculosis succeeded only in 3 cases in producing inoculation tuberculosis. Therefore it appears that in spite of a generalized tuberculosis when udder tuberculosis is not present, tubercle bacilli are not always excreted with the milk; the possibility of elimination however, that is, that the milk of such a tuberculous cow may contain tubercle bacilli, should at all times be given consideration.

Is milk liable to be affected when tuberculosis cannot be clinically demonstrated in suspected cows, or when they appear healthy and yet react to tuberculin? Are tubercle bacilli eliminated only with the milk from animals affected with tuberculosis of the udder, or also in cases where the udder is not affected by tuberculosis? These questions may be answered at the present time with great certainty, namely, that tubercle bacilli of cattle are eliminated with the milk as a rule only in animals which are affected with tubercular mastitis. This question is of especial interest in the eradication of tuberculosis in the dairy herd, since it is well known that calves and hogs are highly susceptible to the tubercle bacillus of cattle. It will be advisable therefore to refer to the more important publications on the elimination of tubercle bacilli with the milk, before entering into the question of the harmfulness of such for man.

Delepine, Ravenel, Rabinowitsch and Kempner, Gehrmann, Gehrmann and Evans, Moussu and Mohler obtained positive results from milk of animals not clinically affected, but reacting to the tuberculin test, through inoculating or feeding of test animals.

Other investigators, as Martel and Guerin, also Hirschberger, aimed to solve the question by inoculating the milk of slaughtered animals or glandular substance from udders of reacting animals. Their work also frequently gave positive results. All of these authors therefore, conclude with great certainty that tubercle bacilli may be eliminated with the milk even from animals which are not clinically affected with tuberculosis.

Other views are supported by Ascher, Muller, Ostertag, Stenstrom, McWeeney, Pusch and Hessler as the result of their negative findings, namely, if the experiments were conducted under the most painstaking requirements and all contaminations through infected straw, etc., were avoided as much as possible, they failed in spite of numerous experiments in producing tuberculosis with milk from a tuberculin reacting animal, and frequently not even with the milk from an animal elinically affected, but free from tuberculosis of the udder.

From the standpoint of milk hygiene the fact is important that in the work of tuberculosis eradication by the agricultural societies the examinations for tubercle bacilli in the mixed milk of individual herds were mostly negative after the clinically affected tubercular animals had been eliminated. According to the works of Muller and Hessler until July 1907, 2,949 samples of mixed milk of individual herds were examined; all of these herds were subjected to the Siedamgrotzky-Ostertag method of eradication. From 30 to 200 cows participated in each test and 156 herds gave milk free of tubercle bacilli. As eliminators of tubercle bacilli were found:

Two cows with udder tuberculosis, 8 times each.

One or more cows with tuberculosis of the uterus, 16 times. One or more cows with tuberculosis of the uterus, 6 times. Once kidney and uterine tuberculosis.

Once pulmonary and intestinal tuberculosis.

Once a cow whose saliva contained tubercle bacilli and

19 times open pulmonary tuberculosis.

In five positive tests there was no clinically demonstrable form of tuberculosis, and the subsequent tests of immediately drawn control samples remained negative. These five cases were observed in the beginning of the eradication work.

The remaining 2,793 milk samples were free of tuberculosis in spite of the fact that among the animals of these herds there were surely a great number which would have positively reacted to tuberculin tests.

The five cases observed at the beginning of the eradication work, in which the milk contained tubercle bacilli, although elinically open cases of tuberculosis could not be found on stable examination, are explained by Hessler in that the milk became contaminated with particles of the feces from pulmonary cases of tuberculosis, which had not yet developed elinically. This is also suggested by the smaller number of bacilli found in the milk.

Tubercle bacilli therefore occur in the milk in great numbers when animals with open tuberculosis, and tuberculosis of the udder stand in the stable. Milk from animals which manifest their tuberculosis by a positive tuberculin reaction, will usually be free from tubercle bacilli. Such cows belong to the least dangerous class. Nevertheless the investigations of Rabinowitsch, Kempner, Ravenel and others, who obtained positive inoculation results with milk from reacting animals, prove that such milk may at times contain tubercle bacilli in small numbers. Ordinarily however this will not be the case, and the milk of such animals may, as a whole, be considered free from tubercle bacilli.

Therefore it appears evident that under present conditions of milk production the ingestion of tubercle bacilli with milk is possible at almost all times.

# What Danger Threatens Man Through Ingestion of Milk Which Contains Bovine Tubercle Bacilli?

In order to answer this question it is necessary to refer to the development of tuberculosis in man, and to consider the factors which are necessary for an infection of his body.

These factors in addition to the toxicity of the infective agent, and the quantity in which it has the opportunity to enter the body, depend upon the avenues of infection which it takes and the local and general resistance of the attacked individuals towards the specific infective agent.

There is perfect agreement relative to the virulence of the bovine tubercle bacillus for man. The bovine tubercle bacillus is a strain of the tubercle bacillus with such pregnant characteristics that it is almost invariably possible to classify it separately from other strains when obtained in culture, that is, to distinguish the bovine tubercle bacillus from the bacillus of the human type.

These bacilli are distinguished as the **typus bovinus** and **typus humanus** (only these two types need to be considered from the standpoint of milk hygiene) which are characterized by the following peculiarities:

# Typ. Bovinus.

The growth is delicate and in the thin film small wart-like colonies develop; on bouillon a mesh-like fine membrane with wart-like prominences develops proliferating downward, or a membrane of tissue-paper thickness results; the bouillon is probably neutralized and finally becomes alkaline.

The bovine tubercle bacillus is as a rule of greater toxicity for smaller mammalia. Rabbits develop through intravenous injections of the bacillus (0.001 gm.), a generalized tuberculosis from which the animals succumb inside of three weeks. When 0.01 gm. is injected under the abdominal skin, it produces in a short time, generalized tuberculosis.

Cattle succumb readily to

# Typ. Humanus.

The growth is a luxuriant, uniformly thick and wrinkled membrane, which proliferates on the wall of the tube. The growth is the same in bouillon; the degree of acidity of the bouillon is usually at first diminished, later increased.

0.001 gm. of bacilli of the human type intravenously injected produces after months only a chronic form of tuberculosis (joints, kidneys, lungs, testicles).

The injection under the abdominal wall produces only local lesions.

Inoculated into cattle the bacilli of the human type (0.05 gm. subcutaneously), produce only slight or no pathogenic action. The process remains local, and extends only to the

infection with the typus bovin- | neighboring glands; a great us, from an extensive form of tuberculosis. Guinea pigs die more quickly from an inoculation with boyine tubercle bacilli than from an inoculation with the bacillus of human type.

healing tendency prevails.

Intravenous injections of 1 mg. of the typus humanus into the vein of a mouse will show it to possess a greater resistance than when inoculated with the typus bovinus.

In the last 10 years about 2000 strains of tuberele bacilli from man and cattle have been cultivated and studied. In these studies even further differences were found which, however, are not as constant as those given above; for instance, the bacillus of the human type in glycerin bouillon cultures is delicate, slender, slightly curved, and of beaded staining qualities, whereas the bovine strain is regular, plump, thick without granular differentiation in staining, and frequently with swollen ends. The pigment formation on glycerin potato is more typical of the human type than the bovine type. The former when placed on serum in hermetically sealed glass tubes remains viable for twelve months, the latter for over a year.

Contrary to the views of many investigators of tuberculosis, Nocard, Hueppe, Von Behring, Römer, de Jong and others support the theory that the tubercle bacillus adapts itself to the infected animal and becomes transformed as a result of its environment. Rabinowitsch, Dammann and Eber have also supported this transformation theory. The latter especially attempted to prove by extensive experiments and investigations that Bacillus humanus, by passage through cattle, changes into the bovine type. This question however appears at the present, to be decided in favor of the stability of the bacillus.

It has at least been shown with six various strains of the human type, that in passage experiments through 2 to 7 cattle, in from 247 to 512 days, the character of the bacillus was not changed (English Commission and Weber), and that bacilli of the human type by eight subsequent passages through goats, in 516 days, and by four passages through cattle in 685 days, were not influenced in their typical characteristics.

The immunization experiments which were undertaken with the bacillus of the human type on cattle showed no changes whatsoever in the human type after the presence of the bacteria in cattle for a year and seven months (Baldwin) in spite of their propagation in the udder of the cow. The same results were obtained in three experi-ments by Weber, Titze and Joern, who allowed the bacillus of the human type to exist in the body of cattle for two years and one month and for two years and six months.

Eber's experiments found no confirmation in the Imperial Board of Health; the experiments however are being continued in strict co-operation with Eber.

## On the contrary it is shown that the bovine bacillus constantly retains its characteristics within the human body.

From a boy who was affected since his second year with tuberculosis of the fourth digital bone of his hand, it was possible during surgical interference to obtain material from the same place at five different times, during his age from 8 to 13 years. The infection was caused by the bovine bacillus.

In spite of their existence for ten and a half years in the human body these boyine bacilli had retained their characteristics. A marked influence in their virulence was manifested, however, since the bacilli, from the second operation, in quantities of 2 mg. could no longer kill rabbits even after intravenous inoculations. From the subsequent operations it was found that the virulence was again increased.

Griffith obtained the same results with bovine cultures from lupus from which the bacilli were isolated six months, two and three and a half years, respectively, after the first examination. It is true that the virulence was several times lower than is or-dinarily the case with the bovine type; the other characteristics of the strain however were tenaciously retained. In one of the cases the bacillus persisted in the human body for  $18\frac{1}{2}$  years. Passage through rabbits and cattle again increased its virulence. Although through animal passage a change of the virulence is possible, nevertheless this change results only inside of the borders of the type and in the direction of the type. The bovine bacillus therefore does not pass into the human type, nor the latter bacillus into the bovine type.

Only a brief statement will be made relative to the so-called atypical strains. It has been demonstrated that there are cultural strains which cannot be classified as belonging either to one or the other type (Kossel, Weber and Heuss, Lydia Rabinowitsch, de Jong and others). These strains proved to be mixed cultures of both types. In the same person not only mixed infections of both types may exist in the affected organs, but also a double infection may occur in such a way that in one organ the Typus humanus, and in the other organ the Typus bovinus, may be found in pure culture (Weber, Weber and Taute, Griffith, Park and Krumwiede, Steffenhagen).

In 1901 Koch explained at the International Tuberculosis Congress at London, that tuberculosis of man is produced by a tubercle bacillus which differs from the bovine tubercle bacillus, and expressed himself as opposed to the general prevailing opinion of that time, regarding the great danger of the cattle tubercle bacillus for man, and as believing that the transmissibility of bovine tuberculosis to man was so slight compared with the danger which threatens man from tuberculous human beings, that its practical importance was negligible.

Although Koch's statement cannot stand in the directness of his declaration, nevertheless at the present time it is generally accepted from the above mentioned differential characters, that marked differences exist between the bacillus of bovine tuberculosis and that of man, and it is a fruitless work to dispute whether they are differences of varieties or peculiarities of the different strains, which lead to the variations, if we accept the fact that the differences of the strains are obstinately retained.

The results are of especial value in differentiating the two types of tubercle bacilli. In association with Shütz, Koch undertook some experiments to establish points of differentiation.

Nineteen calves which were infected intravenously, subcutaneously, intraperitoneally, by inhalation or feeding experiments with the *Bacillus humanus*, showed no manifestations of disease, increased in weight, and on autopsy conducted several months after infection, showed only caseous purulent changes at the point of inoculation. On the other hand, after the inoculation of bovine tubercle bacilli, severe febrile symptoms and extensive tuberculosis, especially of the lungs, liver and spleen resulted. The same results were obtained from the experiments of Kossel, Weber, Heuss. Bacilli of the human type were retained in the regional lymph glands; the changes induced by them gradually retrogressed, whereas infection with the bovine type of the bacillus led to a progressive tuberculosis. Inhalation and feeding experiments showed the slight virulence of the human tubercle bacillus for cattle.

In the experiments of Nocard, Meyer, Calmette and Guerin and Zwick, the inoculation of tubercle bacilli of bovine origin into the milk ducts resulted in a tuberculosis of the udder with rapid emaciation of the animal, terminating in death; whereas the bacilli of human origin produced only a passing inflammatory irritation, and an interstitial atrophy of the udder. Calves which nursed on these latter infected udders remained healthy (Zwick and Maier), or on the other hand (in one case of Zwick) intestinal tuberculosis, with tuberculosis of the mesenteric lymph glands, developed. At autopsy undertaken 20 weeks after the infection, the udder of the cow showed atrophy with miliary tuberculosis, without however typical tuberculous changes in the regional lymph glands.

From these results the conclusion may be drawn that tubercle bacilli of human origin are only very slightly dangerous for cattle. It should be considered however that occasionally after artificial infections the bacilli may persist in the infected region, with or without marked local or at times even generalized changes. Calves may develop intestinal tuberculosis or tuberculosis of the mesenteric lymph glands as a result of ingesting a large amount of tubercle bacilli of the human type.

Almost the same relation exists in man towards the bacillus of bovine tuberculosis. The principal dangers threatening man are through the possibility of infection from affected human beings, and less so to the possibility of infection with diseased products of animal origin, as for instance milk. The possibility of tuberculosis infection through animal products is presented with remarkable frequency, as may be seen from the above statements; still the rarity of infection with the bovine type is quite striking.

Hogs which become readily infected with the bovine type are very frequently affected by the ingestion of skimmed milk containing tubercle bacilli. In northern Germany some of the herds show an infection of 50-60, occasionally

In northern Germany some of the herds show an infection of 50-60, occasionally even up to 90%. The experience at the tuberculosis eradication stations indicated that by the elimination of cattle affected with open tuberculosis a marked reduction was obtained in tuberculosis of hogs, and that this measure in association with pasteurization of the skimmed milk, offers a certain remedy against the spread of tuberculosis of hogs.

The same opportunity which is afforded hogs to contract tubercle bacilli from the feeding of skimmed milk, would apply to man. The relative infrequency of the infection of man with the bovine type of tubercle bacillus is not the result of a milder virulence of the bacilli but is due to the previous boiling of the milk. Convincing observations have also been made on this point.

However before entering into a discussion of these, it will be advisable to illustrate further the possibility of infection for man from the standpoint of the port of entry, and also show the relative condition existing between the necessary infective quantity of bacteria and the establishment of the disease. The development of the affection depends on the most varied conditions, on the quantity of the introduced virus, condition of the port of entry, general resistance, etc.

# Frequency of Tuberculous Infection Through the Alimentary Tract.

If the lesser virulence of the bovine type for man, as compared to the human type, is left out of consideration, which fact is considered satisfactorily proven, the experiments of Ostermann, Schroeder and Cotton show what great quantities of infectious material are necessary in order to produce tuberculosis by ingestion.

Schroeder and Cotton fed milk artificially infected with tubercle bacilli, and proved that infected milk which invariably produced tuberculosis when inoculated intraabdominally in 5 c. c. doses, could be fed for 30 days without causing the disease in the experimental animals.

The dilutions were prepared (1) by adding one platinum loopful of a cloudy suspension of tubercle bacilli to 10 c. c. of milk. (2) by adding one loopful of the original suspension to 10 c. c. of sterile water, and of this dilution one loopful was placed into 10 c. c. of milk, (3) by adding one loopful of the original suspension to 100 c. c. of sterile water and of this dilution one loopful was placed into 10 c. c. of milk. It was not possible to produce ingestion tuberculosis with either the second or third dilution during the period of the experiment, although the dilutions were not as high as they occur in the milk of tuberculous animals (with the exception of tuberculosis of the udder).

Ostermann by comparing the average number of tubercle bacilli in cow's milk with the minimal dose necessary for producing ingestion tuberculosis in guinea pigs, rabbits and goats, came to the conclusion that an alimentary infection is exceedingly rare.

Nevertheless the danger of an alimentary infection with bovine tubercle bacilli, even in high dilutions of the tuberculous material in market milk, cannot be disregarded.

The danger of infection to which small children are exposed from the ingestion of food (without attempting to distinguish "bovine tuberculosis" from "human tuberculosis") is best illustrated by the clinical cases and also those cases of intestinal and mesenteric tuberculosis which are found on autopsy.

Edens, from October 1, 1904, to September 30, 1905, found 12%, and from this time until September 30, 1906, 13.6% of the bodies of children which he autopsied at the ages of 1 to 15 years, affected with primary intestinal tuberculosis or tuberculosis of the mesenteric lymph glands, whereas in man from 15 to 19 years of age only 3.8% and 2.6%, respectively (all autopsies), showed the disease.

The intestinal tract of children appears therefore to be a prominent port of entry for the tubercle bacillus, which is also proven by the works of Orth, Henke, Chiechanowski, Hamburger, Nebelthau, Lubarsch, Bruning, Fibiger and Jensen, Symes and Fischer, Price and Jones, Kingsford, Harbitz, Ogyia, Edens, Wagener and Heller, who demonstrated primary intestinal tuber-

## Tuberculosis.

culosis in varying proportions, up to 47.6% of the tuberculous The frequency, however, with which tubercle bacilli children. actually pass through the intestinal wall without producing demonstrable changes in the intestines and mesenteric lymph glands cannot be stated. The intestinal tract may be the avenue of infection without itself or its regional lymph glands becoming in-The percentage given above should therefore be higher. fected. The works of McFadyean, MacConkey, Harbitz, Weichselbaum, Bartel, Rosenberger, Rabinowitsch, Ipsen and others offer proof for this contention, as they mention cases in which apparently healthy mesenteric lymph glands contained tubercle bacilli which although appearing to be in a latent form at the time of finding, produced tuberculosis when inoculated into experimental animals. It appears also to be proven experimentally that through the feeding of tuberculous material tuberculosis of the lungs may develop without the presence of intestinal tuberculosis or tuberculosis of the mesenteric glands (Bartel, Bongert, Kovacs and others). In this regard the question may be raised as to whether there is any possibility of the tubercle bacilli working up from the intestines into the esophagus, and into the buccal cavity, from which inhalation tuberculosis could result (Uffenheimer, Dieterlen).

This objection would not enter into consideration for the purpose of milk control, since it is immaterial for the hygienist working along practical lines, whether the infectious agent causes disease in the body by way of the circulation or through inhalation. In this instance it is only necessary to keep in view preventive measures, which should completely prevent the body from coming in contact with producers of the infection.

## Alimentary Infection of Man With Bovine Tuberculosis.

After the supposed cases of transmission of the bovine tubercle bacillus cited in former years failed to withstand critical observations, Koch at the International Tuberculosis Conference, held in Berlin, in 1902, urged the following up of all cases of established tuberculosis of the udder, the determination of how long the disease persisted, who consumed the milk and milk products from these cases, whether the milk had been boiled and whether the respective persons became affected with tuberculosis.

This request was fruitful of results, and in 1910 Weber published the results of his compilation investigations, which were carried out by the aid of official statistics from Prussia, Bavaria, Saxony, Wurttemberg, Baden and Hessen.

The investigations extended over the time between the beginning of 1905 to April, 1909; the investigation of some of the individual cases however is still being continued, since in the chronic courses of tuberculosis it must be considered that the results of infection with bovine tubercle bacilli may under certain conditions only manifest themselves after years have elapsed.

In the given period 113 cases were reported, of which 68 were from Prussia, 14 from Bavaria, 6 from Saxony, 6 from Wurttemberg, 10 from Baden, and 9 from Hessen.

At least 628 persons came under consideration in the ingestion of such milk, possibly even more, since at times only the term "family" is designated, and the milk was not infrequently delivered to dairies with a large patronage. These cases were not included, although every person is exposed to an infection who partakes of such milk and dairy products.

In the case of 9 persons no age is given; 284 were children, and 385 adults.

The value of the individual cases must of course be judged in different ways.

In 44 cases it is stated that the milk had been consumed only as an addition to coffee, or mixed with milk of healthy animals, or the data were otherwise not accurate.

Of especially great interest are those cases in which it was emphasized that the milk was consumed in a raw state, unmixed, mixed with milk of only a few cows, or in which such milk was used in the preparation of butter, buttermilk, sour milk, or had been consumed for a long period. In such cases tremendous numbers of tubercle bacilli must have been taken into the digestive tract. According to Bang and Wall the milk from tuberculous udders may retain a normal appearance for months, being used as food without any objection, and yet such milk contains millions of tubercle bacilli. Bang found in smear preparations of such milk, in a single field as many as 200 bacilli.

In all 69 cases were reported, in which it was stated with certainty that raw milk of animals with udder tuberculosis, or products prepared from such milk, were consumed.

The milk was taken for a longer or shorter time, in large quantities, by 151 children, 200 adults, and 9 persons whose age was not mentioned.

These persons are divided by Weber according to the results of the investigations, into four groups, namely:

1. Cases, in which an infection occurred of a bovine type.

2. Those in which a suspicion of an infection exists, but on account of insufficient bacteriological examinations has not yet been determined.

3. In affections in which the bacteriological examination relative to the suspicion of tuberculosis was negative, or in which the human type was found exclusively, and

4. Cases in which no affections whatsoever have been demenstrated up to the present time.

In Group 1 an infection with the bovine type was demonstrated in two families, affecting one child in each. In both cases it was possible to trace the consumption of milk containing the bacilli up to the nursing age. In one case it lasted for one and a half years, in the second case one year, and in the latter case the udder affection had been recognized for three months during the period that the child had been using the milk.

In both cases the respective cow was affected with a severe tuberculosis of the udder in all four quarters; the milk had been consumed at all times mixed with the milk of a second cow, in the first case boiled or raw but in the second case only raw.

The other members of the family remained well in spite of the consumption of this milk; in both instances only the youngest child became affected with tuberculosis of the cervical glands.

In the first case another child of four, and one of five years, was included in the family; in the second case children of the age of 3, 4, 7, 8, 9 and 12 participated in the consumption of the milk, all remaining normal.

The tuberculosis of the cervical glands healed in the two youngest after abscess formations, leaving several slightly enlarged small glands in the surrounding parts. One of the boys appears to be in the best of health, the other is somewhat behind in his development (at the age of 2.34 years he weighs 251bs.); in the last six weeks however his weight increased slightly more than 1 fb.

In the cases of the second group there exists suspicion of a bovine type of infection.

In six children and one adult there are swellings of the cervical lymph glands and in four children and one adult a suspicion of abdominal tuberculosis is given. One child is affected with scrofula. In the four children the manifestations of disease retrogressed, while in the adult it appears doubtful, according to Weber, whether the affection is of a tuberculous nature.

Forty-one persons are included in Group 3, who consumed milk in a raw state from cows affected with tuberculosis of the udder. This was at times mixed with milk of other cows. A girl of 16 years of age and a boy four years old, who died of tuberculosis were included in this group. The producers of their infections were bacilli of the human type. A man and a woman who were affected with pulmonary tuberculosis (human type), a boy with suppuration of the middle ear and cervical lymph glands (not tubercular); an 18-year old boy with rheumatism of the joints and valvular heart trouble, chronic diarrhea and pulmonary symptoms (not tubercular); a woman with catarrh of the apex of the lungs (not tubercular); a woman with swelling of the glands, diarrhea, cough, night sweats and emaciation (inoculation of sputum without results), and a woman and a man with pulmonary symptoms (not tubercular) were also in the total of forty-one.

The cases in which a boy and a girl died from tuberculosis, are of especial importance. In spite of the prolonged consump-





tion of raw milk from a tuberculous udder by the children who were already infected with the human type of the disease, it was impossible to isolate from the tuberculous glands of the neck and mesentery any bacilli of the bovine type. Weber concludes from this that a body already infected with the human type of the disease is resistant rather than susceptible towards an infection with the bovine type.

The fourth group contains by far the greatest number of cases in which children and adults consumed raw milk from cows affected with tuberculosis of the udder, or milk products prepared from the same, and includes those cases in which no disturbances of the health resulted from such consumption. It was especially stated relative to the children that they all appeared thriving and healthy. Among these persons are included those who for a long period ingested especially great quantities of bovine tubercle bacilli; thus a 13-month old child has been brought up exclusively on raw and boiled milk from a cow affected with udder tuberculosis, and up to the present remains healthy.

Other cases may be considered as presenting complete experiments with the necessary controls, since the persons who drank the milk remained healthy, whereas calves and hogs fed with the same milk developed severe ingestion tuberculosis. The observations of such cases may be traced back incompletely for 3 or 4 years.

A 25-year old waitress, and a 28-year old dairy hand drank mugfuls of freshly drawn tuberculous milk, frequently without any other milk being added without becoming affected; the calf of the cow which produced the milk had to be slaughtered after four weeks, and showed tuberculosis of the mesenteric lymph glands, liver, lungs and kidneys, a severe ingestion tuberculosis.

The milk of another highly affected tuberculous cow also suffering from udder tuberculosis, was mixed with the milk of two other cows, and was consumed frequently in a raw condition, by two adults and a 13-year child; a child 1½ years of age was given the milk only in a boiled condition. The adults remained healthy; the calf from this cow had to be slaughtered after five weeks, and showed generalized tuberculosis.

From the stable of a herdsman the milk of a cow affected with udder tuberculosis was mixed with the milk of three other cows, and the cream and butter prepared from this was consumed by four persons, aged 31 to 59 years, without producing any ill effects. The five hogs of the herdsman were found on postmortem to be tuberculous.

Similar results were reported from the ingestion of milk from a tuberculous udder of a goat, which was consumed as raw and boiled milk by three adults and four children of ages from 5 to 16 years. The persons remained well while a hog became affected with ingestion tuberculosis.

In two other cases the milk was consumed in a raw or unmixed state, as milk, buttermilk and butter. It was consumed by seven adults, in one case for a period of four months, in another case even longer. In spite of the fact that the family has been kept under observation for four years no disturbance in health can be detected.

It is proven by the collected material of Weber that even though tremendous quantities of tuberculous material are consumed, still more favoring accessory conditions are necessary in order to produce an infection with the bovine type of tubercle bacilli. Of course it is not yet known, as indicated by Weber, how many of the children which show swelling of the cervical lymph glands and symptoms of suspected abdominal tuberculosis, are affected with the bovine type of tuberculosis, or how many of the persons who fail to show any disturbance of health may harbor one or more infected mesenteric glands; likewise it is not known how many children with a latent form of the disease may through a special weakening, or under the influence of other infections, break down later with tuberculosis, possibly even with a fatal termination.

Through the compilation investigations we know only of the time (which extends over a period of 1-3-4 years in the individual cases), the opportunity and the immediate results of the infection, and not the further development of the same, but we do know that in two children a true bovine type of tuberculosis existed.

Therefore, although a possibility of infection was present in a great number of persons, the infection has positively occurred up to the present only in two children in infancy. This constitutes proof that "the danger which threatens man from the consumption of milk and milk products from cows affected with udder tuberculosis is very slight when compared with the danger of man affected with open pulmonary tuberculosis to his fellow men."

This conclusion of Weber may be supported without further consideration. Nevertheless the danger still prevails, and although it is slight in comparison with the danger through infection with the human type, it should be by no means under-estimated; it should be considered that the danger of infection with human tuberculosis is amazingly great, and the opportunity of ingesting the bovine type of tubercle bacillus with milk is similarly great.

# Bovine Tuberculosis in Man in General.

Although the attention of pathologists of all countries has been directed for the last ten years to infections of man with bovine tuberculosis, up to the present time there are collected only 117 certain cases of bovine tuberculosis in children and 21 cases in adults (over 16 years of age).

Of the 117 cases in grown children 105 are accurately described, and involve the following organs:

60 cases of abdominal tuberculosis

- 25 cases of tuberculosis of the cervical glands
  - 4 cases of tuberculosis of the tonsils
  - 7 cases were generalized
  - 3 cases were localized in the bones and joints
- 6 cases represented lupus

Two cases should also be included in which bovine bacilli were found in unchanged lymph glands.

The 60 cases of abdominal tuberculosis are again divided into 34 severe cases in which the mesenteric lymph glands, the intestines and the peritoneum showed changes. Thirty of these after generalization of the affection, terminated in death. Twelve of the 60 patients had tuberculosis of the mesenteric glands, slight intestinal tuberculosis, and tuberculous meningitis. The 12 cases were severe fatal affections.

In 14 cases the autopsy revealed tuberculosis of the mesenteric lymph glands with the bovine type of bacilli, but this was found accidentally following other causes of death, as diphtheria, scarlet fever, measles, and pneumonia.

In the 21 adults the bovine type of the disease was established three times in pulmonary tuberculosis with expectorations, once in a primary abdominal tuberculosis and pulmonary tuberculosis, once in an infection of the buccal mucous membrane and cervical lymph glands, once each in tuberculosis of the knee joints, the kidneys and the peritoneum, and finally the bacillus of bovine type was isolated from three cases of lupus, two cases of skin tuberculosis, and five cases of *tuberculosis verrucosa cutis* in butchers. Besides these instances the bovine type of tubercle bacillus was isolated three times from the mesenteric glands of adults.

In two cases of phthisis the bovine tubercle bacillus was found in association with the human type.

Of the total of 138 cases, 56 were fatal, and 89 could be explained with certainty or with the greatest probability as ingestion tuberculosis. The other forms of tuberculosis, with the exception of the skin tuberculosis of the butchers and of one milker, may also probably be traced to the same mode of infection.

Weber deduces from his findings that the danger of becoming infected with tubercle bacilli of cattle is great for the individual, but is only slight for the human race as a whole.

Kossel reports in the German Medical Weekly relative to the number of cases of animal tuberculosis in man as compared with the human type of tuberculosis, and observed that in 1602 cases of human tuberculosis the bovine type appeared as the infective agent in 126 cases, the human type alone in 1464 cases, the human and bovine type in association nine times, and the avian type of tubercle bacillus three times. Therefore in about 8.6% of human tuberculosis, bacilli of animal origin were found, and in about 8% of these they were of the bovine character. If however the most frequent form of tuberculosis of man is considered, namely pulmonary tuberculosis, then the bovine type can be demonstrated only in about .6% of the cases, whereas in the other forms of tuberculosis it may be found in 16% of the cases.

Tuberculosis of bovine origin occurs most frequently in children in which tuberculosis of the cervical glands is caused in about 40% of the cases from infections with the bovine type, and tuberculosis of the mensenteric glands may be traced to the same type in 40 to 50% of the cases. A portion of these affections, as has already been mentioned, may terminate fatally. Among the fatal forms of tuberculosis in children 76% are caused by the human type and 24% by the bovine form. The meningitis type of the disease is brought on in only about 11% of the cases by the bacillus of animal origin and in 89% by the human type of the organism.

In tuberculosis of the bones and joints the figures are 5% and 95% respectively.

Gaffky, Rothe and Ungermann found in 400 bodies of children, 76 infections with tuberculosis, in which they succeeded in establishing the variety of the bacillus. In one case they found the bovine type (1.32%), and among 171 other autopsies on children, of which 39 were tuberculous, two (5.1%) cases of bovine infection were observed.

The results of tuberculous infections among children of the population of Berlin were therefore 95 to 96% of human origin, while only 4 to 5% were of bovine origin, in spite of the fact that during infancy the danger of bovine infection is the greatest (Kossel).

[According to figures compiled by Park of the New York City Board of Health, the frequency of bovine tuberculosis in man as collected by various investigators is as follows:

In adults, 955 cases have been examined of which 940 showed human infection and 15 bovine infection. In children from five to sixteen years of age, out of 177 cases investigated, 131 were human infections and 46 bovine infections. Among children under five years old there were 368 cases of which 292 were found infected with the human type and 76 with the bovine type of tuberculosis. Furthermore Park mentions the very suggestive results obtained from nine children under 6 years of age who were fed exclusively on cow's milk at the Foundlings' Hospital. Five of these children died of bovine infection and four of human infection. On the other hand in the Babies' Hospital where the infants are nursed or fed on prescription milk, out of 63 children dying of tuberculosis, 59 proved to be human infection and 4 bovine infection.

The figures taken from clinical work in England indicate that from 23 to 25% of the fatal cases of tuberculosis in children are due to bovine infections. Stiles of Edinburgh has presented interesting statistics to illustrate how bovine tuberculosis particularly affects young children. Of 67 consecutive tuberculous bone and joint cases, the bovine bacillus was present in 41, the human bacillus in 23, while in 3 cases both types were present. In those affected children under 12 months old, only the bovine bacillus was found. Of the 12 children between 1 and 2 years of age, 8 owed their disease to bovine infection, 2 to human infection and 2 to There were 15 cases in 2 to 3 both bovine and human infection. year old children, 11 of which were bovine, 3 human and 1 both infections. The 10 cases from the 3 to 4 year period were 6 bovine and 4 human infections, while the 4 to 5 year period included 3 cases of each type of infection. Stiles further reports on 72 cases of tuberculous cervical glands operated on at the Children's Hospital in Edinburgh, in which the disease was due to the bovine bacillus in 65 cases, while in only 7 patients was the disease caused by the human bacillus.—Trans.]

## Conclusions.

If we compile the results of this chapter the following conclusions may be established:

Although tuberculosis of cattle is less dangerous for man than tuberculosis of man, the danger from the enormous spread of the disease in our herds, and especially among the dairy cows, should in no way be under-estimated. Theoretically the possibility of infection is afforded in all cases in which the ingestion of living tubercle bacilli with the milk takes place; from a practical standpoint however this possibility of infection comes into consideration only when the bacilli enter the individual in great quantities, and the resistance (of a local or general nature) of the body is not equal to this quantitative attack. This disposition, or these relative conditions between the injurious agents and resistance, appear to be especially unfavorable in children; therefore the requirement of the elimination from dairy herds of all tuberculous animals which pass tubercle bacilli with their milk, appears to follow as a matter of course. According to the experience at the tuberculosis eradication stations only those animals must be considered as eliminators of tubercle bacilli which are affected with open tuberculosis, and expel the tubercle bacilli with their secretions and excretions, especially animals affected with tuberculosis of the udder, open pulmonary tuberculosis, tuberculosis of the uterus, intestinal tuberculosis, and furthermore animals with tuberculosis of the liver, kidneys, skin, eyes, and larvnx.

# Measures Against the Danger.

The elimination of animals passing tubercle bacilli should also be energetically encouraged on general economic grounds. For this work three methods may be followed:

1. Treatment of the disease and curative attempts.

2. The immunization of healthy herds.

3. Energetic sanitary police eradication measures, reduction of the possibilities of infection, and protection of young animals from infection, together with favorable conditions for bringing up young stock as a preventive measure against their accidental infection, toward which we are powerless.

The curative measures in affected animals may be left out of consideration as measures of control, since—excepting the uniformly bad or only slightly favorable results—the methods of treatment for veterinary practice are too complicated, and are not practicable in consideration of the value of the animal. For the sake of completeness the experiments with iodipin should be mentioned here (Hauptmann). Creosote has also been employed.

Of the specific remedies, tuberculin, tulase, tulase-lactin, tulon and tuberculase could be considered in the treatment of affected animals. These bacterial preparations, however, according to Römer and Arloing, are ineffective, since the results were negative.

Better results were promised at the onset, from the specific immunization methods, which aimed at a systematic preliminary treatment with slightly virulent strains, or with attenuated boying tubercle bacilli, to increase artificially the resistance of the immunized animals, that is, to protect them against a later accidental natural infection. As a matter of fact cattle immunized with tubercle bacilli prove for a time to be immune, or at least manifest a considerable resistance against a subsequent artificial infection with bovine tubercle bacilli, when compared with non-immunized control animals. For immunization purposes there have been used:

Dry tubercle bacilli of the human type (bovo-vaccine, Von 1. Behring's method). The injection is made into the blood circulation and is repeated. Animals treated in this way after 3 to 4 months, resist an intravenous injection of bovine tubercle bacilli. to which untreated animals invariably succumb. This increased resistance however lasts only a short time. According to the investigations of Rossignol and Vallée and Hutyra it diminishes towards the end of a year, and after another six months it practically disappears. Against the slight practical success of this method the disagreeable fact should be considered that the injected tubercle bacilli of man are retained alive in the body of the cattle for years, and may even produce in the udder local tuberculous processes. from which the bacteria of human tuberculosis may enter into the milk (Lignieres, Weber and Titze).

Titze found that following an intravenous injection of human tubercle bacilli, they were eliminated from the udder even 16 months after the injection. In this regard the various individuals manifest an entirely different behavior. In three other cases bacteria were eliminated after a single injection, from the fourth week up to the 144th day. In a second cow which received an injection of tubercle bacilli of human and bovine type the elimination commenced after the third injection, and in a third cow as early as 24 hours after the injection. All three animals eliminated the bacilli from only one quarter, without this showing tuberculous changes. Bongert found in 186 bovo-vaccinated cattle, 36 which passed tubercle bacilli

with their milk.

The protective vaccination of Von Behring therefore is not only of little practical value, but grave dangers must be considered in connection with it, since the vaccinated animal may eliminate tubercle bacilli with the milk for 21/2 years and longer.

Koch and Schütz, Neufeld and Miessner recommended for the immunization of cattle a single injection of 0.01 gm. tubercle bacilli in suspension, which vaccine they termed "tauruman." The above statement applies equally for tauruman as it does for Similar results to the immunizing value of the inbovo-vaccine. travenous injections, according to Baumgarten, Lignieres and

Klimmer may be derived from the single subcutaneous administration of human tubercle bacilli. According to Lignieres even in such cases the bacteria may remain alive for as long as two years.

According to Von Behring, Calmette and Guerin, Roux and Vallée, cattle may become immunized by feeding with slight quantities of bacilli from tuberculosis of the horse (or bovine tuberculosis). Arloing attempted to immunize with homogenized cultures of strains which had

Arloing attempted to immunize with homogenized cultures of strains which had been cultivated in 6% glycerine bouillon (human type and bovine type). Better results were obtained from the intravenous than from the subcutaneous applications and this again proved superior to administration per os.

Klimmer eliminated the danger of the vaccination for man by heating the human tubercle bacilli to 52-53 deg. C., or by rendering them avirulent by continuous passages through the crested newt. Both these vaccines are no longer pathogenic for guinea pigs, and they cannot regain their virulence by means of passages through animals. The results of immunizations are supposed to be favorable (Klimmer on 10,000 cattle), especially if the vaccination is carried out together with general protective measures, such as raising calves on milk free of tuberculosis, and the elimination of animals with open tuberculosis. Glockner even believes that vaccination has a favorable action on the curing of animals which were already affected with bovine tuberculosis prior to the vaccination, whereas Eber attributes the improvement of the vaccinated herds to the simultaneously executed prophylactic and hygienic meas-Friedmann aimed to produce immunization with his tubernres. cle bacillus from cold blooded animals (turtle). Other authors however failed in producing an effective immunization with such strains (Libbertz and Ruppel, Weber and Titze, Orth).

Heymanns attempted to immunize cattle by the introduction under the skin of cattle of a closed sack of vegetable fiber, containing living tubercle bacilli (human or bovine in origin). The supposition is that these vegetable sacks will confine the bacilli at the seat of inoculation, and that the treated animal will be immunized by protective metabolic products, that continuously form in small quantities within the sack and pass outward from it into the animal's system generally, by an osmotic process.

The vaccination, which is carried out with the aid of a trocar to insert the capsule under the skin of the back, must be repeated annually, since the bacilli may die.

Heymann's method has been successfully used by its discoverer on more than 20,000 cattle, and the percentage of reactors to the tuberculin test diminished from 45 to 21 (18 herds with 188 animals). Animals which have formerly reacted may appear free at the subsequent test.

Good results were obtained by Vallée from passive immunization. He inoculated young cattle with 100 to 200 c. c. of a protective serum, which he obtained from a horse treated with slightly virulent strains from horses, and then with strains from men. With this method he succeeded in rendering the animals resistant to artificial infection with bovine tubercle bacilli.

Since immunization methods have not offered uniformly satisfactory results, and since they must be prohibited on the ground of milk hygiene, therefore results may be expected only from proved sanitary police measures.

The methods which must be followed in the eradication of bovine tuberculosis are:

1. Diminution or elimination of the sources of infection,

- (a) By removal of the animals passing bacilli,
- (b) By separation of healthy and suspected or diseased animals,
- (c) By bringing up tuberculosis-free young animals.

2. Improvement of the general methods in the care of young stock, by introducing conditions which approach the natural mode of living:

(a) Proper care and feeding in well ventilated and lighted stables,

(b) Dividing the pastures so that the animals may be separated (according to whether they are suspected or healthy) and kept in accordance with their age and with the use for which they are later intended.

Measures for eradication must be applied in accordance with the rules here outlined.

The most effective **method of eradication** was worked out by **Bang**, and consists in the elimination of clinically recognizable diseased animals, the separation of reacting animals, and the bringing up of calves on milk free of tubercle bacilli.

The remarkable value of Bang's methods has been proven fully in practice by the results obtained since 1892.

It is important for the results to separate completely the animals which fail to react to tuberculin, that is the healthy cattle, from those which harbor the disease and which react to the tuberculin test. This should be done in such a way that the healthy animals are placed in a freshly disinfected stable or in a portion of a stable provided with a separate entrance, and separated with a board wall, from that part in which the reacting cattle are housed. The attendants of the healthy herd should not come in contact with those of the diseased herd. Animals of the reacting group which after a time become affected so that they may be clinically recognized, should be slaughtered as soon as possible.

Young stock which react should not be permitted to breed, or at least should be immediately placed with the reacting group, providing their breeding value is such that this procedure is deemed advisable. All reacting animals under six months of age should be slaughtered, that is they should be utilized for meat.

Young stock and work oxen should also be included in the

segregation, and the healthy ones must be kept from contact with reacting animals.

Of the calves which are born after the separation, those from non-reacting cows remain with their mothers; the calves from reacting cows, after receiving the colostrum from their mother on the first day after birth, should be placed in the stable of healthy animals, and should be fed with the milk of healthy cows or should be brought up on sterilized milk, or they may be allowed to suck from healthy nurse cows. As soon as possible after weaning the calves should also be subjected to the tuberculin test, and those giving a reaction should be immediately removed. From 1 to 2%of these calves react.

It is proper to place the healthy calves in a stable of healthy young stock, and they may pasture with them, or if this is not possible they should be placed with the older non-reacting group of animals. Before the first breeding the heifers again should be subjected to the tuberculin test, in order to place them in the proper group of cows.

The tuberculin test is annually repeated in the healthy herd, in order to eliminate the animals which in the course of the year have had a possible opportunity of becoming affected with tuberculosis.

Newly purchased animals are clinically examined and tested with tuberculin, and are added to the healthy herd only when the results are entirely satisfactory.

The male animals which are to be used for breeding purposes should not react to the tuberculin test. Under unavoidable circumstances, a reacting bull may be reserved for breeding purposes but only under special precautionary measures.

The results of Bang's eradication method, if carefully carried out, are remarkably satisfactory.

It has been adopted to the greatest extent in Denmark, Sweden and Norway, and it has also been successfully carried out in Hungary and Finland.

The report of Regner, in 1911, affords a good review of the results of Bang's method, and in it are described the results of the governmental eradication of tuberculosis in Sweden. Regner divides the eradication work into an offensive one in herds in which the disease prevails, and into a defensive procedure whose purpose is the prevention of the introduction of diseased animals into herds free of tuberculosis.

Of the groups into which Regner separates the herds and the animals, the first group includes those which originally (on the first tuberculin test which in some instances was applied years previously) were found tuberculous. At that time 16,852 animals had been tested with a percentage of 30.2 reactors. In 1908, 18,719 animals in 457 herds proved to be entirely free from tuberculosis.

The herds of the second group, which proved to be tuberculous

at the time of the inauguration of the method and which continued to contain reacting animals, included 375 herds of 21,899 animals, with 41.5% of reactors. At the end of 1908 the number of cattle had increased to 26,181, of which only 1,496, or 5.7% reacted.

The results were not so pronounced when the reacting animals were retained with the healthy animals, when cattle without the necessary precautionary measures were placed in herds free of tuberculosis, when animals which had not reacted in the old herd were removed into the free herd without being previously tested, or when an opportunity was given for the transmission of the infection by a reacting bull causing the infection in the herd to appear to be renewed. Also in cases when the milk used for the feeding of calves was not free from tubercle bacilli, the results were unsatisfactory.

In the interest of systematic eradication, it is necessary, especially at the commencement of the eradication work, to subject the animals to the tuberculin test quite frequently, with short intervals.

As a third group Regner included 436 herds containing 7,835 animals at the beginning of the work and 9,114 cattle in 1908, which at the first examination, and again in 1908 were free from reacting animals.

The fourth group contains the herds which originally were free from tuberculosis but were not so at the test in 1908. The 98 herds included at first 2,526 and in 1908, 3,720 animals, of which 265 or 7.1% reacted.

Regner concludes from his tabulations: that on the first tuberculin test in 1366 herds, out of 49,112 animals tested, 14,175 or 28.9% reacted; that in 1909 the same herds contained 57,734 animals, of which 1761, or 3.1% reacted; that Bang's method is the strongest factor in the general promotion of breeding, and of stable and milk hygiene.

In other countries the results were similarly favorable.

Bang succeeded in Denmark, from 1893 to 1908, in gradually reducing the percentage of reacting animals from 40 to 8.5. Mahm in Norway from 1896 to 1903 reduced the disease from 8.4 to 4.9%. Hojer in Finland in 1894 to 1900 caused the infection to drop from 24 to 10.1%.

Hutyra reports on experiments carried out on the government farm of Mezohegyes. In this herd the first tuberculin test in 1898 showed 44.8% of reactors out of 329 cows or 26.6% of the entire herd (647 animals), whereas in the fall of 1903 out of 502 cows only 2.8%, and out of the total of 1,132 animals only 1.8% reacted to the tuberculin test. The herd had been increased in this period by 75%, without purchasing additions to it, and the percentage of reactions had dropped 88%.

The stringent measures of Bang have been somewhat modified in certain cases for economic reasons, or when the strict execution of Bang's method has presented peculiar difficulties. On the other hand the requirements have been accentuated in cases where favorable considerations prevailed. Thus for instance in a herd in which only a few animals react it would be advisable to dispose of them without further consideration, and after a thorough disinfection of the stable the defensive work against tuberculosis may be instituted, through the introduction of only non-reacting cattle, and by the disposing of all animals which prove tuberculous on the following tuberculin tests.

The Siedamgrotzky-Ostertag method consists of immediate disposition of all animals with open tuberculosis (by this means the animals eliminating tubercle bacilli are excluded), and in bringing up the calves free of tuberculosis by feeding them with pasteurized milk or with milk from healthy cows. The calves are subjected to the tuberculin test after they are weaned, and the reacting animals are not bred. The herds which are included in this method of eradication are subjected semi-annually to a clinical examination, and the clinically suspected animals are removed and disposed of. Further than this, the mixed milk of the herd, as well as the suspicious secretions and excretions are examined bacteriologically.

The results of the Ostertag eradication method of course can not be compared with that of Bang. Since there are retained in the herd all tuberculous animals which show no clinical form of tuberculosis, or in which there is a suspicion of open tuberculosis but whose secretions and excretions fail to reveal the presence of tubercle bacilli. Therefore a constant danger of infection for the animals free of the disease prevails, as tuberculosis may at any time develop into an open form. But since it is required that the calves should be brought up free of tuberculosis, and that the eliminators of tubercle bacilli should be determined by periodical clinical examinations as well as by the testing of the entire mixed milk of the herd and the individual secretions and excretions of suspected animals, Ostertag has obtained relatively very good results, where his requirements have been conscientiously carried out.

This method has an advantage in that the stock owners who offer great objections to radical methods of eradication on account of the immediate economic losses which they entail, are willing to work intelligently and with pleasure with a system of eradication such as is offered by Ostertag's method.

This assertion is best proven by the tabulation of Rautmann, which shows the increasing popularity of this method. The method was voluntarily adopted in the following cases:

1903-1904, 1,457 animals; 1904-1905, 1,372; 1905-1906, 5,333; 1906-1907, 5,395; 1907-1908, 5.193; 1908-1909, 8,839; 1909-1910, 18,822; and 1910-1911, 19,828 animals. The following data illustrate the results obtained with the method:

Open tuberculosis was present in the province of:

East Prussiain	1900	in 2.7 %	out of	10900	examinations
East Prussiain	1904	in 1.3 %	out of	17500	examinations
Pommeraniain	1902	in $2.93\%$	out of	8808	examinations
Pommeraniain					
Brandenburgin	1903	in $3.46\%$	out of	5200	examinations

#### Tuberculosis.

Branden	burg			.in	1907	$\operatorname{in}$	1.5	%	out	of	5810	examinations
Schleswi	g-Holstein	n		.in	1903	$_{in}$	2.8	%	out	of	2435	examinations
Schleswi	g-Holstein	1		. in	1905-6	in	1.93	%	out	$\mathbf{of}$	11000	examinations
												examinations
												examinations
In these	statistics	s it sh	ould be	e co	nsidere	d t	hat e	ever	v v	ear	new.	unexamined herd

have been included, and further that the experts continually gained more skill in making the examination.

A proof of the reduction of the dangerous forms of tuberculosis is first of all indicated by the above figures, and also by the marked reduction of tuberculosis of hogs. Thus for instance according to Stier the percentage of tuberculous creamery hogs which amounted to 40% was reduced to 4% after the elimination of six cattle with tuberculosis of the udder, although none of the skimmed milk fed to the hogs had been sterilized.

In 1907 out of 38,454 animals examined in Schleswig, 1.4% were found to be affected with pulmonary and udder tuberculosis. Udder tuberculosis alone was demonstrated in 0.124%. In spite of the great advantages of the method, the results eventually come to a standstill, as may be seen from the more recent reports of the eradication stations. The number of the dangerous forms cannot be reduced below a certain percentage, since latent forms continuously change into the dangerous forms, and it is therefore impossible to eliminate the sources of infection from the herds.

The method of Ujhelyi also deserves mention. In this method the cattle are divided into a healthy herd and those which react to tuberculin. The newly born animals of the group giving a positive tuberculin reaction are allowed to remain only in emergency cases with the reacting mothers, but if possible they are nursed by healthy cows.

ases with the reacting mothers, but if possible they are nursed by healthy cows. After weaning the calves are tested with tuberculin. This method differs from Bang's method only in that sterilized milk is not used (prevention of calf diarrhea) and further the calves are allowed at times to remain with the reacting cows. According to Ujhelyi's report this method has given irreproachable results. It has to be considered however that of the weaned calves a greater proportion of animals must be eliminated when this method is employed, than when Bang's method is followed.

Unley's report this method has given irreproachable results. It has to be considered however that of the weaned calves a greater proportion of animals must be eliminated when this method is employed, than when Bang's method is followed. Before the introduction of Ujhelyi's method, out of 1,031 adult cattle, 884, or 85.7% reacted. Out of 626 young stock 333, or 53.2% reacted. After a period of eradication for 4½ years Ujhelyi succeeded in reducing the infection to 4.1% among the adults, and 2.6% in the young stock. He succeeded in the periods from 1898 to 1902, and from 1904 to 1905, in reducing the number of positive reactions among 1,715 cattle, of eight state farms, from 59% to 3%.

The eradication of tuberculosis has been subjected to official control for several years in Denmark, Sweden, Norway and Finland. Thus Denmark in 1893 contributed \$13,500.00, later \$27,-000.00 towards the eradication of tuberculosis, furnished the tuberculin free of charge at first for young animals, later for adults, and finally since 1898 took upon itself the total expenses of eradication (Hutyra). The skimmed milk is permitted to be returned from the creameries to the stock owners only after being heated to 80 deg. C. Cows affected with tuberculosis of the udder are destroyed, the owners being reimbursed. There are about 600 such animals paid for annually.

Similar results were obtained in Sweden, which adopted legislative measures and made a contribution of \$225,000.00. In the years from 1897 to 1908 in 1,370 herds with 48,576 animals, of which 14,225 or 29.3% reacted, the amount of infection was reduced by their work of eradication to such an extent that out of 57,660 cattle only 3.1%, that is 1,765 animals, reacted.

Although the statistics of individual countries having strict measures of eradication (Denmark, Sweden, etc.) appear to show the splendid effects of carefully executed control work, based on scientific principles, and in spite of the fact that the milder modifications, as for instance that of Ostertag, by no means show the same good results, nevertheless measures of too strict requirements cannot be absolutely approved.

Thus for instance Belgium in 1895 required the destruction within a certain time of all clinically affected and all reacting animals, and in 1896 out of 19,004 cattle examined 9,280 were slaughtered. The difficulty of the execution was lessened by the law of 1897 which required that only the visibly affected animals should be destroyed, a measure which resulted in the destruction of 10,269 cattle with reimbursement amounting to \$300,000.00, in 1902 (Hutyra and Marek).

Theoretically, the most radical eradication measures may possibly be considered as the quickest and most effective, and therefore from an economic standpoint as the best methods for the control of tuberculosis. Owing to the extraordinary spread of this disease in almost all herds, drastic measures however may result in the sudden infliction of such heavy economic losses, not alone through the animals destroyed, but through changes of values for breeding, dairy purposes, meat production, etc., that the stock owners, dealers, consumers, etc., would have good grounds to protest against the execution of such methods.

Therefore it is advisable to adopt Ostertag's method at the initiation of the general work of eradication, and after the stock owners have been convinced that the idea is rational following the favorable practical results obtained, then Bang's method may be introduced, unless it is possible to persuade them to employ, at the beginning, the rational execution of Bang's method. In no instance however should destruction of the reacting animals be required in connection with Bang's method. From the standpoint of milk hygiene it does not seem to be justifiable, according to the present status of the question of the infectiousness of the milk of reacting animals, to require their exclusion from the production of milk, unless they show clinical evidence of the disease.

In spite of the separation of the reacting from the non-reacting animals, the milk of the reacting group could be marketed, from the standpoint of milk hygiene, with the milk of the other group, without interference, as has been previously practiced, so long as there is no substantial proof offered as to the danger of marketing such milk.

With the new **law on diseases of animals** the initiation of eradication, based on uniform legislative measures, has been instituted in Germany, and thereby serves as a stimulus to extensive private activity in matters of eradication. The law requires that clinically affected tuberculous animals, or those in which tuberculosis probably exists to a great extent, may be ordered destroyed by the police authorities.

If this is not carried out, or if the destruction is postponed, sanitary police protective measures should be inaugurated against further spread of the disease, by branding the animals.

The police measures against the spread of the disease consist in separation, observation of police control of the affected, suspected and susceptible animals; if necessary restriction of traffic of both man and animals, and special limitations relative to the use of affected or suspected animals, and their carcasses, and finally the usual requirements of disinfection.

For animals which are destroyed by the requirements of the police, and those which after destruction has been ordered, die of the disease on account of which they had been ordered destroyed, the government allows corresponding reimbursement.

Of great importance in tuberculosis eradication is the requirement prohibiting the return of skimmed milk and other milk residue to the milk producers, as food for other animals, unless the same has been heated.

This clause is included in the general requirements of the measure. Centrifugal slime, which has formerly caused the development of ingestion tuberculosis in hogs, must be destroyed by burning or burying.

The measures differentiate three danger classes in tuberculosis: (1) the simple suspicion, (2) the great probability of its presence, and (3) the actual existence of the disease. In the presence of the clinically recognizable classes of tuberculosis, it is required that the milk from such affected animals should not be sold or otherwise utilized without being previously subjected to a required temperature for a certain length of time.

The milk from cows affected with tuberculosis of the udder cannot be used for human consumption even after subjecting it to the required heat, nor can it be utilized for the preparation of dairy products.

The requirements in Bavaria order the destruction of an animal only when it belongs to a herd in which cattle breeding, or raising of cattle is industrially followed, and an appropriate voluntary method of eradication of cattle tuberculosis may then be carried out in the herd under veterinary supervision.

This ought to result in a considerable improvement of the tuberculosis question, and with the elimination of animals which principally enter into consideration as distributors of bacilli, a point is gained which temporarily should thoroughly satisfy even the milk hygienists.

With such measures the stock owner is pleased, as the professional direction of rational breeding in connection with eradication is shown to be for his advantage. This constitutes the basis on which the entire milk production in all its relations may be elevated, and will be elevated, since the voluntary intelligent co-operation of the owners constitutes the fundamental principle on which the state bases its allowance of reimbursement.

Without the voluntary co-operation of the producers, the elevation of milk hygiene is practically impossible.

It will take years before the conditions will markedly improve, but the improvements will surely come, and they will not confine themselves alone to the tuberculosis question.

# Other Forms of Mastitis.

The other forms of chronic mastitis, with the exception of tuberculosis of the udder, are of slight importance for practical purposes when compared with streptococcic mastitis.

Thus for instance the mastitis produced by the Bacillus pyogenes bovis is relatively rare, and the author has had the opportunity on only three occasions to attribute the development of chronic mastitis to the Bacillus pyogenes. Glage, Nielsen, Kuhlmann, and Sven Wall, however, have observed the infection frequently, and even describe an epizootic extension of the infection. Mixed infections of staphylococci and colon bacteria, with the Bacillus pyogenes, appear to be more frequent and in these cases a severe mastitis is produced. It results in abscess formation and necrosis of the affected parts, with an induration of the tissues. The secretion is sanio-purulent, and mostly of an offensive odor. Künnemann found the bacillus at first in suppurations of cattle, and Grips in suppurating processes of hogs. They are small, delicate rods of the size of the swine erysipelas bacillus, growing better anaërobically than aërobically, forming dew-drop like colonies on agar, or serum agar. Milk coagulates to a uniform clot. The bacillus does not take the Gram stain, but it may be stained by Weigert's method.

Very little is known relative to the behavior of the *Bacillus* pyogenes bovis towards man. According to the author's observations it appears to belong to the group of pseudo-influenza bacilli (Pfeiffer). Such rods were found in influenza-like pneumonias, in bronchitis (Pfeiffer), in suppuration of the middle ear (Kossel, Hartmann, Pielicke and Cantani), also in whooping cough (Afanasieff, Szewetschenko, Wendt and others). Friedberger discovered a similar rod in the mucus of the prepuce of a dog. Frank describes it in the pus of a hog. Frosch found it in the blood of geese, and Beck in an infectious pneumonia of rabbits. It belongs to a widely spread bacterial group.

The *Bacillus pyogenes* is non-pathogenic for small, experimental animals and pigeons.

Since rods similar to those of the *Bacillus pyogenes* have been found in man it is not impossible that affections of man may be produced by milk from udders with this form of infection.

## Mastitis.

It has not yet been possible to demonstrate this bacillus in mixed milk, since there occur too many bacteria of similar morphology in stable manure, in the air, and under the epithelia of the teats.

At any rate the milk must be considered as spoiled when it contains secretion from udders with pyogenic infections, and should be excluded from the market, since its injurious effects upon health seem to have been demonstrated in the sense of the pure food act.

Infections with bacteria of the **coli-typhus group** frequently occur when the cows are kept in filthy condition, with unclean bedding, and also when manipulations are undertaken by milkers in order to dilate the milk ducts (penetration with straws, quills, and

# Fig. 23.

contaminated milking tubes). Representatives of this group of organisms were described by Jensen and Streit, Guillebeau, Kitt, Freudenreich, Lucet, Sven Wall and Weichel, as the cause of highly acute forms of mastitis. The bacteria are short rods with rounded ends, mostly motile; they do not take Gram's stain. According to their biologic characteristics various varieties may be distinguished, which at times approach more closely to the colon group, at other times more to the ærogenes group, and at times even to the enteritidis group, which cause meat poisoning.

Milk becomes coagulated with gas formation. The acidification and coagulation occur earlier with some varieties than with others. The colon group always ferments galactose, glucose, laevulose, mannose, lactose, maltose, arabinose, rhamnose, xylose, mannit and sorbit, frequently also sorbose, saccharose, raffinose and dulzit, but not erythrit and adonit. Their action is different towards saccharose, raffinose, sorbose and dulzit, and this differentiation is utilized to separate the groups into those which do not attack any of the mentioned bodies, those which ferment all four, those which split up dulzit and sorbose, and finally those splitting up saccharose and raffinose. Of course bacteria cannot be strictly separated by their fermentative action, since in the cultivation of colon strains in sugar-containing media they may acquire the faculty of fermenting a kind of sugar towards which they formerly were refractory (Twort, Massini). Through the first group the colon bacteria approach the more dangerous group of *Bacillus paratyphus B., Bacillus enteritidis* of Gärtner and paracolon bacteria with their related organisms, for instance the *Bacillus ratin*. *Bacillus suipestifer*, *B. typhi murium*, etc. These dangerous groups may be separated by agglutination into three classes, the *Bacillus enteritidis* Gärtner group, the *Bacillus paratyphus B.* group, and finally the *Paracolon* group.

Weichel succeeded in isolating from two cases of severe septic mastitis an organism belonging to the group of *Bacillus enteritidis*, and another to the *Bacillus paratyphus-B*.

Excluding the inflammatory products which may also possess disease-producing properties in this group of mastitis forms, it is necessary to exercise special care in the inflammations of the udder caused by the *Coli-enteritidis-paratyphus-paracoli* organisms, since among the representatives of this group of bacteria there are those which may produce severe forms of enteritis in man, with symptoms of poisoning, which are known in general as meat poisoning. True paratyphus bacteria may also enter the milk in other ways than with the secretion of an infected quarter, for instance through bacilli-carriers who are employed for handling the milk, through the rinsing water, and also from other sources. It will be of interest to mention here the results of examinations of market milk for the occurrence of *Bacillus paratyphus-B*.

Uhlenhuth and Hübener twice found paratyphus in 100 samples, while Hübener in 40 samples of market milk noted this bacillus 4 times and in 30 other samples of market milk, observed it 3 times.

Klein in 39 mixed milk samples found the *Bacillus enteritidis* 9 times.

The occurrence of coli-aerogenes bacteria in milk would be something very ordinary, and would be considered less injurious for the health than the presence of varieties which are known as toxin producers.

Nevertheless the coli-aerogenes infections of the udder should be considered with the greatest care; although in general the enteritidis and paratyphus varieties produce severe septic inflammations with ichorous secretions and frequently with a fatal termination, the severity of mastitis and the appearance of the secretion are by no means a certain indication of the character of the infection.

Mixed milk to which the secretion from animals with acute affections of the udder has been added, is spoiled according to the pure food act, and should be considered as capable of injuring human health.

The milk of healthy quarters from such infected udders is also

#### Mastitis.

suspicious of being contaminated with the infective agents, and therefore should be prohibited from entering the market. According to Weichel reports on paratyphus and enteritidis

According to Weichel reports on paratyphus and enteritidis infections which may be traced to milk are rare, and no publications can be found which absolutely trace affections in man to a coliparatyphus mastitis.

The Dairy Journal of Berlin reported in 1900 that according to "Dag. Nyheter" nine families in Stockholm became affected with symptoms of meat poisoning (fever, depression, fainting spells, nausea, vomiting, diarrhea, nuscular cramps). The milk, to the consumption of which the affection was traced, originated from 14 cows, one of which suffered from an inflammation of the udder. In the secretion of the affected udder the same bacteria were found as in the feces of the affected people.

Two female attendants of the stable from which the injurious milk was obtained also became affected with similar symptoms.

The observation of Moro also belongs here. Moro observed in six persons after the consumption of milk from a goat suffering with a gangrenous inflammation of the udder, chills, nausea, headaches, and 11 hours later colic, vomiting and thirst. The milk was consumed mixed with coffee.

Weigmann and Gruber report a case of injurious effect (vomiting), from cream which had been prepared from mastitis milk, and they traced the affection to a bacillus of the colon group (immobilis). Weichel fed a six-weeks old dog with the milk of a goat which was artifically

Weichel fed a six-weeks old dog with the milk of a goat which was artifically infected in the udder with a paratyphus strain from septic mastitis. The feeding was undertaken after the appearance of the mastitis in the goat. Three hours after the consumption of 200 c. c. of the secretion the dog showed marked symptoms of restlessness and barked frequently; lachrymation and later repeated vomiting appeared. He soon recovered but refused to partake again of this milk.

Only after 60 c. c. of this fluid had been mixed with 200 c. c. of good milk would he touch it; he then took a small quantity but with apparent distaste. Within five minutes he showed pain, and manifested similar symptoms as the day before, but again recovered before the following day.

In a second feeding experiment on a seven-weeks old dachshund the affection commenced only on the fifth day of the experiment. The animal became listless, refused food, whined, and in addition lachrymation, nasal discharge, and periodical chills appeared. This dog also recovered on the second day.

Weichel also reports a case in which the wife and daughter of a dairyman became affected with a diarrhea after the ingestion of inflammatory products of a cow with coli-mastitis. The dog of the owner also showed similar symptoms after drinking the milk.

As milk offers very favorable conditions for the multiplication of bacteria of this group, the danger from milk containing coliparatyphus bacteria must be considered greater than in the case of meat bearing the same infection. Various data exist relative to the resistance of these bacteria towards influences of heat.

According to Fischer heating to 60 deg. C. for a half hour does not suffice to kill all paratyphus germs; likewise some of the bacteria remained active after heating the milk for 10 to 35 minutes at 70 deg. or for five minutes at 75 deg. C.

Although Kolle states that the typhoid, paratyphoid, and enteritidis bacteria are without exception destroyed when subjected to a temperature of 59 deg. C. for 10 minutes, nevertheless it must be remembered that the conditions in milk are markedly different than in suspensions of culture, and that some of the varieties are capable of producing a heat-resisting toxin. According to Gärtner the toxins of the meat-poisoning organisms withstand 100 and even 120 deg. C. These facts were confirmed by Van Ermengem, Drigalski, Fischer, Hoffmann, Pœls, Holst, Dhant, Riemer, and others.

In practice therefore it is necessary to consider the mixed milk of the affected cow and all dairy milk to which such milk has been added as injurious to health, whenever it is proven with certainty that it contains secretion from acutely affected quarters.

If it is proven with certainty that the secretion contained bacteria of the paratyphoid or enteritidis group such milk may even destroy human health.

Of course the danger which threatens man from such milk must not be overestimated. The changes in the udder and in the milk are pronounced and striking, and usually appear very suddenly, especially in the colon inflammations, somewhat less in paratyphoid and enteritidis infections. Nevertheless in the presence of carelessness of the milker such infections may enter the milk.

According to Fauss the duration of the elimination of the bacteria from affected udders persists for 12 to 30 days, in fatal cases until death. The number of the eliminated bacteria and the duration of the elimination are proportional to the severity of the case, and they cease when the milk again approaches its normal condition.

In other cases of acute mastitis **staphylococci** have been demonstrated. Guillebeau isolated the *Staphylococcus mastitidis*, *Galactococcus versicolor*, *Galactococcus fulvus*, and *Galactococcus albus*. Experimentally it is also possible, as proven by Kitt with the *Botryococcus ascoformans* (a staphylococcus), to produce an acute mastitis, with a tendency to chronic development.

The staphylococci infections of the parenchyma of the udder are relatively rare, but occur more frequently as mixed infections with the *Bacillus pyogenes*. While the course of the staphylomycosis of the udder is mostly acute, with a favorable prognosis, yet in the presence of a mixed infection with the *Bacillus pyog*enes it frequently results in abscess formation and sequestration of the udder.

The staphylococci are small round microbes, separated into two or four parts by division. They take the Gram staining. They are easily cultivated on all media and are frequently chromogenic. They liquefy gelatin from the surface down, since they grow better aërobically than anaërobically.

Staphylococci corresponding to their ubiquitous distribution are present in almost all milk during its first phases of decomposition; but although they possess pathogenic importance as pus-producers in man, from the standpoint of market milk hygiene, they are of no special importance under such conditions. However when the secretion of a cow with staphylomycosis of the udder contains staphylococci, the milk may be injurious to health. Karlinski, for instance, reports a case of pyemia in a child in which infection

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resulted from the milk of the mother containing staphylococci. At least the cocci which Karlinski isolated from the milk, and from feces and blood from the child were identical. The secretion therefore must be considered as spoiled food, and must be excluded from consumption.

The same judgment as stated for staphylococci infections also applies to botryomycosis of the udder. This represents a chronic form of a staphylomycosis, in which the single cocci that grow in colonies are compressed by swelling of the cocci lying on the outer borders, forming capsulated spherical colonies. The central cocci continue to grow, burst the capsules, and the process of the swelling of the bordering zone is renewed until mulberry-like fungoid colonies result. A method of distinguishing Botryococcus ascoformans from staphylococci has not yet been devised. The botryomycotic formations develop mostly in the horse which is probably proof of certain immunity strength of the horse (that is of equidia), against staphylococci infections. In other animals and also in cattle the disease is extremely rare. Mohler, Czokov, Immelmann, and Reinhardt have observed botryomycosis in the udder of cows. Botryomycosis in cattle is of no practical inportance in the judgment of milk.

Actinomycosis of the udder is also of slight importance from a practical standpoint.

The purulent fibroplastic actinomycotic mastitis occurs in cattle with less frequency than the actinomycosis of other organs. It has been described by Rasmussen (four times), Jensen (20 times), Maxwell (once), Bang, and Johne, and represents a chronic suppuration with nodular cicatrization of the udder. After the infection, nodules from a bean to a hen's egg in size, with softened centers and fibrous borders develop, or a diffuse inflammation with a tendency toward cicatrization and hardening of the entire udder results. Actinomycosis of the udder may be primary (McPhail, Williamson) and develop from the introduction of barley beards into the tissue, or possibly from pasturing on stubble fields, or again it may develop by metastatic formations from other lesions in the body.

McPhail believes that some cases of so-called udder tuberculosis are in reality actinomycotic infections of the udder.

Should an actinomycotic process soften in the udder and the abscess burst into the secreting tissue, the finding of actinomyces in the milk is possible. The latter appear as colonies of ray-like fungi (streptothrix). The branching threads form a mesh-like mycelium with spherical or club-shaped enlargements on the end of the threads. The fungous threads proliferating in the animal tissue are influenced by the action of the body fluids. The sheaths swell and club-shaped bodies result, arranged in a radiating manner, which later become adherent to each other forming rosettes in which the mycelium, protected from the immune bodies and leucocytes continues to proliferate or to degenerate and calcify. The actinomyces are widely spread forms of the higher bacteria with true branching, and stand between the lower bacteria and hyphomycetes. They almost invariably occur on grain, hay, straw, fruit, manure, soil, flour and milk. Most of the actinomyces are harmless provided a foreign body does not facilitate their colonization in the animal body. Splinters of wood, and especially beards of barley are frequently the carriers of the infection.

Transmission from man to man or from animal to man is not known up to the present time. The basis of an infection always lies in wound infection either through the above mentioned foreign bodies, or by the fungi gradually becoming accustomed to existence in necrotic tissue of the animal body (caries of teeth). Johne succeeded in producing actinomycosis of the udder through the injection of actinomycotic cultures into the milk cistern.

Although transmission to man through milk from actinomycotic udders is not to be feared, prohibition of the sale of such milk is required since it must be considered as spoiled on account of the presence of pus and other associated changes.

Contrary to actinomycosis, "actinobacillosis" first described by Lignieres and Spitz in Argentina, and which clinically resembles actinomycosis, is of a contagious nature. Therefore although actinobacillosis has not yet been described in man it should be more carefully judged than actinomycosis. In Germany cases of actinomycosis have been reported which from the bacteriological findings, should be classed as actinobacillosis, and these cases occur sometimes in an enzootic form or as stable outbreaks.

Thus Imminger in Oberpfalz and Preusse in Western Prussia, described an enzootic extension of actinomycosis, and Schulze mentioned a case in which the disease affected most of the animals in the stable (of 30 steers 27 were affected). Of 87 newly purchased animals more than half of those placed in the stable became affected, while 12, which had been stabled on other premises and which received the same feed, remained healthy.

Milk from udders affected with *actinobacillosis*, and mixed milk which contains such secretion must be considered as spoiled and prohibited from consumption.

Mixed infections of the udder with these described bacteria and others, should be similarly judged, and likewise infections with malignant edema bacteria, *Bacillus necrophorus*, etc.

### Chapter VII.

## EXTERNAL INFLUENCES WHICH ACT UPON MILK.

- (a) Their effect upon the body, thereby influencing milk secretion;
- (b) Their effect upon milk after its secretion.

Although our knowledge, relative to the development of the individual components of milk from the substances in the blood, scarcely extends beyond the border of hypothesis, nevertheless it is established that milk formation is dependent to a certain extent upon the feeding, although only within limits defined by the breed, family, individual, lactation period and age.

Through outside conditions, those factors of production are especially influenced, which are themselves subject to variations, especially the quantity of milk and fat content, less so the proteid and sugar content, and only very slightly the salt content.

The influence of **feeding** could be explained by reasoning that the gland increases its activity at the moment in which a larger quantity of nutritive substances circulates in the blood, after the ingestion of large quantities of easily digested food. This supposition could be even enlarged upon by considering that the activity of the cell is stimulated by specific substances in the food in such a way that it assimilates to better advantage and in increased quantities the necessary constituents which it draws from the blood.

From practical experience and scientific experiments it must be considered as established that the milk produced is dependent both in quantity and quality, upon the quantity of digestible food and on the presence of specific substances which stimulate milk formation.

This view has been accepted for a long time by practical dairymen, who for instance have observed that clover hay, in spite of its greater nutritive contents has not come up to the value of good meadow hay; that meadow hay cannot be replaced by a mixture of straw and concentrated food mixed in a way to make its nutritive value equal to the meadow hay; further that sweet hay proves a better milk producer than sour hay with equal nutritive value, etc. The value of individual pastures also shows wide differences in the production of milk, although examination of the grasses of the pastures gives similar results. In these investigations however it was found that beyond certain limits the influence of nutrition was no longer usable, and that with sufficient feeding of wholesome and tasty food no influence could be exerted upon production through increased rations.

If animals are allowed to starve, the change in the quality of the milk will result only after the reserve deposits of the body have been utilized to their fullest extent, or completely exhausted. In the state of starvation the milk fat shows an approach in its composition to that of the body fat.

If experiments are started with starving animals, or with animals which only receive small rations, the milk yield, according to Kellner, increases with the added increase of feed. Such cows after an increase of rations yielded: With an increase of 1.5 kg. bean bran, the increase of milk

amounted to 0.92 and 0.53 kg.

With 3 kg. bean bran, the increase was 2.40 and 1.01 kg.

With 1 kg. malt, the increase was 0.84 and 0.3 kg.

With 2 kg. malt bran, the increase was 1.31 and 0.40 kg.

The increase in yield however was not parallel with the increase of the ration, but the closer the quantity of milk produced approached the maximal production of the individual, the slighter became the increase in yield. In attempts to increase the production of the cow, the last liter of milk is the most expensive. It requires for its production the largest addition of rations.

In general it may be said that sufficient quantities of digestible proteins are the fundamental requirements for normal milk production, and that although other food substances are present in sufficient quantities the yield of milk diminishes rapidly when the protein content is decreased below the amount necessary for maintaining the body weight. For 1000 kg. of body weight 1.212 kg. of digestible proteins must be figured, together with a sufficient addition of fat and carbohydrates. Fat and carbohydrates and nonprotein nitrogenous substances in sufficient quantities act as economizers of proteins. For continuous milk production an excess of about 0.40 to 0.55 to 0.65 kg. of digestible proteins is required for 10 kg. of milk (Schmeck and Kellner).

Experiments of Morgen and Fingerling proved that while feeding tasteless non-stimulating food consisting of straw, cut straw, starch and oil, the yield of milk may be increased by the addition of substances which by themselves cannot be utilized in the production of milk, and therefore they are considered stimulating substances which principally stimulate the gland to activity.

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The percentage of fat increased 0.25 to 0.32%.

In order to produce an increase of milk it was sufficient to introduce into the food small amounts of fennel, or to sprinkle it with distillate of hay. If in the experiments good meadow hay was fed in sufficient quantities with other food-stuffs, the addition of the stimulating substances was without effect. These observations are of special importance in view of the swindles carried on with milk powders, by which money is still extorted from the farmers. Through the addition of salt to tasteless food an increased yield in milk and fat was obtained amounting to from 20.6 to 21.9%.

Hansen reports on the influence of **concentrated foods** on the milk yield, which he investigated extensively for seven years. This author divides the concentrated food into four groups:

1. Foods which increase the milk yield and diminish the percentage of fat, as for instance farina, corn, oats, and possibly also soja beans.

2. Those which do not influence the quantity of milk but increase the fat content, as for instance palm-seed oil and cocoanut oil cakes (the specific action of cotton-seed meal is less pronounced) peanut meal, corn-slop and bread flour.

3. Those which do not change the yield of milk but reduce the fat content, as for instance, poppy seeds, flour of rice and other concentrated food, beneficial for fattening, as for instance cake of sesame (Ramm).

4. Those which have no specific action, as for instance wheat bran, and malt sprouts. Such food substances are especially desirable for the use of fattening dairy cattle.

From the experiments of Hansen it appears as a matter of fact, that certain food substances possess a **specific action**. In this regard the composition of the food is of course of importance, since the action of a certain food may be checked by feeding counteracting substances. It has long been known from practical experience that the quality of milk may be greatly influenced through the method of feeding, and not only as far as the constituents of the milk are concerned but also its odor, taste, etc. To what extent food bacteria play a part in this, will be discussed in the chapter devoted to that subject.

Summer butter, mountain butter, and stable butter, are richer in fatty acids with low molecular weight, than fall butter or butter from cows which have been kept on low land pastures, or pasture butter in general prepared in the same manner. Feed rich in carbohydrates produces a soft milk fat. If abnormal constituents of fats are artificially added in experimental feeding, or if fats are fed which are otherwise not found in the body, such constituents are again found in the milk, for instance sesame oil (Engel), linseed oil, hemp seed oil (Gogitidse), iodin and iodipin (Caspari and Winternitz), Sudan III, a specific fat coloring matter (Gogitidse).

According to Schrodt and Hansen **pasture milk** on account of its greater contents of casein, contains more phosphoric acid than stable milk, which on the other hand is richer in chlorin. According to Sanson, Hesse and Schaffer the feeding of phosphate also increases the content of phosphoric acid; this however according

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to Neumann is not immediate but appears only after weeks and then in insignificant proportions. Jensen succeeded in finding only an insignificant influence on the milk from the feeding of considerable amounts of lactates of iron, calcium sulphate, disodium phosphate, dicalcium phosphate, dimagnesium phosphate, potassium chloride, chloride of sodium, and nitrate of potassium. Nitrates appeared in the milk only after 75 gm. of saltpetre had been fed. The administration of from 30 to 40 gm. of saltpetre failed to result in the presence of nitrates in the milk. The salt content of milk therefore changes only insignificantly provided normal conditions are present. According to Henseval and Mullie, the health of the animals plays a part when salts pass into the milk. If these authors fed from 5 to 25 gm. of saltpetre to 20 healthy and 8 diseased animals, the milk of the sick animals always contained nitrates, whereas the milk of the healthy animals did so only exceptionally. Definite quantities of sulphuric acid are supposed to occur in milk after the administration of Glauber salts.

Of the various foods, meadow grass, green clover, rowen, green alfalfa, and peas in which a large amount of young grain has been sown are recommended, for instance, vetch with oats, barley or rye, plants of the white mustard, rape, sainfoin, Kohlrabi turnips, etc., with which oats, barley or rye have been grown. Fodder or straw should be mixed with the green feed. In the winter instead of green feed, mangels, chopped roots, ensilage, grain, potato slop and corn slop should be fed.

Relative to the injurious effects of the various bacteria found in feeds and pastures, see the chapter on milk abnormalities. Good hay and good fodder may be recommended as dry feed. As concentrated food the substances mentioned by Hansen as indifferent, or those food substances of the first and second group which are recognized as milk and fat producers, will be found satisfactory.

Rough fibrous foods cause a loss of energy, and are not well utilized on account of the increased work of mastication and because the intestines are too greatly burdened by this feed. Individual feeding according to the milk yield appears advisable, and the best milkers may be allowed additional rations corresponding to their heavy production. In this regard of course the yield and quality of the milk should be established by sample milkings and examination of the secretion. In cow-fattening dairies the fattening of the animals should commence only in the last three months since fattening foods and fattening of the animals diminish the yield of the milk.

Pure drinking water has a great influence on milk production, and the animals should be enabled to partake of it freely according to their needs. Heyken mentions a case in which each cow yielded one-half liter of milk per day more, when instead of hard marshy spring water containing iron, good well water was substituted. Backhaus observed an increase of milk and fat content after the introduction of an automatic water supply.

Milk of poor quality is known to have resulted from the use of poor drinking water. Stagnant waters give the milk a repulsive taste.

Taken as a whole all foods and all food mixtures which are partaken of and digested by the animals without disturbance in their general condition are adapted to the feeding of milk animals. Food which in continuous feeding causes diarrhea or other intestinal disturbances should be avoided. Intestinal disturbances which quickly subside and which sometimes develop as a result of sudden change of food are of no consequence in the judgment of the food. They may cause considerable fluctuation in the yield of milk and fat content, which however subsides in a few days. From the above statement it will be seen that under certain conditions, especially when a heavy production of cream is necessary, the effects of a change of food must be considered. If, when considering evidence of adulteration, methods of examination are used which take note of the approximately constant factors in milk, that is, such as pertain to the protein-free milk serum, the influence of a feeding method, or a sudden change of feed should be taken into consideration in regard to its effect upon the milk of each individual animal.

**Spoiled food** injures the taste and odor of the milk and butter, and its effects may last for a long period after the time of feeding such food. The feeding of large quantities of beet or turnip tops should be guarded against, likewise over feeding with fresh or sour chopped roots, potato slops, residues from starch factories, brewer's grains, rape seed cake, flaxseed meal and poor straw.

The taste is improved by feeding on pasturage, red clover, meadow grass, carrots, oats and rice flour.

Firm tallowy butter is derived from grass of acid soil, from grass from fall pastures, late hay made from sour grasses, leaves of sugar-beets, or red beets, chopped roots, potatoes, peas, palm seeds, cocoanut and flaxseed cake, and cotton-seed meal.

Soft butter results from the feeding of oat hulls, corn bran, wheat bran, rice flour, rape-seed cake and sunflower-seed cake.

Clover pastures are not suitable for the production of milk for cheese making since the cheese becomes permeated with small holes, and has a sharp repulsive odor. This condition is probably brought about by bacteria which vegetate on the clover plants of the pasture.

Changes from one feed to another should not be made too suddenly if it is desired to prevent an effect on the milk production. Newly introduced food substances should not be fed in large quantities at first. In changing from dry food to pastures a diminished milk yield first results, then a period of normal yield and finally an increase in the quantity, together with an improvement in quality. Pastures or green cultivated forage containing many buttercups should be avoided, since these plants are supposed to produce red and bitter milk, especially before blooming. Meadows or pastures in which Euphorbia plants are growing exert a bad influence; they may produce enteritis with a fetid diarrhea, also paralysis of the bladder and hematuria, and may even cause abortion. The milk turns thin and bluish.

Bluish milk may also result after feeding upon plants of the Polygonum species, the ox tongue (Anchusa offic.), the cat's tail (Butomus umbellatus), the euphorbia (Mercurialis), the marsh marigold (Rhinanthus major), the forget-me-not (Myosotis), and after feeding upon poppy-cake and green alfalfa.

Red milk is produced by feeding blood root (Galium verum), madder (Rubia tinctorum), species of Karex, Skirpus, Equisetum, Ranunculus, Euphorbia and after the ingestion of young sprouts of both deciduous and coniferous trees.

Yellow milk results from the elimination of plant coloring matter after the feeding of carrots, rhubarb, yellow and red mangels, and crocus.

A garlicky taste may also result from feeding large quantities of poor straw, and according to Werenskiold after feeding of flax seed meal which contains large quantities of weed-seeds, pennyroyal (Thlaspi arvense). The taste of the milk may also be changed by the ethereal oils of the following plants:

Garlic (Alium ursinum), mint (Teukrium), hyssop (Gratiola offic.), true camomile (Matric. chamomilla), and by rape, rapecake, oil cake, turnip tops, lupins and orchids.

Milk may become **fishy** from feeding fish meal and through pasturing on marshy fields which have been inundated.

Milk turns bitter from feeding kale, rutabagas, turnip tops, lupins, pea-straw, lupin straw, and sorrel.

A bitter substance from chicory passes into the milk; the milk may coagulate more readily after the ingestion of thistles or sorrel. The ingestion of euphorbia, hellebore, rushes, and hemlock twigs should be prevented on account of the poisonous qualities of these plants. The secretion of the active poisons of these plants has, however, not been proved. Hop leaves, especially those sprayed with copper sulphate, cause a diminution of milk secretion, or even a cessation of the flow.

Concerning the elimination of **medicinal agents** with the milk, or the influence of medicinal agents on milk production the following may be stated: The passage of iodine into the milk after feeding potassium iodide has been proved by Peligot and Stumpf; if however the iodine is fed in alkaline compounds, or combined with proteins and starches, even when fed in large quantities, it does not pass into the milk. In the latter case only the plasma of the milk contains the halogen in the form of a salt, whereas in feeding iodized fats the milk fat contains iodine.

According to Rosenhaupt and Bucura the same applies to bromine.

According to Stumpf, Baum and Seeliger, in feeding compounds of lead small quantities (0.02%) of this substance pass into the milk. The ingestion of such milk was found harmless for animals. The lead was eliminated for a longer period than the time during which it was fed. Milk which contains salts of lead could produce severe injurious effects if taken for a long time (chronic lead poisoning).

Feeding of salts of copper results only in the appearance of traces of copper in the milk.

The feeding of iron preparations does not to any noteworthy extent influence the contents of the milk.

Mercury may pass into the milk (Bucura); likewise arsenic when administered per os or injected in any form subcutaneously (Bucura, Ittalie and Jesionek).

Substances like aloes, senna leaves, rhubarb and croton may influence the milk in color and taste, and will be partially eliminated with the milk.

According to Rost and Wiley boracic acid may pass into human milk; likewise after the ingestion of Glauber salts the  $SO_{\circ}$  content of the milk is supposed to be increased (Hess and Schaffer).

According to Baum tartar emetic is not found in the milk of cows treated with this drug, whereas Harms claims it is eliminated with the milk.

The feeding of large quantities of alcohol effects a diminution of the specific gravity and an increase of the fat content of the milk. The quantity of the milk appears somewhat increased (in goats). Elimination of alcohol with the milk does not occur. Weller and Teichert proved that alcohol would pass into the milk of cows after feeding them with large quantities of incompletely extracted distillery slops.

Although Horder and Herdegen claim the secretion of salicylic acid with the milk, Richter, Pauli and Stumpf disclaim its elimination in large amounts. Pauli and Stumpf succeeded in detecting small quantities of salicylic acid in the milk of nursing mothers treated with this substance, and also in the urine of their babies, as well as in the milk of experimental goats. In this regard individual peculiarities must also be considered since in one nursing mother the presence of salicylic acid was demonstrated, whereas the examination was negative in another case.

According to Pinzoni the same applies to antipyrin. Salol does not appear in the milk after its administration.

Chloroform and ether are found in considerable quantities in the milk after anesthesia (Nicloux). Landsberg failed to detect morphine in the blood, urine or in the organs, either after subcutaneous or intravenous injections, and Stumpf and Pinzoni do not believe that after therapeutical administration of morphine it will pass into the milk in demonstrable quantities. This was found by Ittalie to be the case with opium.

Oil of turpentine is not eliminated with the milk (Ittalie), and the same is true of santonin (Coronedi).

Stumpf undertook experiments with pilocarpin without however being able to find the pilocarpin in the milk, although his methods were unsatisfactory.

Atropin and fluorescin administered subcutaneously, according to Fugin and Bonanni, and Ittalie, may be demonstrated in the milk.

It should also be mentioned here that according to Ostertag meat of poisoned animals may be eaten without harm to the health. He established the fact that meat from animals which have received medicinal agents for therapeutic purposes may be consumed without any possibility of danger. The harmlessness of the meat of poisoned animals has been established by Fröhner and Knudsen for strychnia, eserin, pilocarpin and veratrin; by Harms for nux vomica and tartar emetic; by Feser for strychnine and eserin; by Spallanzini and Zappa and Sonnenschein for arsenic; by Gautier for cottonseed cake; by Feser for apomorphine; by Peschel for colchicum; by Warnke for morphine; and Albrecht for litharge.

Of course milk may contain certain quantities of poison since the udder has a special function as an excretory organ. The question of elimination of medicinal remedies, however, is not of practical importance since the medicinal doses are relatively small and their elimination occurs only in traces.

In this entire question milk inspection is powerless. Through educational advice by the consulting veterinarian the producers may be reminded of their duty corresponding to the prohibitive measures, not to include with milk for the market that produced by animals which are under treatment with certain drugs. From a hygienic standpoint only those remedies deserve mention which are eliminated for a long period after their administration, as for instance lead and medicines whose prolonged ingestion may produce disturbances of health even in the smallest doses.

Considering the fact that in normal feeding with good feeds of any kind the individual influence is paramount in milk production, it becomes evident that in establishing regulations for procuring children's milk more stress should be laid on the health of the animals, on good attendance and care by healthy milkers, and on thorough cleanliness of the stable, and cleanliness in procuring and handling the milk, than on rigorous regulations for feeding which cannot be satisfactorily carried out by the owner on economic grounds, since he must utilize the by-products or refuse of any industry of his vicinity.

There is no reason why pasture milk, or milk obtained after feeding green food should be excluded from the market as certified or children's milk, especially if from a dietetical standpoint the advantages of green feeds for cattle are considered, and the favorable influence which the pasturing exerts on milk production and metabolism be regarded.

Spoiled feed should be prohibited, and also foods which are readily subject to decomposition (fresh residues of breweries, sugar refineries, etc.). Food which is obtained through fermentation processes (hay, grass, clover, mangels, potatoes, green corn, stored in pits in the ground) should if possible be limited, since substances of odor and taste are eliminated with the milk and especially food bacteria which diminish the keeping qualities of the milk. Although they might not have a direct harmful influence in the human organism nevertheless they may spoil the taste of the milk.

The beneficial influence of pasturage cannot alone be attributed to the advantage of change of feeding, but is also the result of the stimulating action of the light and air on metabolism, and of the mild form of exercise. Therefore in the absence of pastures it would be advisable to provide exercising paddocks for the animals. According to Munk moderate exercise increases the yield of milk and its proportion of solid substances.

Excessive exercise of cows should be avoided.

Although moderate exercise on rich pastures in connection with other factors which increase metabolism, produces more abundant and richer milk, increased exercise reduces the quantity of milk but increases its fat contents. In over-exertion however the quantity and quality of the milk are reduced, and the milk and butter both develop an irritating taste (Dolgich).

Exhausting transportation changes the milk production considerably, especially when during that period the cows are milked irregularly, or for advantage in selling the cows the udders are allowed to become engorged with milk. Stasis mastitis results, which may be cured only by repeated and thorough milkings.

Excitement of any kind, such as fright, taking away the calf, change of surroundings (new purchaser), and change of feed, may for a longer or shorter period cause a diminution of the quantity of milk and a change of its quality.

Backhaus observed an increase of over 7% in quantity of milk production and 8% of the fat content after the cows had been **curried**; in other cases it amounted to 4 and  $2\frac{1}{2}$ %, respectively.

In pasturing cows, sheds should be provided for shelter from the strong rays of the sun and rain; otherwise according to Schwenk the yield becomes diminished. Kirsten observed a diminution of the production of milk after prolonged rain. According to Ingersoll and Duncanson, marked **changes in the weather** may even be of significance during the season when the animals are stabled.

A rise or a fall in the temperature may cause a reduction in the fat content. In the morning following rainy nights the milk may become richer. The influence of weather and pasture on milk production has been observed by various German investigators, but the results differed considerably. Some observed a diminution of the fat content, others an increase, while some noted a diminution of the milk yield, and a number of others detected no reaction whatsoever. Following the passage of a heavy thunder shower a diminution of the milk yield and an increase of the fat content were observed which corresponded to the increased activity of the animal in the equalization of the body heat.

If herds which are pastured at night are compared with those which are stabled at night, no favorable influence of the stabling at night is observed, neither regarding the quantity of milk nor its fat content. In animals kept uninterruptedly out of doors the fat content increased more rapidly than in those kept in stables. In the former the live weight increased more rapidly than in the latter; in other experiments, however, the results remained the same. Wychgram in his experiments in East Friesland found the milk yield in stabled animals increased, but the fat content diminished as compared to milk from cows at pasture.

The cows which furnish the milk supply of cities as a rule are kept in large stables. The **stabling** of course should be such that the health of the animals does not suffer, and means should be provided for a pure milk production.

It is not so difficult to comply with these two requirements as some farmers believe. They may be attained without great additional cost, since the increase of expense for proper stabling is amply covered by the increased income from the animals.

In equipping so-called model stables, an extraordinary amount is usually expended for luxury in the equipment and furnishings, so that the practical farmer on visiting such stables is frequently disheartened, instead of being encouraged to change his more primitive place of milk production to comply with these models, since a simple calculation of the expense of such buildings for keeping cows shows him that a change of his stable conditions to equal those of the models is impossible.

A really valuable modern stable however may be built at the present time without considerable additional cost, and may be equipped so that the additional expense of milk production due to the wearing out of the building is not greater than the cost of repairing an unsanitary stable.

For the erection of a new stable a dry building site should be chosen if possible, or at least the penetration of dampness from the ground should be prevented through separation and isolation of the ground and walls. Only under such conditions can the requirement of clean walls be attained.

The floor of the stable must be water tight and without cracks and holes, and should permit of ready cleansing and disinfection. It is to be regretted that such water tight floors are frequently too cold for milk cows, and the action of the cold must be diminished by the provision of wooden planks. The floor surface must be rough enough to afford the animals a solid footing.

The walls of the stable should be provided to a height of 6 feet with an unpenetrable, washable covering, which however should not be dark as was customary in the past, in order to hide the accumulated dirt, but should be light in order that dirt may be readily seen and removed.

The stable ceiling should be separated from the feed loft situated above it, by an air space, and should be whitewashed, the same as the walls. In order that it should be impervious to the stable odors, the ceiling on the inside of the stable should be covered with minerally treated pasteboard. The air space between the ceiling and feed loft should communicate with the outside air.

Angles and corners should be rounded off, in order to prevent the accumulation of dust.

In large herds the erection of several small separated stables should be given preference to a large single stable for all animals. Each of the stables should be made for 16 to 20 animals. The advantages of the smaller buildings are manifested in better possibilities of ventilation, the easier removal of manure, cheaper construction of the roofs, less excitement for the animals, and better possibility of caring for and feeding the individuals. Against these advantages the absence of close supervision, which is afforded by the large stable, is not material (Schuppli).

The animals should be placed in rows in such a way that the light may strike them from the side or from the rear.

In order to provide a great amount of light, high, broad windows and transparent instead of only translucent window glass should be installed, the total lighting surface of which should amount to at least one-twentieth of the floor space of the stable (according to Schlossmann the comparison should not be much less than one-fifth).

Placing the animals face to face should be avoided on account of the danger of infection with tuberculosis, or this danger should at least be diminished by broadening the passages.

The windows should commence from 5 to 6 feet from the floor. Artificial illumination should be provided for; transoms assist in the airing of the stable by allowing ingress of natural atmosphere.

The ventilation should be calculated so that the air of the stable should not contain more than 3:1000 (Marker), or 1:1000 (Schlossmann) carbonic acid. According to Schlossmann a cow weighing 1,100 lbs. produces 12.71 cubic feet of carbonic acid, which would have to be diluted by 1,000 times its quantity of introduced air in order to contain only 1:1000 of the required quantity in the air. This introduction of air is made possible by three changes of air per hour, without permitting a disturbing draught. Therefore, according to Schlossmann, the air space in a stable for cows weighing 1,100 lbs. must be 12,710:72=176.5 cubic feet. Generally however, on account of economic grounds a much smaller air space has to answer the purpose. Schuppli even believes that a reduction of air space below the ordinary 70 to 88 cubic feet of air per animal would be permissible when the ventilation system is working properly, and is satisfied with 42 cubic feet per animal when the ventilation provides for sufficient renewal of air.

The supply of air is provided by wide shafts which take the air from the outside at a height of three feet, lead it up through the wall and expel it from the stable ceiling into the stable. The foul air escapes through an opening close to the stable floor of one or more discharge shafts, which are carried to the highest point of the stable ceiling, or sideways from the median line upwards and outwards. The total capacity of the discharge shafts should be somewhat smaller than the capacity of the supply shafts.

In intensive ventilation, especially when the air space provided for each cow is considerable, heating of the stable might become necessary, a provision which of course could not be considered for the ordinary, medium-sized or even larger establishments.

Well-installed transom ventilators, if sufficient attention is given to their operation, would supply the desired change of air even without heating, and at the same time maintain the desired temperature of 16° to 18° C. If heating is provided the air supply shafts should open over the heaters.

In providing stalls, from the standpoint of cleanliness only the so-called Holland type of stable floor should be recommended for dairy stables. The principle on which these are built consists of rather short standing space with broad, deep drainage trough in the rear. The urine and manure falls into this trough, and soiling of the animal is thereby prevented while the contamination of the bedding is minimal.

Among objections to the Holland type of stables, it is sometimes claimed that the animals cannot move sufficiently and that such stabling constitutes a cruelty to the animals, etc. The best proofs against these objections without doubt, are the facts that in countries which are in the highest state of agricultural development this method of stabling has been practiced for a long time, and the animals soon get used to this method of stabling without suffering in their general condition or being affected in their milk production.

In the Holland method the tails of the animals are tied with a cord in such a way that while the animal stands its tail hangs in a natural position, but when lying down the tail is kept elevated so that it cannot be submerged in the contents of the drain.

The cords are tied to a rod which runs near the ceiling, parallel with the row of cows, or the cords, with weights attached, are allowed to hang over this rod.

The shortness of the stalls of course requires a low feeding trough, over which the animals may extend their heads while lying down. In order that the animals may not annoy each other, the individuals are separated by means of partitions, which extend upwards to the height of the head or the shoulder and at the same time have fastenings which are used for tying the cows.

To prevent the cows from backing into the drain, a moulding of one-half inch is provided along the upper border of the drain trough. This moulding holds the slipping foot and makes possible the placing on the floor of wooden slats when pregnant animals are about to calve. The fundamental principle against the possibility of slipping is the above-mentioned provision of a sufficiently rough stable floor.

The width of the stalls should be about  $3\frac{1}{2}$  feet, the length  $5\frac{1}{4}$  to  $5\frac{3}{4}$  feet.

The most satisfactory **feeding troughs** are those which correspond to the conditions of natural feeding in the pasture, and they should be so constructed that they will serve for animals of all ages with the possibility of providing partitions in order to separate the individual rations. For watering the animals it is advisable to provide each stall with automatic water supply.

The accumulated litter in the **drain** trough should be mechanically removed as often as possible into liquid manure pits which terminate in a tunnel with collecting canals, or the litter may be thrown into a chute through a shaft leading to a water-tight liquid manure pit the size of which should be so arranged that 88 to 106 cubic feet of manure space are allowed for each animal.

Over the liquid manure pit on wooden lattice frames, or alongside of it, should be placed the manure pile, with 30 square feet of surface for each grown animal. On account of the desired decomposition of the material the first-mentioned arrangement of the manure over the liquid manure pit is most desirable. There should be a separation between the stable and the manure pile of at least 20 feet and the latter should be placed on the side opposite to the principal direction of the wind in that locality. The outlets of the manure drains should be closed from the stable by trap or sliding doors.

Good straw should be selected for the **bedding** of animals. The question of straw which is very important in localities where but little is grown is favorably solved by the Holland method of stabling, since by this method a great deal of straw is saved by the short stalls with but slight soiling of the animals. Forest and shade leaves are not recommended, since it is claimed that milk troubles result from their use. Turf straw, shavings and sawdust should be avoided if possible on account of the formation of dust, but should not be excluded if good straw bedding cannot be obtained. The use of bed straw should be prohibited in milk stables. The feeding of the cattle must be performed after milking, on account of raising the dust. The removal of the manure **a**nd the cleaning of the animals should take place at least one hour before the milking.

Complaint is frequently made to hygienists that the require-

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ments which are made by them relative to stable hygiene must increase the cost of keeping the animals, and thereby increase the cost of the milk. This view is only justified to a slight extent. Stable hygiene if satisfactorily adjusted will result in a considerable increase in the yield of the dairy animals.

The economic losses which are induced by udder affections, which spread with especial rapidity in filthy stables and from unclean milking, have been discussed in a special chapter. Attention should only be directed here to the increased production following proper attention to cleanliness of the animals, and to the findings of Bloymeyer and others, according to which cows in well ventilated stables, all other things being equal, yielded from 450 to 480 liters more milk per head each year than cows kept in unventilated stables.

The favorable influence of exercise and light work has also been discussed above. If possible the animals should be given an opportunity to run out of doors in a paddock for at least one to two hours daily, even in the winter months.

Of all outside influences, regular and complete **milking** constitutes the most prominent stimulant for inducing the activity of the udder. It is known that cows which are milked three or four times in each twenty-four hours give more milk than those which are milked only twice (Backhaus). The increased yield from milking three times amounts to from 10 to 15% more than the production obtained from two milkings; in four milkings the increase amounts to from 6 to 8% as compared with three milkings.

The quantity and composition of the milk at each milking depend somewhat upon the time which has elapsed since the last milking. According to general experience the morning milk is of greater quantity with a smaller amount of fat, in comparison with quantity and fat content of the evening milk. During the night absolute rest prevails, whereas during the day the influence of light and motion causes an increase of metabolism which is also manifested in the variations of the body temperature shown by the animal in the morning and evening.

The differences in milk, which are obvious in irregular or so-called broken milkings, may be explained in a different way; that is while the milk at the beginning of the milking contains 0.5 to 1.5% fat, in the middle of the milking it shows 2 to 3 to 4%, and again rises towards the end, during the last strokes of milking to 8 and 10% (Melander, Kaull, Cotta, de Vrieze). The fat-free solid substances are subject to slight changes (according to Boussingault the fluctuation amounts to from 0.2 to 0.28%). The conditions in the milk when the calf sucks are similar.

A truly plausible explanation can hardly be given to the supposition of a separation of cream in the udder (Zschokke); likewise it hardly seems reasonable to suppose that the thin plasma particles flow towards the cistern, while the fat globules as a result of greater fusion and friction are retained, and are only pressed towards the larger milk ducts and the cistern by the newly formed milk which is secreted during the act of 10 milking. The principal cause lies probably in the fact that the separation of the fat represents a greater expenditure of energy than the secretion of the plasma. If the cell is exhausted by previous milking it then secretes milk during the period of rest which is especially rich in plasma and poor in fat. Through this period the alveoli and milk duets are dilated, and the gland cell becomes flat and is at rest. In this position of rest it recovers and is ready for renewed action when, through renewed milking operations, the fluid is withdrawn and stimulation of the secretion is applied through the teat.

If the milk is removed without this stimulation of secretion, with the aid of a milking tube, only milk poor in fat flows from the eistern and the larger milk duets, and the flow ceases as soon as pressure is no longer exerted on the secretion.

If, however, through milking (or other stimulation) new secretion takes place, the rested gland cell engorges with nutritive material, and converts it into fat, which is separated during the milking in increased quantities until the secretion of plasma and the separation of fat cease, which marks the height of these two processes in the secretion of milk. Through an increased stimulation by additional milkings the cell may be further stimulated to a special production, which consists in an increased fat formation (Hegelund). Henkel succeeded by this procedure, in increasing the quantity of milk by 2.4%, and the fat content by 6.2%.

As already mentioned Hegelund's method requires additional work, which may possibly lead to the hiring of additional help and must be considered (Kirchner), when estimating profit and expense. The principal factor in the various methods of milking lies in the thorough milking out of the udder, which will retain its maximum of production only by such practice. Henkel succeeded in demonstrating the extent to which the milk production depends on the **thoroughness of the milker**. The production of a cow when milked by a thorough milker amounted to 8.1 kg. (17.8 lbs.) of milk, with 4.2% of fat; by a less satisfactory employee to only 5.6 kg. (12.3 lbs) with 2.7% of fat.

At the same time it is immaterial what method of milking is pursued, that is, whether the teats are milked crosswise, or those on one side, or those of opposite quarters, simultaneously. Milking of a single teat at a time, which of course is not customary, yields less milk, and the last milked quarter is the poorest in fat (Lepoutre and Babcock). The influence of special methods of milking has been more fully discussed in the chapter on the procuring of milk. According to Klinkmüller the milk yield of the right half of the udder is 3.97 kg. (8.7 lbs.), the left 3.65 kg. (8.03 lbs.), with fat contents of 3.65 and 3.31% respectively. The cause of this increased production of the right half of the udder is, according to Klinkmüller, the result of the practice of milking the right half first, and therefore it is advisable to practice alternation in milking, from right and left.

If milking stools are used care should be taken that the milkers do not take hold of the seat with their hands. The most recommendable stools have only a single foot, and are secured around the body by a strap. Switching of the animal's tail must be prevented during milking by tying it up, or by other effective contrivances.

Conditions which prevail in the handling of milk after it has been procured are of special importance in providing milk of the best quality. The changes which milk undergoes have been discussed sufficiently for the purpose of milk hygiene in special chapters. Those points principally should be emphasized which are to be followed during the drawing and preparation of the milk, in order to check or prevent undesirable and early decomposition of the product.

This relates primarily to **cleanliness.** The requirements of milk hygiene go hand in hand in this respect with the purely economic requirements of the dairy industry.

If it is considered how much milk spoils prematurely on account of improper care and the amount of loss which is sustained when the creameries have to discard hundreds of pounds of cheese on account of improper handling of milk, then the economic value of cleanliness in the stable becomes obvious. The Holland method of stabling, cleaning of the cows and especially the udders are quite simple but important factors in such cleanliness.

In keeping the udder clean special attention should be given towards preventing its contact with filth.

Dry cleaning with suitable straw, or rough towel is preferable to moist washing which often consists in spreading the softened dirt over the entire udder. If the dry method of cleaning the udder is used such milking pails should be provided which will prevent the milk from becoming contaminated by the dust originating from the cleaned udder.

If the udders are washed it should be done with lukewarm water without soap. Subsequently the udders should be rubbed dry and slightly lubricated with paraffin salve. Even with these simple operations milk may be obtained containing only very small numbers of bacteria, and would suffice for all practical purposes. Covering the animals with linen sheets, disinfection of the udder in water-tight bags, and washing of the entire animal represent somewhat exaggerated procedures, and besides they require additional expense, which can be afforded only through a special increase of the price of milk.

The **milk pails** should be so constructed that they will prevent dust and dirt from falling into the milk. This is accomplished by using covered pails, which possess a special receiving tube supplied with a funnel for taking in the milk. Between the receiving tube and the funnel an arrangement for filtering through cotton may be placed. The so-called Algäuer milk pails are provided with such arrangements; likewise the Königsförder milk pails and the sanitary pails of Gurler and North. The funnel should be rinsed and provided with a fresh piece of cotton after the milking of each cow.

After milking is finished the milk should be immediately taken from the stable. This is frequently accomplished by pouring it into a funnel arrangement fastened to the wall through which the milk passes into a suitable tin lined tube to the milk room. This tube should be removable in order that it may be properly cleaned. In the milk room the milk is further treated by another straining and cooled by simultaneous aëration after which it is either directly filled into bottles, cans or collected in a vat in order that it may be thoroughly mixed.

The milk should be handled as little as possible, since each manipulation not absolutely necessary, means a poorer condition of the product from a hygienic standpoint. The producer therefore after straining the milk through cotton strainers should **cool** it and fill it into clean bottles or well-galvanized and properly cleaned cans.

The straining of milk through straining cloths which have been carelessly cleansed by rinsing in cold water, and which in most instances fail to answer the purpose on account of their large meshes, is, it is to be regretted, in most instances merely a pretense, which only tends to further spoil the dirty milk. Milk which is obtained in an unclean way cannot be deprived of its poor qualities by any mechanical means, since the filth dissolves and the bacteria pass through the straining cloths and the cotton filter. If the accidentally contaminating bacteria are removed immediately during the milking (cotton filter in the funnel of milk pail), a considerable improvement of the milk results. The value of artificial cleaning, however, will continue to decrease in proportion to the length of time elapsing between the time of milking and cleaning. If the cleaning of dirty milk is accomplished only hours afterwards at the collecting places and creameries it should be considered as direct fraud, which gives the product the appearance of good quality without however improving it in any way. In such cases filtration and centrifugalization only serve as means of deception. Filthy milk which has been subsequently cleaned, must in spite of its cleaned condition be considered as spoiled in the sense of the pure food law, even if no changes are yet apparent in it.

In milk control work there are frequent opportunities for confiscating dirty market milk, and not infrequently the examination reveals that the contamination of the milk consists in dustsized particles and cow manure, all of the same caliber, which indicates that the milk has been subjected after milking, to a straining process which permitted the manure particles which had been disintegrated during the process of milking to pass through the strainer. Unstrained milk obtained under filthy conditions usually shows the presence of course straw particles, manure, bits of fodder and cow hair.

After straining, the milk is allowed to flow down over the outside of a double corrugated surface, or a series of parallel horizontal pipes for the purpose of cooling and simultaneous aëration; these surfaces are kept cool through pipes containing running water, ice water or brine. Especially practicable and serviceable are the so-called round coolers which are provided with spiral pipes, covered with tinned-copper sheets, over which the milk runs in a thin layer.

#### Aeration.

It will be proper to describe here very briefly the changes which milk undergoes through **freezing**. The freezing of milk occurs with remarkable frequency in the winter time, when the milk is subject to long transportation. There is no change in the number of bacteria which were present at the moment of freezing until after the thawing of the milk. There is neither diminution nor marked increase.

According to the data of Vieth, Kaiser and Schmieder, Henzold, Bordas and Raczkowski, Fritzmann and Mai it may be seen that in the freezing of milk a marked separation takes place. Mai found that such milk under certain conditions may appear at the first glance like ordinary milk, although it is really frozen. Crystal needles of ice make their appearance in such milk. If the freezing continues layers of ice appear at the sides of the milk cans and on the surface, thus enclosing a central fluid portion. The upper part of the milk containing the cream layer freezes more loosely, in a spongy leaf-like manner. After thawing, the milk has its original consistence and its original odor and taste. The peroxidase content also remains unchanged.

The milk inspector must consider the separation of milk during freezing. In taking a sample, special care should be taken to determine whether the milk cans or other vessels already contain ice. Frozen milk should not be sold to customers until thoroughly thawed.

The **aeration** of milk permits the escape of carbonic acid, hydrogen and sulphide of hydrogen, and supplies the milk with air, so that in all probability the development of certain bacteria is checked, which otherwise, if the milk had been filled into containers in a warm and un-aerated condition, would have imparted to the milk a sharp, disagreeable animal taste and odor; the milk would have been "suffocated."

The corrugated surface coolers are especially suitable for use in small dairies.

The **cans** into which the milk is filled after cooling should be tinned in a satisfactory manner. It is to be regretted, however, that this is the case only with new cans. The tin covering especially on the places where the outside strengthening bands are placed, is very imperfect, and after a shorter or longer time defects in the lining develop, which soon result in an extensive formation of rust. The oxidation of the iron finally results in tears and holes which produce deep, sharply circumscribed depressions in the side of the can in which rust, decomposed milk and slime accumulates.

The transportation of milk in rusty cans, or those in which the lining has become damaged, gives it a disagreeable tallowy taste.

Milk should be protected from bright light. Sun rays and indirect daylight may give the milk a tallowy rancid odor and taste, in the same manner as is the case with the prolonged action of ultra-violet rays.

It is important during transportation that the vessels be closed in an **air tight** manner, and with a cover consisting of non-porous material.

All milk utensils should be cleaned with hot soda solution, with subsequent rinsing in fresh pure water, and if possible combined with steam sterilization and rapid drying in places protected from dust.

The transportation of milk should be rapid, and where possible it should be shipped after each milking.

In creameries the treatment of milk after its receipt should be principally confined to cooling. This cooling is carried out in deep cooling appliances or double coolers in which the abstraction of the heat takes place through water at the point at which the milk flows into the cooler, and the lower part is further cooled with ice water or with brine. All further manipulation and attempted improvements of milk for drinking purposes are of no use. Spoiled milk should be excluded from the market and not be subjected to renovating processes.

Thus in some creameries it is customary to clean the milk not only by renewed filtration, but also by **centrifugalization**, which is frequently done on the supposition that the bacterial content of milk becomes reduced through such treatment. This, however, is impossible; on the contrary, such milk often becomes contaminated again by bacteria from the non-sterilized centrifuges, and even if the milk is centrifuged in a sterilized apparatus only those bacteria will be eliminated which adhere to the courser bodies having a higher specific gravity (pus, fibrin, filth, casein coagulum, etc.).

The separator slime therefore contains principally fodder and manure bacteria, lactic acid bacteria, species of milk moulds, and bacteria of those fermentation processes which take place in the residue of milk cans and transportation vessels, and further the specific causative agent of mastitis occurring in pus.

Therefore, although a great number of bacteria are removed, the bacterial count through plating of the centrifuged milk discloses a considerably larger number of bacterial colonies than was the case in the milk prior to centrifugalization, although the short time of the centrifuging process does not permit of an actual increase of the bacteria. Severin observed an apparent increase in bacteria up to 70%. This may be explained by the fact that through centrifugalization, bacterial clumps and colonies floating in the milk, and the clumps of pus and fatty leucocytes which have embodied bacteria, are broken apart, and the bacteria are thereby distributed in the milk. Therefore, in spite of the removal of considerable numbers, there is an apparent increase. This distribution is such that the separator slime and cream are considerably richer in bacteria than the skim milk. The richness of the cream in bacteria may on one hand be explained by the fact that large quantities of bacteria are dragged upward with the fat globules, and on the other hand by the fact that leucocytes containing bacteria, inflammatory products, etc., which possess a lower specific gravity, through fatty changes or fat enclosures, are taken up with the fat globules into the cream.

The most important factor in the spoiling of milk in creameries may usually be found in its being kept for too long a period before it is marketed.

## CHAPTER VIII.

# BACTERIA IN MARKET MILK; THEIR ORIGIN AND ACTION.

Before milking is commenced the udder should be cleansed of all adhering dirt. Cleanliness in milking is one of the most important factors in giving the milk good keeping qualities. Subsequent cleansing through straining, filtration, centrifugalization, etc., is of little purpose after the dirt particles (straw, manure, dirt) have once imparted to the milk their soluble constituents, and an actual inoculation has been accomplished with the bacteria of filth.

The tail of the cow should be tied, in order to prevent bacteria from the skin being thrown into the milk by its switching. If left free it may even subject the milk to contamination with coarser substances.

The importance of the effect on human health of bacteria which fall into the milk, and which multiply therein when milk is improperly procured, is not known, but the thought is at least repulsive when it is considered that milk consists of a manure suspension of a bacterial culture, and on this ground alone absolute cleanliness in milking should be insisted upon. In order to attain this it is again necessary to provide a properly ventilated and well kept stable, as well as milk room. The veterinarians can in no way obtain a better recognition of the milk problem than by always pointing out to the farmer the necessity of keeping healthy cows in properly constructed and well-kept stables, and in impressing upon him the fact that the procuring of pure milk and its proper handling constitute the fundamental principles of a prosperous development of the milk industry in general, and not for the milk supply of the city alone. Many farmers, especially the small ones, can only be convinced by practical demonstrations of the advantages to be derived from proper stabling and care, and therefore it is our duty to win over reasonable and progressive farmers to the erection of model dairies, and to offer to the smaller farmer the aid and advice by which he can improve his condition with the least expenditure. Even if nothing more than diligence, attention and a feeling of responsibility are aroused these alone would mean a tremendous improvement over the conditions prevailing at the present time.

It is evident that if no special milk rooms are provided every-

thing should be avoided during milking which would cause stirring up of the dust, such as removal of manure and feeding.

As long as the milk gland is in a healthy condition the cells secrete a sterile product, which becomes contaminated with bacteria only in the lowest part of the teats, in their ducts, or during the process of milking, etc.

Bacteria are always present in the lowest parts of the ducts of the cistern, as a result of contamination from the litter. These bacteria and also those which fall into the milk during milking, and the massaging of the quarter, render the procuring of sterile milk practically impossible, even when the strictest care is taken to prevent as far as possible the subsequent contamination of the milk.

In spite of opposing views, it may be considered proved at the present time that the milk in the udder is sterile as long as the animal is not affected with diseases of the udder or severe general affections. Lister, Miessner, Escherich, Kitt, Trommsdorff, Rullmann, Seibold and others succeeded in procuring absolutely sterile milk. This, of course, was only in small quantities, and drawn with special care, such as washing the udder, disinfection, protective covers, etc.

The first streams of milk are of course always contaminated with bacteria (Schulz, Luz, d'Heil); the subsequent ones may be sterile, but frequently they also may contain bacteria, as confirmed by the works of Boekhout, Ott de Vries, Ward, Koning, and Freudenreich. In practice the procuring of milk with a moderate number of bacteria must be considered satisfactory. Schulz, for instance, found that the first milk procured contained 55,566 up to 97,240 bacteria per c. c., while during the middle of the milking it contained only 2,070 to 9,985, and in the last from 0 to 500 bacteria. In an interrupted milking, that is, when the milk was obtained in four portions, Backhaus and Appel counted in the first part 170 to 950, in the second 60 to 255, in the third 10 to 70, in the fourth 0 to 45 bacteria per c. c.

For procuring sterile milk the following measures are recommended:

Washing the udder with soap and water, disinfection with alcohol (Kitt, Kolle); disinfection with mercuric chloride solution (Fauss, Klimmer); mercuric chloride solution and rinsing with boracic water and formalin (Boekhout and de Vries); washing with a 2% mercuryoxycyanide soap (Freudenreich), followed with rubbing with sterile cotton (Ostertag); salicylcotton (Eichert); sterile cloths (Freudenreich); and then after cleaning and thoroughly disinfecting the hands of the milker with soap, water and the same disinfecting agent which has been used for disinfection of the udder, the milk may be carefully drawn by the usual method, which is termed "fisting."

Backhaus after a coarse cleaning of the udder, covers it with a bag which con-tains disinfecting fluids. After a short action of the disinfectant the fluid is allowed to escape through a stop-cock attached at the lower part of the bag and the udder is rinsed with previously boiled warm water. Other authors cover the body of the animal with sheets, leaving only the udder exposed. Boekhout, Ott de Vries and Trommsdorff used sterile milking tubes for taking

samples. In this operation it is to be regretted that even in the most careful manipulation with sterile milking tubes, infections of the udder sometimes result. Rullmann therefore prefers direct milking. He also rubs white paraffin salve into the skin of the surrounding part of the udder.

Through such protective measures individual authors obtained the following results:

Freudenreich: 200 to 300 bacteria per c. c.

Szasz: 2 sterile, 11 with an average of about 2,700 bacteria. Hesse: 1,600 bacteria per c. c.

Marshall: 295 bacteria per c. c.

Lux: 0 to 97 to 6,800 bacteria per c. c.

Kolle: 80 to 15,000; in 33% of the experiments the counts were below 300 bacteria per c. c., 50% below 500, others up to 800 per c. c. Only 4.7% yielded 700 to 800 bacteria.

Willem and Minne: 1 to 5 bacteria per c. c.

Willem and Miele: 0 to 37, 4 to 218 bacteria, respectively.

Seibold studied the bacterial content of the milk under the most varied experimental methods, and especially under conditions which correspond most nearly to those prevailing in practice.

1. Without protective measures.

2. After soaping the udder.

3. After soaping and disinfecting with alcohol.

4. After repeated disinfection with alcohol, and procuring through sterile milking tubes.

The poorest results were obtained, as would be expected, by the first method, and the best results by the fourth method, with which it was frequently possible to obtain completely sterile samples.

The number of bacteria by the fourth method fluctuated between 0 and 12, by the third between 0 and 85, and by the second between 0 and 434 per c. c.

The first method produced samples of milk with less than 10 up to several thousand bacteria.

Trommsdorff and Rullmann observed in samples which had been procured without special precautionary measures, such as cleaning of the udder and hands, on an average (96 samples) 6,700 bacteria per c. c., but only 1,500 bacteria when a thorough cleaning of the udder and of the hands of the milker had been undertaken.

Seibold, Trommsdorff and Rullmann found in individual cases an enormously high bacterial content even in freshly procured milk, the colonies on the plates containing mostly streptococci. These samples were obtained from cases of inflammation of the udder, and the milk was already contaminated with streptococci before leaving the udder. These organisms would not otherwise be present in aseptically procured milk (Seibold).

As it is difficult, even under the strictest conditions, to procure sterile milk, or milk with a very low bacterial content, therefore in the wholesale production of milk such results are still more difficult, and in fact impossible. The milk, immediately after leaving the milk canal, becomes contaminated by bacteria which have colonized there. Among the bacteria which may be found in the milk from animals free from udder affections, and which has been drawn under aseptic conditions, the groups of staphylococci, colon bacteria, *Bacillus subtilis* and *B. mesentericus* should be especially mentioned. Seibold also demonstrated acid-fast rods. Rullmann and Trommsdorff found no representative of the colon group, but they isolated staphylococci, a few representatives of spore-bearing species, and especially the anthracoides (mycoides) species.

In ordinary milk production there also come into consideration an army of air and stable bacteria, which adhere to the food, manure and litter, as well as those which vegetate, as saprophytes, on the skin of cattle, especially on the skin of the teats, and on the hands of the milker, besides those groups of bacteria which colonize with special predilection on milking utensils and in the cans.

The number and kind of bacteria found by the different authors vary to a considerable extent, depending upon the degree of cleanliness used in obtaining the several samples.

Russell, in using sterile milk vessels, found 165 bacteria per c. c. in freshly drawn milk, while in case of only ordinary cleansing there were 4,625 bacteria per c. c. in such freshly procured milk. Grotenfeld counted in the milk from well-kept animals, in clean stables, only 106 bacteria as compared with 670,000 per c. c. in milk from dirty stables.

The kind of milk can also has an influence. Backhaus considers enamel cans as the best; tin vessels were found to be almost as good, while milk vessels constructed of wood were unsatisfactory.

The work of Koning shows the influence of the bacterial flora of the air on the bacterial content of the milk. The author counted 500,000 to 700,000 bacteria in the stable air, whereas the outside air contained only 90,000 bacteria. He found that the volume of air between the cows was especially rich in bacteria. Milk which is procured in the pasture contains fewer bacteria than stable milk. If it is customary to change the straw and also feed shortly before or during the milking time, these factors tend greatly to increase the bacterial content of the milk.

If the milk is subjected to the so-called "improving methods" of the most varied kinds, and has to be transported for long dis-

tances, it is obvious that when it finally reaches the consumer it must contain tremendous numbers of microbes of various kinds. The author counted in the market milk of Munich from 13,000 upwards to several millions of bacteria per c. c.

Milk offers to most bacteria which may contaminate it a splendid culture medium, their multiplication in it depending on the character of the container (cans, flat or open bowls), temperature and subsequent treatment.

Freudenreich, B. Meyer, Cnopf and others conducted experiments on the influence of cooling on the number of bacteria, and established definite proof for the statement made in practice that immediate cooling constitutes the best preserving agent for milk.

According to Cnopf the multiplication at 0 deg. C. was remarkably low, at 12.5 deg. it was 4 to 935 times greater, and at 35 deg. 2,200 to 3,800 times greater than at 0 deg. C.

Freudenreich proved that in milk which at the beginning of experiments contained 10,000 (accurately 9,300) bacteria, they scarcely multiplied when kept for three hours at 15 deg., whereas at 25 deg. they doubled, and at 35 deg. they tripled in quantity. After six hours at 15 deg. they numbered 2.7 times, at 25 deg. 18.5 times, at 35 deg. about 1,300 times more than the original number, while after nine hours the number when kept at 15 deg. was 5 times, at 25 deg. 108 times, at 35 deg. 3,800 times as numerous as in the original count; and in 24 hours at—

15 deg. C. the count was 5,700,000, or 613 times 25 deg. C. the count was 50,000,000, or 5,380 times 35 deg. C. the count was 570,500,000, or 61,344 times

The author desires at this place to comment especially on the slight, and somewhat problematical value of bacterial counts, not alone because the results of the different sowing and counting methods show such enormous differences, but because the entire system also depends on a supposition of the development of a colony from a single bacterium which was previously present, a premise which is open to very serious objections. If it is considered how many bacteria attach to tenaciously adhering threads (*sarcina*, *streptococci*), and how many bacteria possess a tendency to proliferate in cultural combinations, and to remain together in the relatively sticky material of milk, then it becomes apparent that the counted bacterial number represents but a small part of the number of bacteria which are actually present in the milk. Of course in general the number of colonies developing on the plate represent a certain initial point for deducing whether and in what degree a bacterial growth has taken place in the milk, but it does not indicate more than the relative age of the milk, since fresh milk number also be rich in bacteria, and besides luxuriantly growing as well as slowly multiplying bacteria may be present in the milk.

A better method for the establishment of the actual number of bacteria in milk is the one suggested by Skar, which consists of a direct count of the bacteria in a smear (see technique).

Through plating a certain impression is obtained of the kind of bacteria occurring in the milk, and corresponding to the growth of the colonies and the morphology of the bacteria it is possible to draw certain conclusions as to the groups under which the bacteria that are present may probably be classified.

Further deductions as to whether the microbes should be considered pathogenic, and whether bacteria are present which confer disease-producing properties to the milk through products of decomposition, splitting up of proteids, etc., can only be possible after an accurate determination of all properties which would allow the recognition of the colony as a certain species belonging to a large group.

This determination of the representatives of a group is not simple, and requires study and continued experimental work of days and weeks relative to fermentation qualities, requirements of growth, pathogenic properties on test animals, ferment-like characteristics, etc. These experiments are only of an optional value in practice on account of the easy decomposition of milk as a food substance. Nevertheless through continued experiments on these problems valuable data and results have been obtained increasing our knowledge of the spread of typhoid fever and the methods for combating this and other diseases.

Therefore, it should be aimed to prevent the entrance into the milk of directly or indirectly injurious bacteria by procuring the milk in a clean and careful manner. Once such bacteria gain entrance into the milk and multiply, their recognition and isolation are too difficult for the practical inspector of milk to consider.

Milk hygiene can produce practical results only if it is inaugurated at the place of production.

The pathogenic bacteria of diseases of animals and man will not be considered here, and only brief consideration will be given to the army of saprophytes which gain entrance to the milk from the air, straw, manure and the milk vessels.

Although from the numerous possibilities of infection of milk a definite bacterial flora can hardly be expected, nevertheless, corresponding with the nutritive material of the milk, and the methods employed in its storage and transportation, as well as the subsequent treatment, conditions are created which are favorable to some varieties of bacteria, while for others they are less favorable or even harmful. Through the growth of a certain kind of bacteria the conditions may be changed in such a way that the requirements of propagation for other groups are produced. Likewise through symbiosis conditions may be developed which are required by certain species of bacteria, or under which certain species may be destroyed, whereas without symbiosis probably neither of the species could exist, since they are dependent upon The growth of certain species is therefore dependent each other. on numerous influences.

According to the thermal limits in which bacteria can live, the species may be separated into those which thrive at 0 deg. C. (up to 15-20 deg.): psychrophile; those which thrive at 10-15-40 deg.: mesophiles; and finally into thermophiles, whose thermal optimum ranges between 40 to 70 deg. C., or even higher.

The species of **psychrophile** propagate even in well cooled milk and at low temperature, and at times change its taste. Reference should be made here to the *Bacillus lactis saponacei* and the *Bacillus sapolacticum*, which give a soapy taste to the milk. This defect of flavor is principally observed in cool weather, and at the beginning or end of the winter. Subtilis varieties, mycoid varieties, vinegar bacteria, yeasts, *Penicillium glaucum*, mucor varieties and aspergilli also grow from 0 to 8 deg. C., as do soil bacteria, fluorescence varieties and bacilli which split up proteids (bitter taste of milk). According to Knüsel, psychrophile bacteria may be demonstrated in sterilized milk, while Bischoff found them in the market milk of Leipsic.

Bischoff found that in milk which had been cooled to about 0 deg. C., the bacterial number gradually diminished from the third to the seventh day; it then multiplied rapidly, without showing a considerable increase in the degree of acidity. A bacterial rennet formed, however, and the milk coagulated on boiling. This appeared as early as the fourth to seventh day, when the nilk was kept between 6 and 8 deg. C. Frozen milk on the other hand keeps for a remarkably long time.

Knüsel found peptonizing bacteria in sterilized market milk which had been kept at 8 deg. C.; as a result of their growth the milk had the appearance of soapy water, and possessed a bitter taste.

Therefore, all milk cannot be protected from spoiling by being kept cool. The milk must be procured at the start with as small a number of bacteria as possible.

The opposite of these psychrophile species are the **thermophiles**, which may be actually isolated from the army of accompanying bacteria by a high degree of heat. They continue to grow even in temperatures of 70 deg. C., and over (Zettnow), a temperature at which most of the vegetative bacteria and to some extent also spores of the mesophiles and psychrophiles are destroyed. Such bacteria were found not only in hot springs by Certes, Garrigon, Karlinski, Teich, Tsiklinsky, but also in river water (Miquel, Tieghem, F. Cohn, MacFadyan and Blaxall, Michaëlis and others); finally they were found almost everywhere by Globig, and in the intestinal content of animals, feces, manure, liquid manure, in the soil and upon fodder by Rabinowitsch.

The thermophile species are not directly pathogenic. This group, however, contains several toxin producers, and peptonizers of milk.

Sporulating bacteria which form spores that resist a heat of 100 deg. C. and over should not be confused with the thermophiles. (Peptonizing species, as mycoides, anthracoides, subtilis, mesentericus and the butyric acid bacilli). Between the psychrophiles and the thermophile bacteria lie the large army of **mesophiles**, to which belong most of the ordinary species of bacteria found in market milk.

Corresponding to their requirements for oxygen they are divided into **obligatory aerobes**, which propagate only in the presence of oxygen, facultative anaerobes, which can vegetate without oxygen, and obligatory **anaerobes**, which can grow only in the absence of oxygen.

They may also be divided, according to the **substances which** they attack, into those which split sugar, proteids and fats, or, according to the **products** which they form during their growth in certain media, into acid producers (lactic acid producers, butyric acid producers, etc.), or into alkaligenic species and gas producers, alcohol producers, bacteria with rennet-like action, pigment producers, slime-forming bacteria, etc.

The varieties of bacteria which are found in milk under general conditions of production, even when conducted under special provisions for obtaining clean milk with unusual precautionary measures (provided that the milk originates from healthy animals and is drawn by healthy milkers), are of special interest to the milk hygienist.

These bacteria split sugar and proteids, and attack fat. According to Flügge, they are separated into:

1. Aërobic lactic acid bacteria, which cause spontaneous souring and do not form spores;

2. The anaërobic butyric acid bacilli, and

3. The aërobic peptonizing bacteria, with remarkably resistant spores.

These bacteria, however, do not propagate uniformly well in milk, but they are subject to influences of the medium, which really constitutes an elective culture medium for some of the species, whereas it is destructive for others. The time during which no increase of bacteria can be noted in milk is known as the **period of incubation** (Soxhlet). In fact there may be not only no multiplication of bacteria in the milk, but under certain conditions during the beginning of the incubation there may even be a diminution of the bacterial number which is first found; the bacteria present in the milk are subject to the injurious influences of the animal secretion; the milk is in the germicidal stage (Koning).

Fokker in 1890 was the first to assert that raw milk (he used for his experiments goat's milk) must have germicidal properties. He proved that raw milk when inoculated with lactic acid bacteria resists spoiling for a longer period than was the case with milk that had been boiled. Prior to his investigations however Wolffhügel and Riedel found in 1886 that cholera vibrios readily multiply in boiled or sterilized milk, whereas in raw milk their growth is rapidly checked. As a result of these findings the question as to whether milk possesses germicidal properties became the subject of dispute. While Freudenreich, Hesse, Park, Cozzolino, Conn, Schenk, Behring, Rullmann and Trommsdorff, Rosenau and McCoy, Sassenhagen and Bab claim that milk possesses inhibiting, or even destructive properties for bacteria, Richet, Hueppe, Heim, Friedrich, Kitasato, Uffelmann, Weigmann and Zirn, Basenau, Schrank, Schottelius, Moro, Heinemann, Rubinstein, Stocking, Sommerfeld, Klimmer, Knox and Schorer, and Kuntze express their belief against this power in the sense of the bactericidal action of blood serum, and think that the germicidal properties exist only towards certain species of bacteria. They also believe that the composition of the milk creates favorable conditions for the propagation of some of the bacteria, while for others this is not the case, just as with elective media, some of the less favored species become injured or destroyed by the multiplication of lactic acid bacteria and their products.

The presence of specific germicidal substances (alexins, amboceptors, leucins) in special kinds of milk, such as colostral and mastitis milk, has been proven by the work of Bauer, Sassenhagen, Rullmann and Trommsdorff, whereas the question of the occurrence of these in normal milk has not yet been sufficiently demonstrated, although the fact of a diminution of bacteria in freshly drawn normal milk has been established by our methods of counting. In order to furnish a few examples, several experimental results will be cited from the work of Grimmer.

Koning found in various samples of freshly drawn milk the following numbers of bacteria per c. c.:

0						
		Milk:		Colostrum:		
	1.	2.	3.	1.	2.	
immediately	107,000	143,000	18,510	77,000	82,900	
after 6 hours		142,000	16,000	71,000	76,400	
after 12 hours	,			74,000		
after 18 hours	120,000	155,000	14,700		56,000	
after 24 hours			13,200		115,000	
after 30 hours	145,000	470,000	13,800		115,000	
after 36 hours		• • • • • •		2,050,000		
after 42 hours			106,000		596,000	

The slight diminution of the bacterial content of this experimental series could of course not be attributed to the existence of a specific germicidal action, but rather to errors in our methods, or to the elective action of media, since the differences are relatively slight.

The results are more apparent in the tables of Rullmann and Trommsdorff: Keeping at room temperature:

Teat	Milking	Imme- diately	1 hour	2 hrs.	3 hrs.	4 hrs.	5 hrs.	19 hrs.	43 hrs.
fr. rgt.	beginning	2,400	2,100	2,300	1,900	2,100	2,500	2,100	1,200,000
	middle	1,400	<b></b>	1,300	1,600	1,800	1,700	1,300	1,400,000
	end	700	800	600	900	700	1,100	700	500,000
fr. lft.	beginning	12,000	900	9,000	7,000	7,000	9,000	6,000	1,000,000
	middle	16,000	14,000	1,800	1,400	1,100	1,600	1,600	450,000
	end	3,000	1,000	2,400	2,400	3,000	2,700	2,200	1,100,000
hd. rgt.	beginning	200,000	118,000	118,000	10,700	98,000	71,000	33,000	420,000
	middle	35,000	56,000	37,000	23,000	38,000	28,000	17,000	250,000
	end	13,000	11,000	10,000	5,000	-2,600	2,400	900	1,400,000
hd. lft.	beginning	7,000	4,400	4,600	7,200	5,400	5,600	7,200	20,000,000
	middle	60	400	160	240	180	240	160	10,000
	end	3,400	3,000	3,600	2,600	5,000	2,200	2,100	230,000

In special samples with small bacterial count the comparison was still more striking:

Cow No.	Teat	At room temperature	Imme- diately	After one day	After two days	After three days
8	fr. rgt.	beginning	160	40	16,000	2,000,000
		middle	80	40	18,000	16,000,000
		end	40	80	17,000,000	20,000,000
	fr. lft.	beginning	11,000	9,000	8,000	40,000
		middle	1,700	720	40	1,200
		end	1,300	600	800	18,000
	hd. rgt.	beginning	120	400	2,500,000	5,000,000
	S	middle	40	400	18,000,000	60,000
		end	180	160	64,000	5,000,000
	hd. lft.	beginning	200	40	78,000	5,000,000
	11.1.6 12.0.	middle	80	500	300,000	6,500,000
		end	240	80	50,000	2,500,000
9	fr. rgt.	Cha	600	360	2,500,000	25,000,000
5	fr. lft.		40	80	43,000	4,000,000
	hd. ret		450	500	2,500,000	25,000,000
	hd. lft.		40	240	9,000,000	innumerable

Trommsdorff and Rullmann conclude from these and other experiments that the bacterial content of milk does not increase at room temperature in the first period following the milking. "On the contrary in some of the samples inside of the first 5 to 7 hours a pronounced diminution of the bacterial number was observed, which was still more pronounced in the following period so that in a great number of cases the bacterial number, after 1, 2, and even 3, and in one case even after 5 days, was found lower than directly after the milking. Where there occurred no diminution in bacteria the bacterial content remained the same as that found after the milking, during 1 to 3 days."

Milk which has been contaminated with numerous bacteria in the earliest periods after milking (dirty milking, filthy vessels), shows only to a very slight extent the germicidal phase. At 37 deg. C. the germicidal substances act more rapidly, but the duration of the germicidal phase is shortened (Koning, Rullmann and Trommsdorff).

Heating the milk to over 70 deg. C. destroys its germicidal properties.

Bauer and Sassenhagen established the absence of complement in ripe milk which gives the impression that in ripe milk the action is elective in the sense that the contained substances constitute food for one microbe and poison for another. Of course, it has been established for even ordinary kinds of bouillon that, depending on their composition, the growth of certain bacterial species upon them has been checked for a time (Basenau). The lecithin contents of raw milk must not be left out of consideration; in certain concentrations lecithin exerts a strong inhibitory action on bacterial growth.

Finally the diminution of the bacterial content may be only apparent, as the bacteria may multiply through their sticking together in agglutinated masses, thereby simulating a diminution, a view which is supported also by Bab for colostral milk.

After the germicidal action of the milk has worn off the various phases of decomposition of milk set in, beginning sooner or later, depending on the original contamination of the milk. Koning distinguishes seven such phases.

The fight of the microbes, their harmonious, or again antagonistic relation to each other, results in a predominance of certain species of bacteria in the various phases.

First (second phase according to Koning, the first being the germicidal) the proteolytes split up the proteid bodies of the milk, and thereby prepare the soil for the acid producers, which dominate the further decomposing phase (third phase according to Koning); the milk coagulates. In the fourth phase the alkali producers partly neutralize the acid again by further splitting up the albumoses of the acid milk with the formation of ammonia. Peptonized casein is also attacked. The principal representative of this decomposing phase is the *Bacillus facalis alkaligenes* (Gram negative, no gas formation, no indol, no spores, colors litmus milk blue). Through neutralization of the lactic acid, certain lactic acid bacteria, *Bacillus acidi paralactici, Bacillus acidi lævolactici* and *Micrococcus acidi paralactici liquefaciens*, again regain predominance (fifth phase).

Up to this stage the higher fungi have played a subordinate part, although they may have multiplied; now they appear in great masses. The degree of acidity does not hinder their growth. The *Oidium lactis* participates principally in the sixth phase of decomposition, although other varieties of moulds, penicillia, and mucors may also play a part. The degree of acidity being diminished by the oidium, bacteria of the fifth phase again commence to multiply, until the seventh phase is inaugurated through the growth of the anaërobic butyric acid bacilli. According to Koning this phase is reached at room temperature on about the eighth day.

Grasberger and Schattenfroh designate the principal representative of this bacterial group, the *Bacillus saccharobutyricus immobilis liquefaciens*, a bacillus which is large, thick and stubby, stains after Gram, and forms spores which are located either centrally or at the end of the bacillus. According to Burri the bacillus may be most readily isolated by boiling the milk for several minutes, and allowing it to ferment at 37 deg. C. Besides this butyric acid bacillus, other motile forms or bacteria related to the first group may be found.

The seventh phase finally passes into the eighth, in which the milk changes into a stinking putrid fluid, in which the decomposition of the food material is completed by the proteus, subtilis, *Bacillus fluorescens*, and *Bacillus mesentericus*, besides mould fungi.

Of these phases of decomposition of milk the first three, especially the second and possibly the beginning of the third phase, are the most important.

In these two phases the proteolytes and the rennet formers are the first to multiply, causing a partly visible precipitation of the casein, but subsequently again dissolving it; further, the precipitated protein is immediately partly dissolved when the dissolving tryptic ferment is present to excess. Weigmann collects these bacteria under the name of **casease bacteria**. On account of their properties of producing peptones from proteid bodies they are called **peptonizing bacteria**. Most of them liquefy gelatin. The group of casease bacteria includes a great number of forms of bacteria, for instance:

Staphylococci, small spherical bacteria which occur ubiquitously and in smears from cultures appear as grape-like conglomerates. They stain by Gram's method. They grow from 0 deg. to about 40 deg. C., and frequently form at 15 to 20 deg. and over, yellowish orange, or lemon-yellow colored colonies. At a low temperature the casease enzymes are especially active. In gelatin stab cultures staphylococci first develop a nail-like growth; the liquefied gelatin may then dry and at the point of the stab a bell-shaped air vesicle forms, or the liquefaction may progress rapidly and a cloudy layer of liquid with a sediment of staphylococci stands above the solid gelatin.

In gelatin plates the small round colony drops into a cup-shaped depression which also results from the drying of the liquefied gelatin. Besides the formation of albumose and peptones the milk sugar is split up; Löhnis therefore classifies these organisms with the lactic acid bacteria.

The growth of sarcina, which collect into two, four or eight members, etc., is similar, thereby forming bacterial clumps of certain forms. They are also Gram-positive.

On account of their occurrence even in milk drawn in the most cleanly manner, their presence in the udder was accepted, and as a matter of fact pathogenic organisms do occur in the group of staphylococci, which produce inflammations of the udder. Ordinarily however, they only inhabit the milk duct.

The putrefactive representatives of the proteus varieties should be classified among the non-spore bearing casease bacteria. They manifest many forms of growth, and include the following representatives: Proteus zopfii, zenkeri, vulgaris, mirabilis, and fluorescens.

They are rod-shaped bacteria, motile, non-spore bearing, and Gram-negative. The forms of colonies and characteristics are quite variable, sometimes showing root-like extensions, and at other times branching outshoots.

The most important spore-forming proteolytes originate from forage, hay, stable dust and the stable air, and are collected, under the name of potato bacilli, hay bacilli and root bacilli.

They are small rods up to the size of the anthrax bacillus, with central or terminal spores, and are Gram-positive.

They grow best under aërobic conditions.

Gelatin is liquefied, milk is precipitated as if by rennet, and is then dissolved. In these instances the rennet action is at times more prominent, at other times the action of the casease appears more prominently.

On solid media the colonies have either a wrinkled, slimy appearance, or they are dry, with a fine map-like drawing on their surface; again they may appear like dull glass, gray, grayish-white, yellowish to brownish, delicate and profuse. On the borders of the culture branch-like shoots or "forms of Medusa heads" similar to the colonies of anthrax (anthracoid varieties) may be seen.

The more important forms are:

The Bacillus mesentericus vulgatus; the potato bacillus (fuscus, graveolens, ruber); Bacillus liodermes; Bacillus subtilis (hay bacillus); the mycoid varieties; the anthracoid

varieties, Bacillus ramosus, implexus, radicosus, tumescens, megatherium, tyrothrix, etc. In fluid media they generally form prolific wrinkled membranes; at times they cloud the bouillon or they may grow in long, stringy filamentous masses. Some of the varieties also form butyric acid; thus for instance the Bacillus mesentericus changes lactic acid into butyric acid. To this class some of the mycoid varieties belong, for instance the Bacillus butyricus Hueppe and the Clostridium polymyxa.

Through the growth of these and similar forms, the formation of albumose and peptones develops, and the further decomposition of the proteid substances is carried out, if possible, with the production of end products such as leucin, tyrosin, ammonia, carbonic acid, indol, skatol, methylmerkaptan, sulphureted hydrogen, toxic toxalbumins, and ptomaines.

For the judgment of milk which is considerably contaminated with bacteria from litter and forage the increased presence of such bacteria is of special importance, since most of them form spores which are not always destroyed at the temperature of 100 deg. C. and higher.

The Bacillus prodigiosus, Bacillus fluorescens liquefaciens, Bacillus amylobacter, and Bacillus putrificus Bienstock, may also split up proteins. Their products vary.

The bacterial substances which dissolve proteins and split them up, exert their action especially in neutral and alkaline material, and they are therefore hindered in their action by the products of the third phase, the second decomposing phase.

The lactic acid producers, however, proliferate only after the necessary requirements for their propagation have been created by the activity of the peptonizing bacteria.

Through the activity of the lactic acid bacteria the milk sugar and other varieties of sugar are fermented to dextro-rotary (rotates plane of polarization to right) lactic acid, inactive lactic acid, and levo-rotary (rotates plane of polarization to left) lactic acid, depending on the species of bacteria or the conditions under which the special species prevail.

The splitting of lactose C<sub>12</sub>H<sub>22</sub>O<sub>11</sub> is accomplished by an inverting bacterial enzyme, the lactase, through the introduction of water whereby it is converted into  $2C_6\dot{H}_{12}O_6$ known as D-glucose and D-galactose, which by further splitting break up into 4C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>.

The lactic acid yield, however, corresponds even in the extreme cases only to about 98% of the contained milk sugar, as in the meantime, depending on the variety of the lactic acid producers, the lactic acid itself is again broken up into simpler acids, acetic acid, valerianic acid, succinic acid, and carbonic acid; alcohol, aldehyde and possibly hydrogen result besides the lactic acid.

In spontaneously coagulated milk mostly inactive lactic acid is found, or a mixture of inactive and dextro-rotary lactic acid; but only in exceptional cases is pure dextro-rotary lactic acid found (Gunther and Tierfelder). Kozai mostly found only the dextrorotary polarizing form.

The lactic acid bacteria may also be considered as ubiquitous micro-organisms; they have been found in straw, hay, fodder, dust, feces and in the air. Proliferating in milk they soon adapt themselves to the nutritive medium which at first is not quite suitable for their propagation, and finally they form standard varieties for which the milk is especially adapted. Through continued growing a weakening of the acid forming qualities may develop, and the coagulation of the milk may not take place in spite of their growth, the bacteria having become "milk tired." Under these conditions they may show other properties, the bacteria rendering the milk slimy instead of sour, which is known to be the case with some of the lactic acid streptococci.

The specific lactic acid bacteria are aërobes, or facultative anaërobes.

Löhnis separates the specific lactic acid bacteria into four collective groups:

- 1. Streptococci.
- 2. Plump short rods.
- 3. Slender, long, lactic acid bacilli.
- 4. Micrococci and staphylococci.

The most frequent of these are the representatives of the streptococcus group. Arranged into wreath-like, shorter or longer bodies, the individual members are characterized by coccus or ovalshaped bodies. On artificial media they frequently manifest vacuolar degenerative forms which change the individual microbes to the size of bacilli. Frequently pure diplococcic forms may be found which at their ends are mostly pointed in a lancet shape. Streptococci grown in milk are composed of individual members mostly in the shape of a figure "8" which lie with their long axis in the direction of the chain proving them to be streptococci that entered the milk after its secretion as compared with the forms from the udder. Some of the representatives form capsules but only under special cultural conditions, as for instance in blood medium, while in other media capsule formation appears to be a constant characteristic and occurs especially in old milk cultures.

Most of the streptococci are Gram-positive. They possess no motility and form no spores. On solid media the colonies usually remain delicate and small; in fluid media

the growth takes place either with general clouding, or with the formation of flakes and tufts and the bouillon then remains clear. The optimum temperature of the growth lies between 10 and 42 deg. C. Most of the varieties are facultative anaërobes.

Coagulation of milk may take place with acid formation, or in spite of acid formation it may not coagulate, or again coagulation may take place with only slight acid formation, which in all probability depends on the formation of substances having the properties of rennet.

Gas formation has never been observed by the author, but it is supposed to occur; slime formation is typical of some of the species.

Löhnis classifies the varieties of streptococci found in milk as:

1. Those which coagulate milk and form gas: Micrococcus sornthali (Adametz), Streptococcus "a" from Kefir (Freudenreich), Streptococcus memelensis (Leichmann), Streptococcus caucasicus (Migula).

2. Those which coagulate milk but form no gas: Streptococcus güntheri, Bacillus lacticus Kruse, Bacillus lactis Lister, Lactococcus beijerink, Bacillus acidi lactici Grotenfeld, and the streptococci from Armenian buttermilk.

3. Those which neither coagulate milk nor form gas: Streptococcus kefir, Streptococcus 'b'' Freudenreich, Streptococcus soya.

4. Those which neither coagulate milk nor form gas: some streptococci of cheese, *Streptococcus inocuus*.

5. Those which form slime; some varieties of the Streptococcus pyogenes, leuconostoc varieties, Streptococcus hollandicus, Bacterium lactis longi, ''sticky milk'' producers, Micrococcus mucilaginosus, Bacterium lactis acidi.

6. Those which in culture form vine or tongue-like shoots.

7. Those which liquefy gelatine.

It is well known that the cultural characteristics of the streptococci may readily change: a strong acid forming variety may lose this characteristic by long cultivation in milk, and may become a slime producing variety, so that the distinguishing feature is not absolute. There are transitory forms between one and the Fig. 24.

Streptococcus lacticus.  $1 \times 1000$ .

other type, and one type at some time may change into another type by changing its characteristics.

Some varieties also belong to those streptococci (collective name streptococcus or *Bacterium lactis acidi* Leichmann), which produce volatile substances and alcohol from milk sugar and which at the moment of their development unite into a fragrant substance, a so-called fruit ester, which reminds one of the odor of a certain fruit or fruits; other representatives of this variety produce other substances with odor and taste, which may be described as straw-like, sorrel-like and especially malt-like. The author never succeeded in producing striking odoriferous substances in sterile milk with the streptococci cultures at his command. He, however, does not wish to refute from his few experiments with about 20 strains from various provinces, the possibility of the production of special odoriferous substances by the *Streptococcus lacticus*.

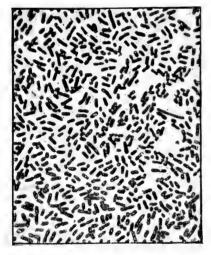
Under certain conditions tremendous quantities of streptococci may occur in market milk from cows which are affected with streptococcic mastitis. Special reference has been made in the chapter on affections of the udder, regarding the similarity of these patho-

genic varieties, to the probably harmless varieties of lactic acid producers.

The collective group of the *Bacterium acidi lactici* is the second of importance in the lactic acid group and is also invariably represented in milk.

While the streptococci produce dextro-rotary lactic acid, among the representatives of Bacterium acidi lactici there are those which produce levo-rotary lactic acid. Since the growth of both species of bacteria depends on the temperature and the method of keeping the milk (in a shallow bowl or in a deep vessel), therefore under certain conditions whereby the Bacterium acidi lactici has better chances for vegetating (aërobe, optimum at about 37 deg.), it produces levo-rotary lactic acid and in souring in deep vessels (the Streptococcus lactis acidi is a facultative anaërobe and grows well at 20 deg.) it produces dextro-rotary lactic acid. These results have been confirmed by the observa-

#### Fig. 25.



presentatives of the coli-aerogenes group, from a culture.  $1 \times 800$  (Bac-terium phlegmasiae uberis, after Kitt.) Representatives

tions of Conn and Esten. The findings of Heinemann, Thiele and Hölling that at incubator and room temperature, in milk drawn under specially clean conditions, "d-lactic acid" is formed only at the beginning, are of interest.

Against the practical utilization of these observations, namely the conclusion that the ex-clusive presence of dextro-rotary lactic acid is dependent on the specially clean procurance of the milk, are the investigations of Pere and Harden, who claim that the nature of the acid formed depends not only on the producer but also in the case of the same producer on the character of experimental procedure; thus for instance one and the same strain of *Bac-terium coli* produced lactic acid showing optically different properties when cultivated under different conditions, aërobically or anaërobically, etc.

The Bacillus acidi lactici (Hueppe) group also does not represent constantly uniform species, but it is a collective name which unites all bacteria with especially strong acid forming properties that lean towards the colon and especially to the aërogenes species.

The Bactcrium acidi lactici Hueppe and the Bacillus pncumoniæ Friedlander are similar to the coli-aërogenes species. They are plump, mostly Gram-negative, from cocci to short rods in appearance, forming individually longer rods and short thread-like filaments; they grow luxuriantly, forming moist or almost dry indented colonies with a slimy or jelly-like consistence. In dextrose media usually a strong acid formation takes place. On potatoes the growth is either luxuriant with gas bubbles, or brownish and thin, or transparent. The odor varies, being either disagreeable or pleasant, or at and thin, of transparent. The otor varies, being either disagreeable of pleasant, of at times even odorless. From this description it may be seen that a great number of bacteria are united in this group which are classed by Löhnis as follows:

Type of the Bacillus acidi lactici Hueppe.
Gas formation with milk coagulation. To these belong the Bacillus aërogenes, Bacillus "a" Guillebeau, Bacillus "b" Freudenreich, Bacillus laevolacticus, Bacillus

acidi lactici Gortenfeld, also the lactic acid bacilli of Fokker and others which possess differing characteristics, as for instance the formation of esterlike odors, cheesy odors, etc.

2. Milk coagulation without gas formation. Bacillus limbatum (acidi lactici) Marpmann, without special tendency to deep growth. Bacillus acidi aromaticus, Bacillus granulosum, crenatum, spirans and ramificans (Weiss) and others. 3. Gas formation without coagulation of milk, producing white, circumscribed,

hemispherical colonies, as for instance the Bacillus pneumoniae Friedlander.

4. Neither gas formation nor coagulation.

Neither gas formation nor coagulation, but slime formation is present, for 5 instance the Bacillus capsulatus, Bacillus viscosus, Bacillus capsulatus mucosus, Bacillus lactis pituitosi, Bacterium ozacna.

 The tendril-shaped colony forming Bacillus aerogenes capsulatus.
 Without gas formation, but with liquefaction, for instance the Pneumobacillus liquefaciens bovis.

A decisive separation of these types cannot be strictly accomplished anywhere in the entire group. Each type has individual or several representatives which show transitory tendencies towards one or the other group, and the entire group is closely related to the colon aërogenes group, but in the latter, there are no such pronounced lactic acid formations. The colon group is motile, the aërogenes group non-motile. While the colon bacilli form indol and split up proteids, these properties are absent in the aërogenes group. Milk is coagulated with gas and acid formation, in which the case in usually remains on the surface of the pressed-out serum in the form of a spongy coagulum. The milk receives at the same time an unpleasant, slightly offensive odor, and a salty, bitter taste. Some varieties change the milk in this manner without the special changes being perceptible. The several groups adapt themselves very rapidly to their surrounding conditions, for instance to the cultivation on cabbage or turnip media, and when transferred to milk they impart to these the well-known changes of taste.

Both bacteria are found in the intestines of sucklings (Escherich).

It is an important fact that most bacteria of this group are destroyed in a short time at 65 deg. C., so that their occurrence in pasteurized milk (in bottle pasteurization) may be an indication that the heating to which the milk has been subjected was insufficient.

According to De Jong and Graaf some varieties of the colon group resist heating to 70 deg. C. for a short time.

The slender "cheese bacteria" of the third group of the lactic acid producers are again divided into:

1. Those coagulating milk with gas formation, for instance Bacterium casei (Freudenreich), Bacillus caucasicus of Kefir (Freudenreich):

2.Those coagulating milk but with no gas formation;

3. Those producing gas but no coagulation;

4. Those showing neither of these characteristics:

5.Those producing slime;

6. Those growing in tendril-shaped colonies.

The bacteria of this third group are almost invariably nonmotile, and sporeless, mostly without capsules. They are Grampositive. The fermentation of sugar varies. In milk they mostly form levo-rotary lactic acid, while the other two varieties are rarely produced. Some peptonize proteids; their growth is favored more by aërobic than by anaërobic conditions.

The bacteria do not grow well in milk but they are found in cheese, in the oriental varieties of sour milk and in sour food. These bacteria are of only slight importance in the ordinary lactic acid fermentation. They prefer higher temperatures and produce fermentation only in the absence of oxygen, although their growth is prolific even in the presence of oxygen.

As representatives of this group the *Bacillus panis fermentati* occurring on sour bread should be mentioned, and the *Bacillus delbrücki* found on sour food.

Some representatives of these "cheese bacteria" are capable at high temperature (40-50 deg. C.) of producing and withstanding large quantities of lactic acid, up to 1.5%, and even to 2 to 2.5%.

Löhnis classifies the staphylococci as the fourth most widely distributed bacterial group of the lactic acid producers, but on account of their peptonizing characteristics they might better be considered with the casease bacteria of Weigmann. Their properties have already been described during the discussion of bacteria of the first decomposing phase. The staphylococci may also be separated into:

- (a) Those coagulating milk and liquefying gelatin.
- (b) Those which only liquefy gelatin.
- (c) Those which only coagulate milk.
- (d) Those which posses neither of these properties, nor form slime, produce gas, nor form tendrils.

The species mentioned by no means exhaust the number of species and groups which are capable of producing lactic acid. Thus the anthrax bacillus splits up sugar and starch into lactic acid, and also forms acetic acid (Napias), and formic acid (Iwanoff). Feinberg demonstrated for the diphtheria bacillus the capability of splitting up milk sugar with the formation of alcohol, aldehyde and volatile as well as non-volatile acids. The bacillus of malignant edema, according to Kerry and Fränkel, in the anaërobic fermentation of grape sugar, produces ethyl alcohol, formic acid, butyric acid and lactic acid; cholera vibrios and related organisms form lactic acid; for instance the vibrio of Asiatic cholera, the Vibrio proteus (Finkler and Prior), Vibrio massauah, vibrio danubicus and others form levo-rotary lactic acid, the vibrio of Deneke forms destro-rotary and Vibrio berolinensis produces an inactive lactic acid. The formation of these lactic acids, however, does not depend on the bacillus alone.

The *Oidium lactis*, a milk mould, and others, are also capable of producing lactic acid from milk sugar. Some of the lactic acid forming varieties are rare in milk, others may accustom themselves to milk so rapidly that they form the typical acidifying flora, the presence of which under certain conditions may be desirable since by their multiplication the vegetation of the harmful peptonizing bacteria and of the producers of butyric acid is inhibited.

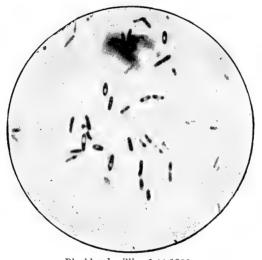
With this we leave the most important varieties of bacteria which are responsible for the normal spoiling of milk, and will briefly discuss those varieties of microbes belonging to the bacteriology of milk and milk production which always occur in market milk.

Milk also invariably contains butyric acid bacilli. Their predominance is inhibited by the lactic acid fermentation. The specific butyric acid bacilli are obligatory anaërobic or facultatively anaërobic, that is they thrive best in the absence of oxygen; there are, however, aërobic bacteria which are capable of forming butyric acid, for instance several of the above-mentioned peptonizing varieties, the hay and potato bacilli, which without specially attacking the milk sugar, form butyric acid from the products of the split proteids.

The individual varieties show varying properties toward the different kinds of sugars, as well as toward the formed by-products, as butyl alcohol, isobutyl alcohol, formic acid, acetic acid, propionic acid, valerianic acid, carbonic acid, hydrogen, etc.

The obligatory butyric acid producers are rods, chains or threads, with either a plump or a slender form; they possess oval or roundish polar or central spores. The bacteria

Fig. 26.



Blackleg bacilli.  $1 \times 1200$ . (After Friedberger & Fröhner.)

frequently form so-called clostridium forms, and especially in starch-containing media they take up granulose; therefore certain parts of their body or even the entire bacillus may be stained blue with iodide of potassium.

The representatives of this group are known to pathologists as producers of blackleg, gaseous phlegmons, malignant edema, bradsot, botulism, and tetanus. These bacilli at times are capable of energetically forming butyric acid, and at other times less intensively; at the same time peptonizing ferments (tryptic) are formed, which become active in the absence of acid.

Generally motile and non-motile forms of butyric acid bacilli are distinguished in milk. The latter form of the granular *Bacillus saccharobutyricus* is considered by Grasberger and Schattenfroh as a developing form of the motile, spore-forming variety which takes up granulose, forms toxins, and attacks lactic acid.

According to their properties they may be divided into those which form butyric acid principally from certain carbohydrates, as for instance the blackleg bacillus, the non-motile butyric acid bacillus (Grasberger and Schattenfroh), and the bacillus of gaseous phlegmons (Fränkel). The others are producers of putrefaction and split up the proteids into forms from which volatile fatty acids develop.

Obligatory fat-splitting bacteria may also, although less frequently, be found in milk, as for instance the *Bactridium lipolyti*cum (Huss), through the growth of which the milk acquires a rancid taste. The *Bacillus fluorescens*, *Bacillus prodigiosus* and others, for instance certain mould fungi, may also produce fat-splitting enzymes.

Actinomyces form the transition organisms which lead from bacilli to higher fungi. These fungi form long threads with true branchings. Widely distributed on grasses and especially grain, as well as in the soil, they are of course also contained in manure and litter, and may occur in milk and milk products, butter or cheese, and multiply therein.

As is the case with all milk bacteria, among the actinomyces there may occur forms which under certain conditions such as wound infection, produce diseases (chronic suppurations).

Some varieties of bacteria classified by Löhnis as lacto-bacilli, as for instance a microbe isolated by Chatterjee from "Dadhi," (Indian sour milk) *Streptothrix dadhi* and several bacteria which were found in Mazun, Yoghurt and in the Montenegrin "Grusavina" and "Kysla varenika," appear from their morphological properties, to belong to the streptothrix (actinomyces).

Finally it will be advisable to discuss the higher fungi, yeasts and moulds which occur in milk. They cause a slight alcoholic fermentation of the milk; not all varieties however attack the milk sugar, although a great number of the most varied fungi and yeast are found in milk, for instance penicilia, mucors, aspergilli.

By far the most frequent fungus in milk is the *Oidium lactis*, under which name are collected all mycelial forms, whose radiating mycelia carry hyphæ, that break up into small, rectangular, cylindrical members, the so-called oidia, which in proper media again grow out into a mycelium. The growth of the oidium varieties gives the surface of the cream layer a yellowish-white, velvety, frequently wrinkled appearance, which later may take up a glassy transparent appearance.

Oidium lactis causes fermentation in sugar-containing media, and develops carbonic acid and a slight amount of alcohol. A pleasant aroma results from cultures in dextrose but in the splitting up of saccharose, lactose and maltose, an intensive cheesy odor develops. Besides sugar, proteids if present are split up. Therefore in the zones of growth of the oidium varieties a peptonization is manifested in the milk. Lactic acid is also produced and later again disappears.

Besides the oidia there may also be found the closely related

#### Buttermilk.

moniliar varieties which at times grow like the oidia with a typical mycelium, at other times it is a sporulating fungus (Mon. variabilis; candicans, etc.); also varieties of mycoderma, which always multiply in a longitudinal direction, by the protrusion of daughter cells which continuously bud out new daughter cells and these continue to grow in the already established direction.

In the preparation of certain fermented forms of milk which are frequently desired in certain sour milk preparations, the sporulating fungi which multiply in all directions of space through sporulations are of importance.

Through their activity, that is through the formed enzymes, the milk sugar is split up into dextrose and D-galactose, and ultimately the dextrose is split up into alcohol and carbonic acid. Milk may contain saccharomyces varieties, which form endospores and torula varieties, whose daughter cells no longer separate in all directions but arranging themselves into rows form mostly spherical shaped or sausage-shaped buds, and have no endospores.

# Milk Preparations, Buttermilk, Etc., Produced By Special Fermentation.

Many varieties of foreign buttermilk or sour milk have recently become known in this country. Especial dietetic value is attributed to them; as to whether they possess advantages over our own buttermilk or not is not yet known. The author believes that our native buttermilk possesses the same advantages provided it is prepared with the same care as the buttermilk known as Yoghurt, Mazun, Leben-raib, Gioddu (Sardinia), etc., besides many of the foreign milk preparations which are marketed under various names frequently contain nothing more than native varieties of our lactic acid streptococci and certain cheese bacteria.

Yoghurt is the buttermilk of Bulgaria. It is prepared by adding to the milk the ferment maya after the milk has been boiled down to half of its volume, and cooled to about 50 deg. C. The mass is then kept at 40-50 deg. and after 10-14 hours the Yoghurt is finished. The necessary fermentation temperature is obtained through cooking boxes, or covering the hot vessels with non-conducting material (woolens). Weigmann in his "Mycology of Milk" quotes the verbal information of Kostoff from which it may be seen that the concentration of the boiled milk is not always carried out in Bulgaria, but a ferment (Maya, in Bulgarian Podkwassa) is stirred up with a small amount of boiled milk, which is added to the milk and kept at 45-48 deg. C. If a sufficient amount of ferment is added the Yoghurt is finished in from 31/3-4 hours. It is cooled for 1-2 hours, and may then be consumed.

According to information obtained by the author there is another method of preparation in Bulgaria which is carried out by the dairymen, and produces a primary Yoghurt. According to the description of Marcoff, to whose kindness I am indebted for this information, the dairymen take a widely grown herb (name was unknown to Marcoff), which they crush up in small linen sacks. A small quantity of the juice is then squeezed out and added to the raw milk, whereupon without further treatment the coagulation of the milk takes place within a few hours. From this preparation the Yoghurt then may be prepared.

According to this description the primary juice action may be attributed to a vegetable rennet. With the plant juice other bacteria also enter into the milk, the product of which is represented by their elective cultivation.

As effective bacteria in the production of Bulgarian buttermilk are considered: 1. A lactic acid long rod, which belongs to the acidophilic bacilli of the intestinal tract: the *Bacillus bulgari*cus. This is the aroma producer of Yoghurt. The Yoghurt also contains streptococci of lactic acid; yeasts are not desirable (see Table IV).

The same conditions exist in the Armenian Mazun, a very aromatic preparation of buttermilk, which is prepared from boiled cow milk, buffalo, sheep or goat milk. Düggeli demonstrated that satisfactory Mazun (Tartaric Katych) contains principally three varieties of microbes, a streptococcus, a long rod-shaped lactic acid bacterium, and yeasts, the activity of which produces the aromatic bodies, besides a slight amount of alcohol and carbonic acid.

Leben-raib, according to Rist and Khoury contains five microorganisms, among them being two varieties of yeast and two lactic acid producers. The buttermilk is prepared in a similar way to Kefir, by using cow, buffalo or goat milk.

**Kefir** has been used for a much longer time and therefore is more generally known. It contains alcohol and is very rich in carbonic acid; it has a pleasant, slightly acid odor and taste. It contains the milk proteins split up to some extent (Hueppe) in addition to alcohol and carbonic acid and a slight amount of glycerin, succinic acid, butyric acid and acetic acid.

Kefir is best prepared from skimmed milk, since in full milk, cream clumps may readily result and the Kefir thereby becomes rancid, which diminishes the consuming value. According to Freudenreich the fermentation is principally produced by four varieties of organisms which include yeasts, two streptococci varieties, and one microbe described as the *Bacillus caucasicus*.

The yeasts are the *Torula kefir* and *Saccharomyces fragilis*, both of which ferment lactose. Investigations which have been recently conducted by Kuntze showed that the bacteria of Kefir consist of an aroma-forming rod which produces casease and alcohol, and a lactic acid long rod which at first acidifies the milk and then renders it alkaline. They are the *Bacillus esterificans* Maassen and the *Bacillus kefir* classified by Kuntze as belonging to the group of butyric acid bacilli (cited by Weigmann). These bacilli inoculated into milk, together with ordinary lactic acid producers, living in symbiosis with yeast, form granules which grow to raspberry sized clumps and nodes. Sponge-like masses of ruffle-like appearance, the so-called Kefir kernels result, in which are included the necessary varieties of microbes. In dried condition these kernels are of the size of millet seeds, but after treating with warm water or warm milk they swell and proliferate in the milk up to the size of a fist. The small young kernels are the best, as the larger readily degenerate, become slimy and crumbling as compared with the elastic granules of more recent development. In order that they may again produce good Kefir they must be subjected to treatment by washing, drying in the sun, etc. The Kefir kernels may be purchased.

The origin of the Kefir kernels, that is the microbe colonies which are clumped in the kernels, is not known, but the primary development may have some connection with the method of fermenting milk in containers made out of goat skins.

If it is desired to prepare Kefir it is necessary to first obtain the kernels which may be purchased. The Kefir kernels are allowed to soak in boiled or lukewarm water, and then they are transferred several times (3 to 5 times) from one warm milk into another, the milk being poured off every 3 or 4 hours.

The utilizable Kefir kernels increase in size during this time through further swelling, and as a result of becoming lighter in weight through absorption of carbonic acid they rise to the surface of the milk, whereas kernels in which one of the varieties of microbes for some reason or other became destroyed and degenerated remain on the bottom of the vessel. If the degenerated variety of microbes recover through longer treatment with raw milk, and if the proper relation of symbiosis again appears, then these kernels are satisfactory for the production of Kefir. This condition is manifested by the kernels rising to the surface of the milk after some days.

If a tablespoonful of these kernels is added to about one-half liter of milk and this is allowed to stand for from 8 to 12 hours at 14 to 18 deg. C., with frequent shaking, then a primary or mother Kefir is obtained, from which through further fermentation in corked bottles the Kefir may be prepared ready for use.

The "millets of the prophet," the Kefir kernels, are strained through a sieve, and the homogeneous fermented milk is filled into bottles, or from the strained fluid a considerable quantity is poured into a bottle, to which boiled milk cooled to about 20 deg. C. is added. The bottle is then closed and allowed to continue to ferment for from 24 to 28 hours, at from 12-15 deg. C. In this process the casein and serum separate but may be readily homogenized by shaking. The Kefir is then ready for consumption, and represents a thick, sour, aromatic fluid of a pungent taste, with a remarkable nutritive value.

A similar product is prepared by the nomadic population of

Southern Russia, Siberia, and Central Asia, which represents a milk wine made from the milk of mares and asses, and which is known by the name of **Kumys**. In the preparation of Kumys, alcohol and carbonic acid fermentations are the principal processes. After long fermentation Kumys contains up to 2% of alcohol and 1% or more carbonic acid.

Bacteriologically Kumys is of similar composition to Kefir containing yeasts, lactic acid, streptococci, and the *Bacillus kumys* (Schipin), which is a facultative anaërobic microbe which splits up milk sugar with the formation of lactic acid and alcohol and peptonization of the proteids. These act together and form after several days a delicious drink of white color, and creamy consistence. Special varieties of milk, containing much sugar, are best adapted for the preparation of Kumys but cow's milk is the least desirable.

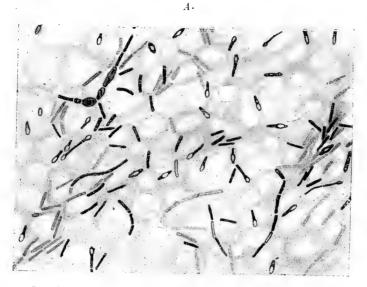
**Gioddu**, the buttermilk of Sardinia, is prepared from boiled milk cooled to about 35 deg. C. To four parts of milk one part of old Gioddu is mixed, the Gioddu being added to cow, sheep or goat milk. The fermentation is produced by the *Bacillus sardous* in symbiosis with the *Saccharomyces sardous*. According to Grisconi the *Bacillus sardous* belongs to the streptobacilli.

The preparation of buttermilk constitutes an important branch of the utilization of milk in all countries. In northern Bavaria the milk is usually set in large earthen pots and allowed to undergo voluntary fermentation. In southern Bavaria and in the Bavarian forests the "fall milk" is utilized for the preparation of the "sour soup." By keeping buttermilk and continually adding sour skimmed milk to it a fermenting product is obtained which is thickened by the removal of the whey (Herz).

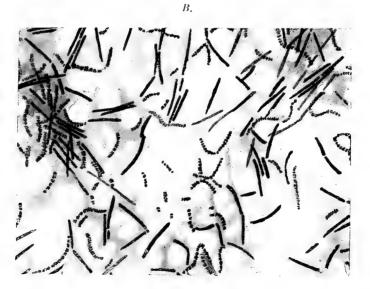
In Sweden and Norway a milk product is known under the name of "thick milk" (Tätmjölk), which is produced by slime and lactic acid producing bacteria which vegetate on the leaves of the butterwort (*Pinguicula vulgaris*).

The leaves of this plant are placed on the bottom of the milk vessel and milk poured over them, whereupon the milk becomes so thick in several hours that it must be cut with a spoon or knife in order to be taken into the mouth (Weigmann). New milk may be inoculated with the residue of old milk.

The necessary preparations of bacteria for the making of special forms of popular buttermilk may at the present time be purchased in the market. In using any of these "ferments" the directions for use should be carefully followed, since at a temperature either too high or too low an overproduction of undesirable bacteria may readily take place which would make good results impossible. Even if the directions are carried out most accurately, the propagation from milk to milk may be a failure since the biological properties of the bacteria are not absolutely constant; the microbes "grow wild" and their pleasant qualities are lost, or they may change, assuming undesirable properties.



Butyric acid bacilli in boiled milk, kept for two days at 37°C.  $1\,{\times}\,1200.$ 



Film of Yoghurt. Bacillus bulgaricus and lactic acid streptococci. Gram-safranin stain. 1 $\times$  1200.

Ernst, Milk Hygiene.



#### Bitter Milk.

Porcelain vessels and bottles made from glass free of lead are most suitable for the preparation of buttermilk, since the buttermilk may extract lead from enameled earthenware and from pots whose glazing contains lead in its composition.

According to Chlopin 0.84 mg. of lead was extracted from 100 gm. lactobacillin-buttermilk; in a second portion (300 gm. buttermilk) which was five days old, the amount reached 7.86 mg. Brückmann obtained similar results: 300 gm. of ordinary buttermilk contained after four days 4.2 mg., and after six days 5.7 mg. of lead, when this product had been prepared in pots with lead-containing glazing.

### Defects of Milk.

Bacteria produce certain modifications in milk which partly on account of their frequency are designated as normal processes, or again others appear which are less frequently observed, occurring only under special conditions and therefore are known as milk defects. The modification, as has been seen, may be even desirable, as for instance in cream souring and cream ripening for butter making, or in the preparation of Kefir, Yoghurt, and buttermilk, or it may be undesirable and injurious, spoiling the milk, and having a disturbing influence on milk utilization, especially in its use for drinking purposes.

Among the changes in milk there are those which appear frequently, and others which are very rare.

Under conditions which favor propagation of peptonizing bacteria (staphylococci, sarcina, anthracoides, mycoides, mesentericus varieties, fluorescens, pyocyaneus, etc.), the milk attains a bitter taste.

For instance if uncooled milk is filled into cans which are immediately closed it "suffocates," acquiring a strong stable odor which may even reach a putrid character, causing a solution of the casein by reason of which the milk no longer coagulates; or the appearance of a bacterial rennet produces a rennet-like precipitation of the casein, and the milk coagulates without turning sour. It is "sweet-coagulating." By the action of peptonizing micrococci, which in part are psychrophilic the development of a bitter taste may occur in thoroughly cooled, and even in excessively cooled milk. The bacteria of the colon group when the conditions of their propagation are favorable may produce an odor in milk ranging from aromatic to rancid, or some varieties of this group which have grown on mangels may confer the odor of mangels to the milk.

A bitter taste in milk may also occur from the feeding of foods containing bitter substances, thus for instance from the feeding of lupins, vetches, mangels, camomile, beet leaves, wood-fern, raw potatoes, mouldy or spoiled hay, straw, etc. It may however be accepted that the development of a bitter taste in milk usually results from its contamination with varieties of bacteria vegetating on food substances, which enter into the milk directly from the stable air or indirectly with the manure and litter. They then convey to the milk this altered taste. Experiments to confirm these views have been undertaken quite recently by Weigmann and Wolf (Kiel).

Defective flavors are frequently present in milk.

Of 1,000 retentions made during 1909 in Munich, 90.50% were on account of souring, 14.6% on account of soapy taste, 18.25%rancid, 2.19% fecal contamination, 8.76% oily, 1.46% bitter, 2.92% granular, 2.19% sweet-coagulating, and 1.46% on account of slimy conditions.

Representatives of the colon-aërogenes group may actually be cultivated until they become aroma producers if they are allowed to grow for instance upon media made from rape leaves. If an adaptation of these and other bacteria to the ingesta within the gastro-intestinal canal is admitted, then an acquisition of certain other properties, depending on the consumed feed, is readily conceivable.

Weigmann and Ritland and Jensen demonstrated such "rape bacteria" in milk having a rape-leaf taste; the milk at the same time had a stale taste and an odor of dish-water. The author observed a distinct phosphorus taste in cases in which the milk was placed without being cooled into unclean or poorly cleaned covered cans.

Animal and fecal odors result when freshly drawn milk is placed into covered cans without airing and cooling. In these cases the vegetation of anaërobic and facultative anaërobic bacteria may play a part, and the temperature may have an effect on the bacterial elective conditions. The milk attains a taste like animal viscera if it contains bacteria of the mycoid, megatherium or fluorescens group.

Fishy taste of milk may result from pasturing cows on marshy meadows which have been inundated. In these instances the presence of various other varieties of bacteria should be taken into consideration.

The multiplication of the *Bacillus lactis saponacei* (Weigmann) and the *Bacillus sapolacticum* (Eichholz), produces a soapy condition of the milk. The milk attains a sharp, rancid, soap-like taste, and when cold it reminds one of valerian; in a warm state it has a sharp, soapy odor. In shaking such milk a fine, vesicular, persistent, tenacious foam results. The change appears in thoroughly cooled and excessively cooled milk, and in the cold season of the year and in cold rainy summers the bacteria are psychrophilic, originating from the feed and straw.

The *Bacillus lacticus saponacei* grows well at room temperature, liquefies gelatin, and produces a slight yellowish shimmering coloring matter; the growth is aërobic. The *Bacterium sapolacti*- *cum* grows similarly. It is not supposed to liquefy gelatin. The nutritive media become fluorescent.

The propagation of butyric acid bacteria causes rancidity of milk, as does likewise the multiplication of bacteria which spilt up fats, for instance the *Bacterium lipolyticum*.

The appearance of the milk defects here mentioned may sometimes be confined to the product of a single individual in the stable. The milk of one or of several cows may manifest these defects which may be retained persistently in spite of changing the feed and disinfecting the stable.

Weigmann mentions a case in which, with uniform feeding and care of the animals, the milk of only one cow developed a fishy odor, and to such a marked extent that the milk of the entire herd became fishy (possibly the udder of this cow was diseased).

The same author mentions another case which occurred on an estate in northern Germany. In that instance the milk of the Montavania cows in the stable was constantly rancid, whereas the milk of the Holstein cows was faultless, although the animals were all kept under the same conditions. The *Bacillus lipolyticum* was found to be the disturbing bacterium. It is noteworthy that the milk of the Montavania cows was frequently blody at the same time. Therefore it is possible that the elimination of the aroma bacteria took place from the affected udders, that is, the same bacterium was also the cause of the inflammation of the udder. However, it is more likely that through the secretion of the affected udders conditions were established in the milk from the Montavania animals which favored the propagation of the *Bacterium lipolyticum* in the milk, or probably the bacteria were present in the milk eisterns of these cows as harmless saprophytes, and the blood content of the milk may be attributed to some affection of the udder (yellow garget), which had no connection with the cause of the rancid milk.

It has also been proved that other changes in milk may persistently occur in the secretion of certain individuals so that it appears as if the causative agents of the changes in the milk may at times exist as saprophytes in the cistern (Schultze), or that they have at least multiplied in the excretory duct of the cistern.

Thus Schultze proved that in the appearance of "blue milk" the defect can only be removed by a thorough cleaning of the stable, animals, milk vessels, and all creamery utensils, and the milk cisterns of the animals must also be treated by antiseptic infusions of the udder.

The "blue milk" is produced by the *Bacillus cyanogenes*, a Gram-negative, aërobic, actively motile, unipolar, flagellated bacillus, with rounded ends, about  $0.4\mu$  thick and  $2.4\mu$  long. It is also known as the *Bacterium syncyaneum* Hueppe (Heim). Growing in sour milk the bacillus produces sky-blue to indigo-blue spots which gradually become confluent. The bacillus attacks the casein, and produces alkali besides a coloring substance, the triphenylrosanilin (Erdmann), which, depending on the reaction of the nutritive media, appears greenish or pale blue, violet or indigo-blue, or blackish-brown. The *Bacillus cyanogenes* in itself is colorless.

The coloring is less typical in sterile milk; a dirty bluish-gray discoloration with a reddish hue of the cream occurs, the color gradually diminishing in the deeper parts. Indigo-blue spots develop only in sour milk (Heim).

The changes which occur in milk appear to be especially frequent in certain localities; in others they are more rare and appear to have a connection with the properties of the soil. Pastures rich in clover are supposed to favor the appearance of the defects while in woodland pastures they have not been observed, or at least only exceptionally. This would explain why the defects occur in certain periods during the feeding of the incriminated feeds, or while the cows are feeding in certain pastures. According to observations they are observed more frequently in the fall, and during wet, foggy weather than during other periods.

These defects persist tenaciously in creameries and dairies and can only be eradicated after a thorough determination of their origin. Disinfection of the milk room and utensils with milk of lime and hot soda solution, and extending this disinfection to the stable in association with cleaning of the animal and possibly antiseptic infusions of the milk cisterns may yield the desired results.

Another organism causing "blue milk" is the Bacterium cyancofluorescens (Zangemeister). It is actively motile, bipolar, flagellated, grows on gelatin in the form of whitish colonies with indented borders and produces a fluorescent coloring matter in the nutritive media. The culture has an odor of trimethylamin and putrid fish. The bacteria produce dark blue spots in milk which change to a sky-blue color after coagulation of the milk. Other blue bacteria are those which occur on hay dust, in water, and in sewage in the vicinity of cheese factories, in ditch-water, and also the bacteria cultivated by Voges, Claessen, and Beijerinck which have been described under the names of *B. cæruleum*, *B. indigan*aceum, *B. cyaneofuscum*.

According to the observation of Weigmann and the description of Hallier certain hyphomycetes may also possess the faculty of producing a blue coloration; this is accomplished by the action of the blue coloring matter which they harbor.

In the zone of the milk supply of Munich ordinary milk defects occur very rarely; the author observed them in only one dairy, and was able to trace the trouble to a certain farm. Another defect of milk occurs much more frequently in the vicinity of Munich, the cause of which, according to the author, has not yet been described. It concerns the production of brownish milk.

The bacterium of brown milk appears to be closely related in all its characteristics to the producer of blue milk; it is  $2.4\mu$  long,  $0.5\mu$  broad, unipolar, flagellated, actively motile, Gram-positive and remarkably resistant to drying. In gelatin it grows especially well aërobically as a fine, iridescent deposit, which later becomes somewhat thicker, turning to a chestnut brown color. The oxygen zone of the lactose gelatin retains a saturated brown to deep brownish red discoloration, the nutritive media becoming alkaline to litmus.

A culture of the brown milk organism may be readily produced in milk by rubbing traces of the culture of milk having such a defect upon the bottom of a large Petri-dish, and pouring over it fresh (not sour) milk. In most instances after 15 to 20 hours ocher-colored to sepia-brown spots develop in the cream layer, which enlarge and coalesce, conveying to the milk and coffee-like appearance. After coagulation the superficial layer of milk again liquefies; whether this is brought on by the bacteria of brown milk alone or by peptonizing bacteria which multiply especially well when mixed with the bacteria of brown milk, which render the media alkaline, has not yet been established. The skimmed milk is not discolored by the *Bacillus fuscogenes*. The brownish color gradually diminishes from the surface down and at a depth of 5 mm. it disappears.

If the milk is allowed to sour the appearance of gray, orangered, red, yellow, green-fluorescent and violet spots may frequently

#### Red Milk.

be observed, which cause a glassy, transparent thickening of the wrinkled yellowish-white velvety layer of the oidium covering, or they penetrate into the depth of the jelly-like layer of the milk.

Thus under certain conditions the Bacillus violaceus, Bacterium janthinum, Bacillus lividus, and Bacterium amethystinus, a water organism, may appear in violet spots (Schroeder, Zopf, Mazé, Flügge and others).

Greenish-yellow spots and discoloration of the entire sour milk are produced by the *Bacillus fluorescens* which varies greatly in its characteristics, at times liquefying gelatin, again only discoloring it. It is a short rod with motility, but without spore formation.

A red coloring matter is produced by the *Bacillus erythro*genes Hueppe, which coagulates milk, but liquefies it later through peptonization, coloring the whey red.

According to Gruber a flagellated short rod, the *Bacillus lac*torubefaciens is supposed to produce a slimy condition of milk with the formation of a red coloring matter. Other bacteria such as the *Micrococcus cerasinum* (Keferstein), the *Sarcina rosea*, the *Bacillus prodigiosus* and others, form red spots. Red varieties of yeasts have also been found.

The author demonstrated through the examination of a dirty and dry milk pail that the layers of color which adhered to different parts somewhat like red varnish consisted of blue-red yeasts which had grown on the dried milk residue. The accumulation of color was present in the yeast cells proper, which on examination showed a reddish transparency. Their attempted cultivation was unsuccessful.

The discolorations of milk may vary from red, and pink, to rust-color and orange.

Yellow coloration sometimes only of the cream, at other times of the entire milk is caused by the *Bacillus synxanthus* (Schroter), the *Sarcina lutea*, the *Sarcina flava*, and *Bacterium fulvum* and others. Wild yeasts and moulds, which have been observed by the author may also cause a yellow coloration of sour milk. The *Bacillus fluorescens*, may at times cause a yellowish-green discoloration.

Other bacteria again show the action of their vegetation by the development of a tenacious slimy consistency of the milk. Strains and varieties of the peptonizing bacteria in which acid formation is dissipated and the peptonizing action of which retracts against the properties of producing rennet-like substances, may in a few hours cause a casein coagulation, and thereby convey to the milk a granular consistence. This defect is relatively rare, and on the contrary the milk may become non-coagulable, slimy and bitter.

More frequently, especially by keeping the milk in a warm place, a change of the milk to a slimy consistence may be observed. The action of the slime-forming bacteria may appear in two forms, and render slimy either the entire milk, or the casein is precipitated and only the whey develops a strong tenacious, stringy consistency.

The cause of the slimy condition may be produced either by a slimy change of the sugar, which is accomplished with the formation of a high molecular weight body, the galactan or the viscose, or by the swelling of the bacterial capsules which form a mucinlike substance.

The best known producer of slimy or stringy milk is the *Strep-tococcus hollandicus*, the cause of the "long whey," which is considered by Weigmann as a degenerated streptococcus of lactic acid fermentation. If cultivation of the producer of the "long whey" is continued at 35 deg. C. it loses the property of producing slime, and changes into a lactic acid producer.

From various groups of bacteria the following have been proved to be slime producers: *Bacterium lactis longi*—a streptococcus—in Swedish thick milk (Troili Petersson), *Micrococcus* (*streptococcus*) viscosus (Schmidt-Mühlheim), *Micrococcus mucilaginosus* from slimy cream (Rátz), and *Streptococcus burri* from stringy whey.

Slime is further known to be produced by the colon-aërogenes group (Emmerling, Schardinger), the *Bacillus guillebeau*, as well as the *Bacillus lactorubefaciens*. Adametz, Duclaux, Gruber, Ward, Eckles, and Marshall have also isolated slime producers from milk, whey, food substances, straw, stable air, and spring water.

Other defects of milk which are associated with change of consistency (and color changes), are produced by milk drawn from affected udders, which subject has been discussed in the section on "Diseases of the Udder."

Considering the living requirements of the special varieties of bacteria, the defects of milk appear to be especially frequent under certain weather conditions and in certain periods of the year. Thus the milk dealers of Munich complained of the appearance of defects of taste, especially in the cool and cold period of the year, and at the time of changing the animals from stable to pasture feeding and vice versa. The cause may lie in the fact that with the beginning of the dry, that is stable feeding, the microbian flora of the intestinal canal and of the forage and the stable air is different from that existing during the period of pasture feeding, and thereby other species of bacteria, aroma producers, contaminate the milk; likewise in certain cold climates and in certain methods of keeping milk the bacteria, excepting the lactic acid producers, find just the requirements which aid them in their propagation.

In 1909, the following defects of milk were found among 1,000 samples examined monthly:

Month		53	63	4	ດ	9	2	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	6	10	11	12
Dirty	289.6	249.9 145.4	145.4	366.24 200.91	200.91	416.35	263.08	830.5	665.5	809.48	765.7	294.
Sour	55.1	22.05	22.05 157.9	61.04	54.3	96.75	21.76	21.76 199.94	15.72	24.78	361.59	246.96
Soapy	48.23	29.40	17.54	•	5.43	12.9	•	•	• • •	8.26	• • •	35.28
Putrid		•	•	•	•	.6.45	•	46.14	5.24	41.30	•	•
Cow taste	•	•		•	•	•	8.68	•	•	•	21.27	•
Rancid	13.78	29.4	8.77	7.63	21.72	45.15	17.36	15.38	•	8.26	•	•
Oily	62.01	7.35	8.77	•	•	•	•	• • •	•	•	• • •	11.76
Bitter	•	•	•	•	• • •	6.45	•	• • •	•		•	• • •
Granular	•	7.35	17.54	•	•	6.45		•	•	•	• • • •	•
Sweet coagulating.	:	7.35	•	•	•	• • •	• • •	15.38	• • •	• • •	21.27	• • •
Slimy	6.89	7.35	•	•	•	•	•	•	•	•	•	-
Red		:	• • •	•	:	5.43	•	13.02	• • •	10.48	16.52	11.76
Brown		7.35	8.77	7.63	•	•	• • •	•		8.26	•	_
Mastitis	62.01	279.36	385.88	488.32	321.23	290.25	368.9	230.70	361.65		289.10   170.16	211.68
				_	- 1							

Various Defects.

At the same time it appears as though the spoiling of milk, for instance by souring, is less influenced by the temperature, which of course may be of importance, than by the atmospheric pressure. It could hardly be attributed to an accident that, except at harvest time when the milking is sometimes hurriedly done, the number of samples spoiled by souring were almost in reciprocal relation to the measured average value of the atmospheric pressure for the month.

Likewise in several months a certain parallel exists between the occurrence of dirty milk and souring, so that the dirt is present in largest amounts during August, September, October and November.

Months in which milk contains a great deal of dirt appear also to favor the requirements for the development of a putrid taste. (Height in June, August and October, harvest time.) During this period the milk is not aired and cooled, so it "suffocates." The cans are not cleaned, and all dairy work is slighted.

Direct contamination with cow manure appears to be of less importance in the development of an animal flavor, than pollution with bacteria from the skin of the cow, which may contaminate the animal while in pasture. These views are strongly supported by the experiments of Wolf and Weigmann, who proved the identity of the bacterial flora of the defective milk with the bacteria which were cultivated from the pasture plants, and by the experiments in which the authors succeeded in reproducing artificially these defects by using special bacteria.

This view is also supported by the observations of the author. A milk dealer complained about the bad taste of milk in a certain delivery. It was noticed that only the evening milk of the farm, and not the morning milk possessed the defect. Before the evening milking the animals were kept in a pasture during the day. It was remarked from the beginning that the morning milk did not have the taste, which was the more suprising since the animals by standing in the stable during the night must have affected the purity of the stable air. Nevertheless the evening milk which was obtained in the stable after a sufficient airing and cleaning, possessed the objectionable taste. Of course the time could have played a part since both the morning and evening milk were delivered at the same time, the evening milk being allowed to stand all night at a temperature of 12 deg. C. The milk was kept in a milk room next to the stable.

The conditions, however, were not changed by removing the milk immediately after milking, to a well-ventilated room, cooled by ice.

The passing of the odoriferous substances into the milk directly from the food could be excluded since the substances could then have been demonstrated in the morning milk as well, and therefore the only explanation which remained was that while lying down the abdomens of the animals became contaminated with the bacteria of pasture plants (meadow grass and clover). These bacteria contaminated the evening milk during milking in the stable, whereas the morning milk was principally contaminated with bacteria from the bedding. All other factors could be given about equal consideration. That the age of the milk did not play a part was proven by the fact that the morning milk in spite of longer keeping during both cold and warm weather in exposed or covered vessels, had never been affected by the disagreeable taste.

It is almost impossible at the present time to establish definite relations between defects in milk and contamination of milk with bacteria, since the propagation of the bacteria causing the defects may be influenced by the most varied factors.

It should be especially emphasized that bacteria of one and the same species may under certain conditions produce different defects in milk, depending on the accompanying conditions, as for instance whether they are associated with one or several other species of bacteria. According to Wolff and Weigmann the Bacterium fluorescens possesses the characteristic of producing by itself an ester-like odor, while together with the Bacterium mycoides and the Streptococcus lacticus it produces a disagreeable aroma, and finally with the Bacterium megatherium, B. mycoides, and lactic acid bacilli it produces a cheesy odor. This of course renders the study of milk defects difficult, since a bacterium cultivated in pure culture may show an entirey different action than when present in milk in a mixed culture, and mixed culture experiments with the entire flora would become necessary.

### Types of Coagulation.

The decomposition and fermentation microorganisms, which develop in milk, are utilized in the examination of milk that is intended for the manufacture of cheese. A fermentation test is made from each delivery of milk, and after a certain time each sample is tested for odor, taste, and in regard to its appearance and visible changes.

According to Jensen the fermentation may be distinguished as:

- 1. A fluid type,
- 2. A jelly type,
- 3. A gaseous type,
- 4. A whey type,
- 5. A cheesy type.

It should be emphasized that milk samples rich in bacteria usually produce a good jellylike type, whereas in milk samples containing few bacteria, whey fermentation frequently occurs.

The jelly type results in the profuse presence of lactic acid formers, which distend and tear the coagulum by the action of aërogenes varieties and cheese bacteria, whose gas production frequently forces the coagulum upwards. The milk becomes whey when true saccharomyces varieties form gas at the moment of coagulation. At the same time sub-types may be distinguished, such as porous, granular and flaky. The cheesy fermentation type develops in the presence of an increased number of rennetproducing bacteria.

These fermentation tests of milk are important to ascertain if it is in satisfactory condition for cheese production. For the determination of its fitness for drinking purposes, however, these tests are of little importance, since the questions relative to the value which these bacteria possess in the nutrition of man, have never been satisfactorily answered.

# Bacterial Reductase, Bacterial Catalase and Lactic Acid Production.

Among the characteristics of milk bacteria, which are of especial interest are those which are utilized in the examination of milk, and which may have a disturbing effect in experiments

conducted for the demonstration of original ferments because of the reactions which they cause. One characteristic is the reducing property of some bacteria and their ability to split up  $H_2O_2$ into water and molecular oxygen, corresponding to the action of catalase.

The reducing action of bacteria as indicated by the presence of reductase, has been observed for a long time. Helmholtz in 1843 proved that putrefactive changes which could not be demonstrated by changes of odor could be proved by discoloration of litmus coloring matter. Subsequently this reducing action was confirmed by many authors to be the property of various anaërobic and aërobic organisms. Thus according to Gayon and Dupetit the anaërobes are capable of forming ammonia from nitrates, while the Bacillus prodigiosus, B. anthracis, Spir. finkler and Staphylococcus citreus form nitrites out of nitrates. Others again reduce sulphur to  $H_2S$  (through "Hydrogenase'').

As an agent for demonstrating the reductase processes some authors, for instance Spina, Cahen, and Wolff, use coloring substances which change into leuco-compounds, as a result of the reduction, but from renewed contact with the air they become re-oxydized, as for instance tincture of litmus, thionin, methylene blue, indigo blue, neutral red, etc. Others again use metallic salts to render the reduction directly visible (Scheur-len and Klett, Gosio), for instance selenite and tellurite, whose sodium and potassium compounds confer upon the colonies a brick-red or grayish-black tinge, by the reduction or indirectly, the transpiring reductive action is shown through secondary reactions, for instance the formation of nitrites from nitrates, through the addition of iodine starch paste which becomes decolorized by the nitric acid.

Methylene blue is used at the present time most extensively for the reductase test; that is, the solution recommended by Schardinger consisting of 5 parts of concentrated alcoholic methylene blue solution to 195 parts of water is best adapted for the examination of milk.

The reducing qualities of various bacteria towards methylene blue vary. Thus Jensen established the reduction qualities of a series of milk bacteria, and proved that varieties of colon, staphylococci, sarcina, and mould fungi, reduce rapidly, whereas acid streptococci do not decolorize the solution.

The findings of Koning, who used cultures 24 hours old in his experiments, were the same. Arranged according to length of time, reductions take place as follows:

D 117 C	-	,	I I I I I I I I I I I I I I I I I I I	201101
Bacillus fluorescens nonliquefac	iens. in.			8 min
Bac. acidi lactici Hueppe, in	,			o min.
Pao and detter inteppe, in		• • • • • • • • • • • •		12 min.
Duc. proutytosus, In			14	0.15 min
Bac, fluorescens liquefaciens	in			10 mm.
Bac. fluorescens liquefaciens, i		• • • • • • • • • • • •	**************	13 min.
<b>1</b> Toteus 20 ppt, III				15 min
Bac. coli communis, in				17
Raa subtilio in	•••••	• • • • • • • • • • • •	• • • • • • • • • • • • • • • • • •	17 min.
Bac. subtilis, in				30  min
Mesentericus, in				60 min.
Mill bactorio T in		• • • • • • • • • • • •		00  mm.
Milk bacteria I, in				80 min.
Streptococci of lactic acid, Oi	dium lac	tis and 2 s	table atmospharic	
bacteria, not in	and the		table atmospherie	
				00

90 min.

Schardinger in 1902 stated that suspensions of the Bacillus acidi laevolactici decolorize in 3 minutes, the Bacillus gasoformans in 3 minutes, the Bacillus lactis pituitosi in 30 minutes, the Bacillus coli in 15 to 20 minutes, etc.

The ability to reduce methylene blue has also been found in anthrax and tubercle bacilli.

In the experiments it was proved that although not all bacteria are capable of reducing methylene blue, the power of reduction in some is very strong, while in others it is diminished and in still others it is practically nil.

Reduction properties appear to be characteristic of the living

bacterial cell, which do not pass into the filtered fluid (Schardinger, Spina, Cahen).

From the above mentioned facts it is evident that milk which contains numerous bacteria has a strong reducing property. Through the works of Smidt, Müller, and Schardinger it has been proved that as a rule the richer the milk is in bacteria the earlier the reduction of the aqueous methylene blue solution at 37 deg. will occur. Milk drawn under sterile conditions fails to reduce the methylene blue solution even after days (Rullmann). Müller proved that freshly drawn and cleanly handled market milk requires 10, 12 or more hours for reduction (mixing 10 parts to 1 part of methylene blue solution), whereas fresh market milk during cold weather requires 6 to 9, and in warm weather only 1 to 2 hours for the decolorization. At the stage when bacteria begin to multiply, which is at the end of the incubation period, the time required for reduction amounts to from 1 to 2 hours. If sour milk or cow manure was added to fresh milk the time of reduction was hastened.

He therefore proved that all factors which favorably influence bacterial growth hasten the reduction.

In the author's first investigation he found that 10 c. c. of milk with about 44,000 bacteria per c. c. failed to reduce one c. c. of methylene blue solution in six hours.

With about	200,000	bacteria	reduction	took	place	${\rm in}\ldots\ldots 4$	to 6	hrs.
With about	500,000	bacteria	reduction	took	place	in		5 hrs.
With about	1,600,000	bacteria	reduction	took	place	in	2	hrs.
With about	6,000,000	bacteria	reduction	took	place	in	70	min.
With about	350,000,000	bacteria	reduction	took	place	in	50	min.
With about	800,000,000	bacteria	reduction	took	place	in	15	min.
Similar res	ults were ob	tained by	7 Jensen a	s foll	ows:			
With about	264,000,000	bacteria	reduction	took	place	in	1	min.
With about	80,000,000	bacteria	reduction	took	place	in3	to 5	min.
With about	50,000,000	bacteria	reduction	took	place	in	10	min.
With about	7,000,000 to	11,000,00	00 bacteria	redu	etion t	ook place in 4		
With about	3,000,000	bacteria	reduction	took	place	in		hrs.
With about	1,600,000	bacteria	reduction	took	place	in		5 hrs.
With about	1,000,000	bacteria	reduction	took	place	in		hrs.
With about	126,000	bacteria	reduction	took	place	in		6 hrs.
	,				<b>T</b>		- /.	-

Since the degree of acidity increases with the growth of bacteria there exists a certain connection between the degree of acidity of the milk and the rapidity of reduction, and since the degree of acidity increases rapidly after incubation, a rapid reduction would be expected to follow a rapid increase in the degree of acidity.

This is also proved by Jensen's experiments. Milk which reduced in one minute had after 12 hours, at 25 deg. C., a degree of acidity of 36. Milk which reduced in—

5	min.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of19
8 :	$\min$ .	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of
6	min.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of
$10^{-1}$	min.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of
8 :	min,	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of
7 1	min.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of
1	hr.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of
1	6 hr.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of25.5
$1^{3}$	4 hr.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of11.5

2¼ hr.	after	12	hrs.	at	25	deg	had	an	acidity	of				 				 	'	15
-3% hr.	after	12	hrs,	at	25	deg.	had	an	acidity	of				 						9
									acidity											
7 hr.	after	12	hrs.	$^{\rm at}$	25	deg.	had	an	acidity	of				 				 		10.5
6½ hr.	after	12	hrs.	at	25	deg.	had	an	acidity	of				 				 		8
7½ hr.	after	12	hrs.	$\mathbf{at}$	25	deg.	had	an	acidity	of				 				 		8
12¼ hr.	after	12	hrs.	at	25	deg.	had	an	acidity	of	• •			 				 		7
8½ hr.	after	12	hrs.	at	25	deg.	had	an	acidity	of	• •	• •	-	 • •	• •	• •		 		7.5

The findings of the author were very much the same as those of Jensen.

Milk which failed to reduce in 20 hours had after 24 hours at 20 deg., from 7.4 to 10 degrees of acidity.

The following table shows the results of the technique employed in testing milk, where the reduction number is understood to mean the number of drops of methylene blue solution which in a given time was completely reduced by 5 c. c. of milk:

Degree of acidity	After 24 hrs.	Time required	
at delivery	at 20 deg.	for reduction	Reduction number
7.0	7.4	20 hrs.	0
6.2	8.6	20 hrs.	0
6	10	20 hrs.	0
6	9	20 hrs.	4
7	10	20 hrs.	0
6	24	4 hrs.	2
6.4	26,5	8 hrs.	10
6,5	26	8 hrs.	6
6.2	24	8 hrs.	8
6.2	27	8 hrs.	6
6.8	26	8 hrs.	4
6	14	8 hrs.	4 4 2 2 2 2
7.8	30	6 hrs.	4
6.5	28	2 hrs.	2
7.2	32	1 hr.	2
6	34	1 hr.	2
6.2	38	1 hr.	10
8	40	5 hrs.	10
10.5	26.4	0.5 hrs.	10
6	30	6 hrs.	2

These numbers were obtained from a great number of samples during work at the milk control station, without any selection. They show that milk which sours rapidly, and is therefore at the end of the incubation period, also reduces rapidly; there exists, however, no absolute constancy in the parallelism, neither with the values of acidity in milk after twelve to twenty-four hours, nor with the values in samples of fresh milk.

After thorough souring the reduction power of the milk again diminishes for a time. This may be due to the fact that the acid reaction inhibits the reduction power—as a matter of fact the rapidity of reduction is again considerably increased by the addition of sodium carbonate or bicarbonate—and also because a nonreducing organism, the acid streptococcus, outgrows the other bacteria.

The addition of an alkaline solution brings about acceleration of the reaction only in sour milk, while in milk with low bacterial count the reaction is retarded, but this may be overcome when through acid formation neutralization has taken place. Antiseptics, such as boracic acid, salicylic acid and formaldehyde, inhibit or destroy the reduction power of bacteria; the same result is obtained by heating, which destroys the life of the vegetating bacterial cells.

Milk which has been heated for 10 to 30 minutes at 80 to 100 deg. C. shows only the slightest reduction power, which increases again only after the recurrence of bacterial multiplication.

It should be emphasized that milk, in spite of being spoiled to a marked degree, may have a slow reducing power, as for instance soapy milk, provided this condition is not associated with extensive bacterial contamination with other species of bacteria. Although the bacillus of soapy milk reduces very rapidly, soapy milk in itself is only capable of bringing on this reaction to a very slight degree, which probably is proof that defective flavors may result even when only a very slight bacterial growth has taken place, although the bacterial action is of tremendous importance.

For the completeness of this chapter it should be mentioned that milk very rich in bacteria, which has been sterilized by heat, reduces also the formalin methylene blue solution as a result of the original bodies in milk, a property which has nothing to do with the Schardinger reaction.

The formalin methylene blue reducing principle in market milk is also a pre-formed substance, which occurs in milk drawn under sterile conditions (original ferments).

### Bacterial Catalase.

Similar to the power possessed by body cells and body juices, bacteria have the ability of splitting gaseous oxygen from hydrogen peroxide solutions. This property may be observed in many bacteria, but it should be mentioned that not all species of bacteria possess it, and that certain bacteria have a specific power in this direction.

Koning and Jensen made confirmatory statements to this effect, having found that the acid streptococci of milk do not split  $H_2O_2$ . The author's experiments confirm this observation. Jensen made an especially interesting observation, namely, that the bacterial flora present in milk during the incubation period of souring usually possess strong catalytic properties.

The following data are taken from a work of Koning, arranged according to the catalase figures: Species of Bacteria Catalase test Reductase test

pecies of Bacteria	Catalase test	Reductase test
B. prodigiosus		15 minutes
B. proteus zopfii	57	15 minutes
Milk bacterium I	55	80 minutes
B. Auorescens liquefaciens	53	13 minutes
B. coli communis	39	17 minutes
B. lact. acid. Hueppe	32	12 minutes
Stable air bacteria II	31	90 minutes
B. mesentericus	30	60 minutes
B. fluorescens nonliquef	29	15 minutes

Stable air bacteria I	28 90 minutes
B. subtilis	
Milk bacteria II	
B. mycoides.	
Oidium lactis	
Str. mastitis longus	0 90 minutes

From these figures it may be seen that frequently with a high catalase number a very rapid reduction time may be present.

Jensen found similar conditions in his investigations; he, however, expresses himself as believing that a parallelism of both factors does not prevail. Arranged according to catalase values expressed in figures, giving the number of c. c.'s of oxygen formed, the relation between catalase and the time of reduction is as follows:

10110 W.S.	Catalase	Reductase
B. proteus vulgaris	27 c. c.	7 minutes
B. proteus zopfii	27 c. c.	5 minutes
B. prodigiosus	27 c. c.	7 minutes
Microc candic.	27 c. c.	4 minutes
<i>Microc. A.</i>	27 c. c.	3 minutes
B. coli	18 c. c.	5 minutes
B. aërogenes	9 c. c.	10 minutes
B. mycoides	7 c. c.	12 minutes
B. dentrificans	1 c. c.	10 minutes

With other bacteria, for instance, butyric acid bacteria, there appears to be no relation between the reduction power and the development of oxygen, whereas with certain lactic acid producers, for instance, the streptococci and cheese bacilli, the inability to develop oxygen coincides with the long time required for reduction.

In unspoiled milk during the incubation stage of souring and at the beginning of souring at the end of this incubation stage, the bacterial catalase will always have to be considered, but in general the bacterial action in slowly reducing milk is very slight. If in the latter instance high catalase values are obtained then usually the catalase originally present in the milk is responsible for it.

Koning further showed that catalase increases with the age of milk, and with a rapid angle of incidence. The line of incidence in fresh milk is at first only slightly bent, later more or less so, whereas old milk uniformly shows a rising line. Spindler's recent experiments confirm this statement. From the investigations of Spindler, however, it may be observed that during the time when milk is fresh enough for drinking purposes the fluctuations are only very slight and the catalase value obtained is always greatly dependent on the original catalase value of freshly drawn milk. Faitelowitz indicates that catalase multiplies many fold after keeping fresh milk at room temperature for 24 to 30 hours.

Through heating to 70 deg. C. the "bacterial catalase" is destroyed, or at least the bacteria are attenuated in their action to such an extent that the oxygen-splitting property becomes almost nil. Chick has already ascertained that this inactivation of the bacterial catalase may be abrogated in a certain time by inoculation of the heated milk with raw milk and Koning states that old pasteurized milk, or milk freshly pasteurized with insufficient heat, splits the  $H_2O_2$ . The catalase test is therefore recommended by Knüsel for the examination of pasteurized milk as to its suitability for drinking purposes. It is to be regretted that the bacterial catalase cannot be separated from the original catalase, so that it would be possible to draw definite conclusions from the catalase findings of market milk, as to whether the catalase quantities which are demonstrated were present in the freshly drawn milk or whether they have been subsequently formed by bacterial growth. Wolf claims that milk which reduces slowly and shows a strong catalytic property by the formation of large quantities of oxygen should be suspected of containing secretions from animals with affected udders. It would be impossible to draw conclusions based on this statement in those cases where rapid reduction occurs coincidently with strong catalytic action.

## Degree of Acidity.

In discussing the original properties of milk it was mentioned that casein, acid salts of milk, carbonic acid, etc., give to milk an acid reaction to phenolphthalein.

Even immediately after milking, in order to produce neutralization of the milk against phenolphthalein, several c. c. of sodium hydrate are required. The number of cubic centimeters of a normal Na OH dilution which are needed to neutralize a certain quantity of milk are known as degrees of acidity. The number obtained varies, depending on the method and dilution employed.

Thus Soxhlet-Henkel, for instance, employed 100 c. c. of milk and  $\frac{1}{4}$  normal Na OH and obtained an average value of about 6 to 7 degrees of acidity.

Jensen, who works with  $\frac{1}{10}$  normal Na OH, uses on an average 18 to 19 c. c.

Thörner dilutes 10 c. c. of milk with 30 c. c. of water and titrates with  $\frac{1}{10}$  normal Na OH.

The degrees of acidity, as determined by Dornic, are higher than those of Soxhlet-Henkel: He uses 10 c. c. of milk and alkali which contains 4.445 gm. Na OH to 1000 H<sub>2</sub>O.  $\frac{1}{10}$  c. c. of alkali, according to Dornic, is equal to a degree of acidity.

Schrott-Fichtl and Dornic suggested as an advantage the dropping of the "scale of degree of acidity" and employing an alkali, 1 c. c. of which would correspond to 0.01 gm. of lactic acid, or to figure the degree of acidity on the basis of lactic acid. Then 1 c. c. of  $\frac{1}{10}$  alkali would correspond to 0.009 gm. of lactic acid and 1 c. c. of  $\frac{1}{4}$  normal Na OH=22.5 gm.

Of course, it should be remembered that the neutralization of the alkali does not correspond entirely to the amount of lactic acid present but depends also on other factors, for instance, on the proportion of acid phosphates, carbonic acid and casein.

Only the increase in acidity which is obtained by a comparative testing of fresh milk and an older sample of the same milk, should therefore be considered as lactic acid, since Henkel proved that free lactic acid is not present in freshly drawn milk. The degree of acidity of milk depends on the lactation period. Colostrum, milk of animals with affected udders, and milk from freshly milking cows have an abnormally high acidity, while milk from animals in the last stages of lactation, and sometimes milk from affected udders, may be lower than normal.

Besides these factors the degree of acidity of milk is also influenced by the growth of bacteria, the species of bacteria, and therefore by all factors which have an influence on the bacterial growth, such as cleanliness in milking, cooling, outside temperature, age of the milk, etc.

Plaut, for instance, demonstrated that milk which has been kept-

a temperature of	showed multiplication of	and voluntarily coagulated
	bacteria after	after
10 deg. C.	48 to 72 hrs.	100 hrs.
15 deg. C.	20 to 24 hrs.	63 hrs.
20 deg. C.	12 to 20 hrs.	48 hrs.
25 deg. C.	8 hrs.	24 hrs.
31 deg. C.	7 hrs.	22 hrs.
37 deg. C.	5 hrs.	12 hrs.
aning has also beat	and the set of a set	

Koning has also kept milk at various temperatures and titrated the degree of acidity after varying periods:

				t at 7 to 9 deg. C.	Kept at 22 deg. C.
			I	Deg. of acidity	Deg. of acidity
				with ¼ <sub>10</sub> NaOH	
		r delivery			13.6
		hours			14.6
Aft	er 29	hours		14.6	20.6
Aft	ter 41	hours		. 16.0	62.6
Aft	ter 53	hours		. 16.0	71.0
Aft	ter 65	hours		16.8	
Aft	ter 77	hours		17.6	
Aft	ter 89	hours		. 18.8	

Since the growth of various bacteria depends on the method of keeping the milk, therefore the acid formation varies in accordance with the same conditions during the same time. Koning's experiments confirm these findings:

Time of	Milk in sh	allow vessels	In tall	cylinders
delivery	<b>at</b> 22 deg.	at 37 deg. C.	at 22 deg.	at 37 deg. C.
Shortly after mill	king.18.6			
After 24 hours.	16.4	18.5	18.8	32.4

The degree of acidity depends, furthermore, upon whether fresh milk is boiled or raw; in boiled milk it is lower than in raw milk, and it also depends on the aeration of the milk.

Milk drawn carefully into bottles 25 minutes after the milking has 17.4; after being aired by pouring from a height of  $\frac{1}{2}$  meter, 16.4; after repeated aeration 16.1, and after boiling only 16 degrees of acidity (Koning). The escape of the volatile carbonic acid seems to play a part in this.

Finally, the degree of acidity depends on the method by which it is tested. For instance, if the milk is diluted with water for the purpose of titration (method of Thörner, Pfeifer, etc.), then through this addition of water, a solution of alkaline calcium phosphate takes place and the acidity becomes less.

At

Since the acidity varies immediately after milking, after lactation, among individuals, and even in milk from different teats. and from interrupted milkings, the immediate measuring of the degree of acidity constitutes no proof of the age of the milk. The periodically continued titration of the same sample may, however, be a good indication as to whether the milk has passed the incubation phase and thereby afford an approximate indication of the "age." By "age" is not understood the difference in time between milking and examination, but a condition which may appear in milk sooner or later, depending on the cleanliness in its preparation and handling, and on the outside temperature. This condition is effectively determined by the reduction of methylene blue. If the milk has once passed the incubation time the curve of acidity rapidly and progressively rises, when the milk is kept subsequently at temperatures at which lactic acid bacilli grow prolifically (20 to 37 deg.).

Koning made a test of milk which at delivery gave a degree of acidity of 15.8 ( $\frac{1}{10}$  n Na OH :100),

And showed after	at 10 deg.	22 deg.	37 deg. C.
1 day		28.8	96.0
2 days	. 16.7	91.1	92.8
3 days		102.4	105.2
4 days		96.4	144.0
5 days		105.6	184.0
6 days		103.2	219.6
7 days		102.8	241.6
8 days		106.0	261.6

Since, however, the amount of lactic acid formation does not depend on the time and temperature alone, but also on the variety of bacteria growing in the milk, only general conclusions as to the aging of the milk can be drawn.

### Subsequent Contamination With Infections of Man.

The occurrence of disease producing agents in milk is of interest from the standpoint of tracing the origin of disease, but from a milk inspection standpoint it is a most thankless field. These disease producers may originate from affected persons, or from healthy bacilli carriers, or they may reach the milk through infected material, as, for instance, infected water used in washing utensils, or as an adulterant, or in the treatment and preparation of milk products.

That milk may become a transmitter of disease has been positively proven.

1. **Typhoid Fever.** The causative agent may contaminate the milk through infected water, through vessels which were returned without cleaning from houses harboring persons affected with typhoid, through affected and convalescing patients who are employed in producing or in the subsequent handling of milk, and through attendants and other intermediate hosts, especially through bacilli carriers. Konradi positively demonstrated typhoid bacilli in such milk. Levy and Jakobstal discovered true typhoid bacilli in an abscess of a cow so that under certain conditions it should be considered possible for typhoid bacilli to gain entrance into the milk directly from the udder of the cow.

2. **Paratyphoid Fever.** All that applies to typhoid bacilli holds equally true for **paratyphoid**, and to other bacteria of that type, for instance the *Bacillus enteritidis* and the *Bacillus paracoli*.

In these affections especial attention should be directed to the animals which are affected with intestinal inflammations, purulent metritis, and acute, severe inflammations of the udder, and also to stables in which white scours of calves and calf-ill occur frequently.

The possibility of the transmission of scours to man has been indicated by Lenz, Jehle and Charleton. Up to the present time, however, its certain transmissibility through milk has not been satisfactorily demonstrated.

3. Cholera.

4. Diphtheria.

5. **Tuberculosis.** Rabinowitsch demonstrated tubercle bacilli of human type in milk.

6. Scarlet Fever.

The sanitary police or the authorities in charge of milk control in all cases in which a suspicion prevails that such diseases have been transmitted through milk can provide that the possibility of the continued spread of such infections should be prevented. The sanitary police authorities should continuously impress upon all persons interested in the production of milk, and in the dairy industry, that there are always possibilities of the transmission of disease; and the attendant physicians should caution the patients and their families as to the danger of allowing it to spread further, and any violations should be dealt with to the extent of the law.

The health authorities of a locality at every appearance of a dangerous epidemic should consider the possibility of the development of the disease through milk consumption, and should trace the places from which the affected persons and their families draw their milk supply. If from these investigations there exists the slightest cause to assume that the milk supply may be the original cause, the attending physicians should cause a further investigation of the matter. In the meantime the suspected milk should be rendered harmless by pasteurization.

With these remarks an intrusion has been made into the chapter upon "the supervision of the milk traffic and milk control," which will be given special consideration.

### The Destruction of Bacteria in Milk.

It is our purpose to discuss briefly the destruction of bacteria in milk, which aims to free the milk from disease-producing germs and add to the keeping quality of the milk.

In practice this is accomplished most frequently by heating, in which the following distinctions are made:

1. Sterilization of the milk;

2. Simple boiling;

3. Pasteurization.

If it is desired to judge the value of these methods of preparation, the question first considered must be, what changes does the milk undergo through heating? Milk is a biological product the properties of which may be considerably influenced by cold and heat.

It is generally known that after heating milk retains a socalled boiled-milk taste, and that this becomes stronger the longer the milk is subjected to a temperature of from 70 to 100 deg. C.

The method of heating is important for the appearance of the cooked taste. Open boiling even for a short time produces a marked change in taste when compared with heating in specially closed utensils or in bottles after subsequent cooling.

The curdling of boiled milk is more difficult than with raw milk; the boiled milk in curdling after a long time forms a loose, coagulum with less uniform consistency. This change is not so pronounced with heating between 70 to 80 deg. as in boiling and in heating to over 100 deg. C.

Depending on the height of the temperature and on the length of time the heat is applied, globulin (at 75 deg.) and albumin (at 80 deg.) are precipitated. Proteids which are precipitated in milk by heating to boiling temperature disappear if the boiling is continued. According to Peiper and Eichloff the intermolecular attachments of the proteids become loosened by heating to high temperatures, and leucin, tyrosin, ammonia, sulphureted hydrogen and phosphorated hydrogen are formed. If the heating has been conducted in poor earthenware or glass vessels, especially new ones, potassium silicate will pass into the milk. Fynn noted the absence of sulphureted hydrogen from heated colostral milk. The reaction became apparent only on the fourth day of lactation. The formation of sulphureted hydrogen and phosphoric acid in milk results from the splitting up of casein.

Hydrogen sulphide can be demonstrated in canned milk even months after heating, whereas in sterile bottled milk, under the influencee of light and in the presence of oxygen, the sulphide of hydrogen is utilized for the formation of water and sulphur.

In higher heating the milk becomes brownish through caramelization of the milk sugar and the lecithin content of the milk diminishes, which, according to Kida, may be seen from the following example:

In 1000 c. c. of milk, lecithin was present as follows:

		a e lo c a e	co romo	7 TT N +	
Unheated samples0.474 gm.	0.474	0.505	0.467	0.462	0.517
Heated to $75 \deg \dots 0.444 \text{ gm}$ .					
Heated to 80 deg	0.420	0.467			
Heated to 95 deg			0.349		
Heated to 100 deg					
Diminishing amt0.030 gm.				0.111	
In percentage6.33	11.39	7.52	25.27	21.22	22.17
• •					

In heating, the proteids also change, peptone is formed and tricalcium phosphate is precipitated.

The original ferments are especially susceptible to the influence of heat. Through heating to a certain temperature the amylase, the peroxydase, the catalase and the aldehyde reductase disappear. The amylase and the aldehyde reductase disappear even at a temperature of 65, that is from 50-65 deg. C. Of course milk which has been changed in this way by heat must naturally be judged differently from a nutritive standpoint than raw milk. Out of 3,462 digestible proteids used in each 100 gm. of milk there remained undigested:

> > 85 deg. C....1.493 gm.

90 deg. C....1.420 gm.

95 deg. C....1.540 gm.

100 deg. C....1.719 gm.

Experiments by Brückler, Reiner and Eichloff showed that dogs fed for months on sterilized milk showed a good nutritive condition, and some of them even manifested a greater gain in weight than the control dogs fed with raw milk, but the latter were brighter, their blood was richer in ash, with diminished salt content; it contained more fibrin, had a higher specific gravity, and the structure of their bones was more dense and richer in ash. The bone marrow of the animals fed with sterile milk was more anemic, the periosteum of the bones separated more easily, and at times hemorrhages appeared on the borders of the diaphyses, such as occur in rachitis.

The nutritive results in children which have for a long time been exclusively nourished on sterilized milk are similar. The infants become affected with infantile scorbutus, a symptom complex, which is known to the physician as "Möller-Barlow disease," and which disappears when raw milk is provided.

From the above it appears that high prolonged heating of milk should be avoided, and if possible the advantages derived from the heating should be obtained by heating the milk for only a short time at a relatively low temperature, which when properly applied will appropriately destroy the bacteria.

The vegetative bacteria may be destroyed by subjecting them to the influence of heat at 60-70 deg. C., for one-half hour, or to a temperature of 85 deg. C. for a half minute; on the other hand it is known that spores of bacteria not infrequently resist a temperature of 100 deg. C. and over.

In practice it is advisable to abstain from the sterilization of milk with high degrees of temperature, and to apply pasteurization, since, through the usual method of sterilization the destruction of all germs is not attained and the disadvantages are too apparent.

The wholesale depots may be provided with outfits for bottle pasteurization and milk heating, in which flowing milk, through the influence of steam on heating surfaces may be brought to a temperature of 85 deg. C. In heating bottled milk it is essential to observe that the milk should become uniformly heated throughout; this is attained by shaking the milk during its pasteurization. Following this, rapid cooling should be undertaken, which is best accomplished by atomizing pipes which cause water to fall upon the hot bottles in the form of a spray.

The apparatuses in which the milk flows over heated surfaces should be so constructed that all parts of the milk will come in contact with the heated surface, making the heating of the milk uniform in all parts. The utilization of the heat in some of the appliances is regulated in such a way that the cold inflowing milk is warmed by the outflowing pasteurized milk, the latter, however, being cooled subsequently. Through the exchange of heat from the outgoing stream of milk about one-half of the required heat may be saved. The efficiency of some of these apparatuses is enormous, since they are able to treat from 5000 to 8000 liters per hour.

From a sanitary standpoint it is apparent that such apparatuses must be so constructed that they may be readily cleaned mechanically, since improperly cleaned places conduct the heat poorly, and may give rise to contamination of the milk with putrefactive bacteria.

In pasteurization, the same as in milk production, the greatest stress should be laid on immediate and thorough cooling, and on keeping the milk continuously cool until its consumption, since otherwise the pasteurized milk will become spoiled, and will undergo a form of decomposition which is very undesirable (especially in bottle pasteurization). Pasteurized milk decomposes through multiplication of protein splitting, peptonizing bacteria whose spores may have withstood the heating. The vegetative bacteria, among these the lactic acid producers, are mostly destroyed, and except for a few resistant forms of spore bearers only heat-resisting organisms will remain viable, but these forms of bacteria are usually harmless (Rullmann, Gerber and Wieske, Burri, Russell and Hastings). These germs decompose proteids and carbohydrates by forming butyric acid with gas production, and peptonizing the proteids. Boiled milk decomposes more readily than raw milk from the bacteria which contaminate it after the heating process.

Relative to the effects of pasteurization, the following should be noted: As a result of the effect of 85 deg. C. the bacterial number dropped from 10,000,000 to 500 per c. c. These remaining organisms however, which consist principally of peptonizing varieties, multiply rapidly to very great numbers if the milk is brought again to 25 degrees C., frequently producing changes in taste, which becomes bitter and irritating, but sometimes without markedly changing either the appearance or taste.

This however does not render pasteurization hazardous, since it is possible through proper handling of the milk to prevent these undesirable processes. The marketing of pasteurized milk becomes dangerous only when the consumer considers that pasteurized milk being free of germs may be kept indefinitely under almost any circumstances and therefore takes less care of pasteurized milk than he would of the raw product; besides this consumers repeatedly heat such milk and thereby diminish its nutritive value more and more. It is for this reason that various authorities have taken action against the indiscriminate distribution of pasteurized milk. It should be required that the date of pasteurization be indicated on each bottle.

A statement from the officials of the city of Leipsic asserts that pasteurized milk is not more valuable than raw milk, but that it appears to be of lesser value on account of the destruction of its raw condition and the consequent changes. Any manipulation of milk which claims to extend its keeping properties by several hours, and which possibly may be used in the establishment of false valuation by statements that the milk has a lasting, keeping quality and a freedom from bacteria, is directly dangerous and injurious to health if the consumer is not thoroughly informed with regard to the effectiveness and limitations of pasteurization. The action of peptonizing bacteria in milk that has been pasteurized is pointed out, and recommendation is made against the purchase of milk which was pasteurized more than three days previously. The official statement also calls attention to Barlow's disease, and to the dangers attending improper keeping of such milk.

Sometimes pasteurized milk which is never free of bacteria is marketed under the attractive declaration of "free from diseasebacteria." What is the relation of pasteurization to such a claim as this? According to the experiments of Forster, van Geuns, de Mann, Ringeling and Koning, de Jong, de Graaf, and Beck, the disease-producing bacteria are affected differently by high temperatures while in milk than when in bouillon or water. Thus for instance heating for a half hour at 70 deg. C. is not always sufficient to destroy colon bacteria. Tubercle bacilli are still more resistant. According to Kolle and Beck they are not destroyed with certainty even when subjected to heating for a half hour at 80 deg. C., especially not when they are isolated from the influence of the heat by the formation of a surface scum and by coagulation. De Jong concludes from his experiments (bottle sterilization):

1. That heating for a half hour at 71-72 deg. C. is not always sufficient to destroy the tubercle bacilli mixed with the milk.

2. That heating even to a higher degree does not always give satisfactory results since the resisting power of the tubercle bacillus varies.

3. The designation "free from disease-bacteria" for pasteurized milk is false.

4. Those who desire milk free from tubercle bacilli must purchase sterilized milk, provided it is not obtained from herds free from tuberculosis. Bang, de Mann and Forster obtained evidence that heating to 85 deg. C., for three minutes destroys the tubercle bacilli, a fact which has also been confirmed by the work of Weigmann and by the experiments of Tjaden, Koske and Hertel under conditions which prevail in large distributing plants with milk from tuberculous udders. Other non-spore-containing disease-producers in milk are also destroyed at this temperature provided that certain conditions do not prevent proper heating.

[According to the experiments of Rosenau, it is evident that the tubercle bacillus in milk loses its infective properties for guinea pigs when heated to 60° C. and maintained at that temperature for 20 minutes or to 65° C. for a much shorter time. It should be remembered that the milk in the tests of Rosenau was very heavily infected with virulent cultures, which was indicated by the prompt deaths of the control animals. Milk would practically never contain such an enormous amount of infection under natural conditions. It is therefore justifiable to assume that if 60° C. for twenty minutes is sufficient to destroy the infectiveness of such milk when injected into the peritoneal cavity of a guinea pig, any ordinary market milk after such treatment would be safe for human use by the mouth as far as tubercle bacilli are concerned. These results are substantiated by the findings of Versin, Bonhoff, Th. Smith, Schroeder, Russell and Hastings and Hesse.

Belative to the thermal death point of other organisms Rosenau found that typhoid bacilli are killed in milk when heated to  $60^{\circ}$  deg. C. and maintained at that temperature for two minutes. The great majority of these organisms are killed by the time the temperature reaches 59° C. and few survive to 60° C.

The diphtheria bacillus succumbs at comparatively low temperatures. Oftentimes it fails to grow after heating to  $55^{\circ}$  C. Some occasionally survive until the milk reaches  $60^{\circ}$  C.

The cholera vibrio is similar to the diphtheria bacillus so far as its thermal death point is concerned. It is usually destroyed when the milk reaches  $55^{\circ}$  C., only once did it survive to  $60^{\circ}$  C. under the conditions of the experiments.

The dysentery bacillus is somewhat more resistant to heat than the typhoid bacillus. It sometimes withstands heating at  $60^{\circ}$  C. for five minutes. All are killed at  $60^{\circ}$  C. for ten minutes. However, the great majority of these micro-organisms are killed by the time the milk reaches  $60^{\circ}$  C.

So far as can be judged from the meager evidence at hand,  $60^{\circ}$  C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. The *Micrococcus melitensis* is not destroyed at 55° C. for a short time; the great majority of these organisms die at 58°, and at 60° all are killed.

Milk heated at 60° C. and maintained at that temperature for twenty minutes may therefore be considered safe so far as conveying infection with the micro-organisms tested is concerned.

Avers states that the best method of pasteurization at the present time, and the one which should be used, is the holder process, in which the milk is held for 30 minutes. For this process a temperature of  $63^{\circ}$  C.  $(145^{\circ}$  F.) is to be advised, since that temperature gives a margin beyond that sufficient to destroy pathogenic organisms, while at the same time it leaves in the milk the maximum number of lactic-acid-producing organisms which cause the souring of the milk. When using the flash process, the milk should be heated to at least  $160^{\circ}$  F. Since there is almost always a fluctuation in the temperature during pasteurization, care should be taken to see that the temperature never drops below  $71^{\circ}$  C.  $(160^{\circ}$  F.) in the flash process.—Trans.]

Exposure at 50 degrees of temperature for 15 minutes or at 70° C. for 10 minutes is sufficient to destroy the virus of foot-andmouth disease. The virus is destroyed instantaneously at 85 deg. C.

All of these advantages may also be obtained from subjecting the milk in the household to heating for a short time without boiling, when through occasional stirring the formation of the scum upon the surface is prevented. Therefore the purchase of raw milk, whose fresh condition can be readily controlled, should be generally recommended, and the destruction of bacteria should then be carried out by simple heating.

The observation recorded by Schut appears to be worthy of consideration, namely that relatively low temperatures rapidly destroy bacteria, when applied simultaneously with a lowering of the pressure. In heating the milk at 70 deg. C. the disturbing scum formation was omitted. As accepted by Schut, in this process the steam penetrates into the body of bacteria, which explains the more efficient action of this method.

Experiments which aim to improve the keeping qualities of milk by the addition of **chemical substances** are very numerous. In addition to improving its keeping qualities the retention of the raw condition of the milk was attempted. This does not refer to the adulterations which are undertaken by dealers for fraudulent purposes, or which are carried out in the household, and it should be considered that all additions to milk without subsequent declaration are equal to an adulteration of food, changing it to a spoiled product, possibly even converting it into material injurious to health. At that the additions do not accomplish the purpose for which they are intended in the dilutions in which they are used (Richterboracic acid), or they diminish the utilization of milk for cheese production because they inhibit the rennet action. Soda or bi-carbonate of soda, boracic acid and borax, more rarely salycilic acid, and recently formaldehyde are mostly used. Adulterations will not be discussed here, but only earnest scientific experiments will be taken up, in which the accomplishment of an actual improvement in milk has been the object sought.

1. Budde succeeded in improving the keeping qualities of milk with the aid of peroxide of hydrogen. The milk is heated to about 50 deg. C., 0.036 to 0.5% H<sub>2</sub>O<sub>2</sub> is added and it is then filled into bottles and kept for several hours at 50 deg. C.

According to Lukin it is possible with pure peroxide of hydrogen, as indicated by Budde, to give the milk a low bacterial count, or render it free of bacteria. Budde's method has not attained an extensive use. According to Chick, Rosam, Gordan, Bergmann and Hultmann, Eicholz, Nikoll and Duclaux the amount of peroxide of hydrogen recommended by Budde is not sufficient for the satisfactory destruction of bacteria in milk, but according to Lukin their failures were due to the use of impure preparations of peroxide of hydrogen. Tubercle bacilli and typhoid bacilli were not destroyed by this method. If the authors used 0.1% of peroxide of hydrogen, the necessary quantity to produce sterilization, then the milk obtained a bitter taste, which disappeared only after the excess of hydrogen peroxide had been eliminated by catalase. According to Utz a small quantity of peroxide of hydrogen is retained in the milk even when used in the quantities recommended by Budde. De Waele, Sugg and Vandevelde, who worked with 0.3 and 0.4% of peroxide of hydrogen, have used in addition small quantities of defibrinated blood for splitting up the retained H<sub>2</sub>O<sub>2</sub>.

Much and Römer employ a similar method of preserving milk which has been obtained under special precautions as to cleanliness. The milk is filled into sterilized bottles, mixed with 0.1%of peroxide of hydrogen, and kept for one hour at 52 deg. C. For the destruction of the  $H_2O_2$  in the milk, hepin, a catalase prepared from liver, is added to the milk before its consumption. Since the hydrogen peroxide milk is very sensitive against the influence of light (when exposed to light it very readily becomes bitter, tallowy and rancid), it is best to keep it in green bottles and in a dark place. Even with these precautions a change in the taste may become apparent after two weeks.

Injurious action of the peroxide of hydrogen if used in these quantities should not be feared; the results in infant feeding are supposed to be favorable.

The milk which is freed from the retained peroxide of hydrogen by the addition of hepin should be immediately used, since it is no longer resistant to decomposition through bacterial contamination, after the hepin has been added.

Years ago von Behring recommended the preservation of milk by formaldehyde. Experiments upon animals showed that the addition of formaldehvde to milk in the proportion of 1:1250 gave it no properties injurious to health by any method of application (even intravenously), and it was further found that animals with a very delicate sense of smell failed to recognize the presence of formaldehyde if it had been added to the milk in a dilution of 1:10.000. The action of formaldehyde in such dilution is quite marked. The addition of a 1:10,000 dilution postpones coagulation for many days (von Behring, Price and Schaps); 1:25,000 and 1:40,000 prevents coagulation from 1 to 4 days (Kolle). The action of formaldehyde was found to be more effective in accordance with the cleanliness of the natural milk and this action according to Rothschild and Metter appears to result from the fact that the lactic acid bacilli chiefly succumb, whereas the other saprophytes are harmed to a lesser degree. Tubercle bacilli are not influenced in their viability by these dilutions.

The feeding of infants for weeks with formalin milk (additions of 1:25,000) may result in an injury of the kidney epithelium of the children, which leads to the elimination of albumin. According to Baudini the rennet pepsin and trypsin action may be considerably inhibited by formalin; the acidity of the milk is increased. In the experiments of von Behring the action of formalin depends upon its effect in checking the development of bacteria, and not on its disinfecting or sterilizing property. A concentration of 1:25,000 up to 1:50,000 has no influence on the typhoid and colon bacteria and staphylococci (Vaughan and Schaps). Diphtheria, colon and pyocyaneus bacilli have not been destroyed even in dilutions as low as 1:5,000. Tubercle bacilli are protected by their waxy covering even against higher proportions of formalin, and as a matter of fact formalin is used, on account of its action on other bacteria, for the purification of sputum for the purpose of cultivating the tubercle bacilli from the saliva. Formalin milk constitutes a food which should be designated as spoiled and injurious to health.

3. Seiffert worked out a method of milk preservation in which the bactericidal action of ultra-violet rays is used for sterilization of milk. The method of action of the ultra-violet rays has not yet been satisfactorily explained. According to Lobeck (cited by Grimmer) the exposure of water to such rays produces peroxide of hydrogen. Grimmer believes that the latter is also formed in milk, but on account of the catalectic factors of the milk it immediately decomposes again. It is possible that the formation of peroxide of hydrogen constitutes the germicidal power of ultra-violet rays. The milk fat is not changed (Lobeck). According to Dreier-Hansen the proteid is coagulated after a prolonged exposure of milk to such rays. Seiffert passes the milk in broad bottles along the illuminating bodies, allowing the rays to act upon the milk for about two minutes. He employed Leyden jars fitted with aluminum or cadmium points, which are charged with a current of high tension through an inductor which discharges mutually. Gerber and Hirschli used for sterilization the uviol light which is rich in ultra-violet rays; he was unable however to demonstrate a marked reduction of the bacterial content by subjecting a layer of milk of 1 mm. thickness to its influence, whereas Finkelstein and Lobeck, Henri and Stodel, Billon and Daguerre obtained good results with the ultra-violet rays from mercury and quartz lamps. According to Billon and Daguerre sterilization may also be accomplished when milk is exposed to white light in violet glasses. The action is the best when the white light is split up by a prism.

Römer and Sames, who also conducted experiments on the bactericidal action of ultra-violet light proved that market milk which has been exposed to the rays of a Heräus' mercury-quartz lamp of 6 ampere strength in a quartz alembic (at a distance of 15 cm. from the source of light the action of which has been increased by a reflector) caused a reduction from 98,900 original bacteria after one hour of exposure to 16,500 bacteria; after 1<sup>3</sup>/<sub>4</sub> hours to 8750; after 2<sup>1</sup>/<sub>2</sub> hours to 2,050 bacteria. The taste of the milk was pronouncedly irritating. In a second test the number of bacteria diminished from 111,800 to 94,000 in 10 minutes, and to 65,500 in 20 minutes. On the surface of the milk a yellowish scum forms. The peroxydase reaction of the milk is destroyed after a prolonged exposure to such light.

[The experiments of Ayers and Johnson indicate that with quartz mercury vapor lamps of the present power and construction it would not be possible commercially to completely sterilize milk by the ultra-violet rays.

It might be possible to obtain bacterial reductions as great as by pasteurization even on a commercial scale by the use of large revolving drums and a number of lamps. However, in milk so treated there would be no assurance of the complete destruction of pathogenic organisms since the rays do not seem to exert any selective destructive action on vegetative cells. Of course since pathogenic organisms might be assumed to be present in a small number in proportion to the total bacteria in milk, if 99.9 per cent. of the organisms present were destroyed, it might be assumed that the pathogenic bacteria would be destroyed. This process, however, would not afford the same security as does proper pasteurization. Then, again, it would be difficult on a large commercial scale to constantly control the factors which influence the bactericidal action of the rays.

It is also doubtful if the lamps could be made to successfully

compete with the present method of steaming milk bottles in order to partially sterilize them.

From these experiments it appears doubtful if ultra-violet rays can be used on a commercial scale to replace the process of pasteurization. However, it may be possible to use the rays, in combination with pasteurization, in the preparation of a special milk with a low bacterial count, provided there is a demand for such milk in limited amounts for the use of infants and invalids.—Trans.]

4. Other methods of preserving milk are its saturation with carbonic acid under strong pressure, its ozonisation, and its sterilization with electrical currents.

The carbonization recommended by Hoffmann, van Slyke, and Bosword, the ozonisation advised by Dorn, and finally the sterilization through alternating electric currents of high tension recommended by Guarini and Samarini have not yet attained any practical significance.

The best means of imparting keeping qualities to milk are cleanliness in its procurance; the only method of preservation which should be generally permitted for milk is proper cooling.

This concludes the theoretical consideration of milk. In the following chapter the method of control of milk in general will be discussed, and finally the method of milk examination will be taken up with emphasis on the points which appear especially important in the examination of market milk and for the examinations of individual samples of milk.

## CHAPTER IX.

#### MILK CONTROL.

The sanitary police control of foods has advanced greatly in importance during recent decades. The study of diseases of nutrition in general and the solution of the etiology of these affections have resulted in a recognition of the necessity for the establishment of measures relative to the quality of food substances, and have led to the formulation of laws, ordinances and regulations.

The most extensive development in this relation is shown by the importance of the meat-inspection law, which has been advanced to correspond with the value of meat as human food. Instead of controlling the marketable meat products in the shops, the as immortant part of the inspection is placed at the point of meat production, that is, in the abattoirs. With the exception of the so-called home slaughtered meats, not a single pound of meat is consumed or used for food products in Germany, without being first subjected to inspection.

The meat consumption per capita in Germany in recent years has amounted to from 103 to 110 lbs. In addition to the value of the meat produced, the amount of milk consumed should be considered, there having been made an approximate estimate of an annual production of 7 billion gallons of milk, the smaller portion of which is utilized as drinking milk, the larger part for the manufacture of milk products as cheese, butter, etc.

According to statistical compilations, in 1905 the quantity of milk consumed per capita amounted

> to 106.5 liters (30 gal.) in Berlin

in Munich to 131.5 liters (37 gal.)

in Hamburg to 137.5 liters (38 gal.)

It is gratifying to note that the consumption of milk in Munich has increased during the last decade, and when its nutritive value is considered its low cost as a food stuff is quite apparent. The amount of milk and meat consumed in Munich per capita is as follows:

> 1900: Milk 130 liters (36 gal.) Meat 81.8 kg. (180 lbs.)

> 1904: Milk 131 liters (37 gal.) Meat 75.1 kg. (165 lbs.) 1908: Milk 149 liters (41 gal.) Meat 85.9 kg. (189 lbs.)

and without doubt milk consumption will still continue to increase

if the cost of all other foodstuffs continues to rise. There are no means by which the nutrition of the people could be increased to better advantage than by increasing their consumption of milk, since it has not yet reached the high point warranted by the value of milk as a nutritive substance.

From the various discussion in this work, the importance of supplying consumers with milk of good quality is apparent. An increase in milk consumption is of equal importance to the interest of the nutrition of the people and to the interest of agriculture. This increase however can only be obtained when, in addition to an educational propaganda regarding the nutritive value and cost of milk, care is taken to rectify the generally existing evils attending its production, by which means the milk will be brought up to a standard, which may reasonably be required of any food. Therefore it should not only be unadulterated but must be produced and delivered in a clean manner, in an unspoiled, fresh condition, and possess no disease-producing properties.

The important significance of healthy milk as food for the people, especially for infants, has been emphasized by physicians, hygienists and veterinarians in numerous special articles, which have argued for and against the desirability of gaining nutrition through the use of market milk. Public interest and private philanthropy have accomplished a great deal of good by the establishment of infant milk depots. Such establishments are frequently attached to the abattoirs, and are conducted under the successful direction of veterinarians. It is impossible to enter here into a discussion of such establishments, since this field constitutes only a small branch of the great question of the milk supply.

While the determination of the causes of the so-called diseases of nutrition may not yet be sufficiently clear, the general and local surroundings of the patient or other conditions may influence the course of these diseases. This is particularly true in summer mortalities of children, as in these diseases various conditions, such as want of natural nutrition, faulty housing, etc., may all play a part as factors. Nevertheless, from the experience of specialists the conclusion must be drawn that a strict sanitary police control must be established in order to protect human health as much as possible from the ills occasioned by dangerous milk.

Such harm may result from the consumption of:

1. Milk from diseased animals,

2. Milk originally wholesome but which has been subsequently contaminated with bacteria pathogenic for man,

3. Milk, which has been spoiled by any kind of decomposition, or which is beginning to spoil,

4. Milk containing chemical preserving substances.

These are briefly the points which in themselves prove the value of hygienic control of the milk traffic, and their elimination, with as much consideration as possible for the economic importance of the various factors, must be constantly kept in mind.

A proper execution of sanitary police regulations governing milk traffic is not only of importance for the health of the people but attains even greater value for the milk industry and general agriculture through the indirect advantages which result from their enforcement, such as the improvement of the herds, etc. Measures which are in perfect accord with the hygienic requirements of milk traffic, are at present enforced by most cities, which have adopted various forms of ordinances and laws to cover this subject. Some of the states and the federal government also provide for certain additional control.

The milk control stations of several cities in Germany have attempted to produce an improvement of the milk traffic by the establishment of proper ordinances. Even the best organized stations confine themselves almost entirely to the control of the finished product offered for sale, and therefore they are limited to the examination of samples. If the existing distribution of milk and extension of deliveries in large cities are considered it would require an army of officials to take samples and examine them, in order to test all the milk delivered to ascertain its value as food.

Even in the eventual centralization of the milk traffic, appropriate examination of market milk from a hygienic standpoint will be impossible, since in each shipment too many questions would have to be solved, and besides this, we have not at our command reliable methods for examining the finished market milk rapidly and thoroughly.

The advantages of market milk examinations, which should not be underestimated, consist in the fact that it is possible to detect gross neglect and wilful violations, by which in many cases guilty parties may be held responsible. The knowledge that he is under observation, and the fear of punishment compel even the most indolent milkman and dealer to give increased attention to production and handling, including transportation. In some cities of Germany a great deal has been accomplished in the control of market milk, but an effective improvement is prevented by the existing methods of milk officials. Whatever has been accomplished through the control of market milk, it is slight when compared with the requirements of the law and regulations. If milk control is confined in a one-sided way only to adulteration, preservation and to the dirt content, or to fermentation tests and acid content, as they are mostly practiced, it is hardly possible to expect proper improvement from a hygienic standpoint.

Food chemists have been the chief officials engaged in milk control up to the present time, since the principal stress has been laid on the detection of adulteration or of attempts to improve milk by the use of preservatives. The author considers it as absolutely essential that this field of control should continue in charge of chemists, since the physico-chemical properties of milk require a great amount of special training if the results obtained by examination are to be subjected to critical judgment. This however does not infer that veterinarians, physicians or other

#### Milk Control.

persons who have obtained special training have not the same right to take up the work against violators. It is immaterial who executes the work if it is only carried out properly. Chemists, veterinarians and physicians have their special sphere of activity in milk control, and all should work in harmony that they may accomplish the desired results, each profession exerting all its power towards improvement of the milk supply from the time of its production until its distribution to the consumer. It is deemed advisable to introduce here a short description of milk control in the City of Munich:

The beginning of control in Munich can be traced back to 1834. Police authorities brought to the police physician samples for examination. With the taking over of food inspection by the magistrate in 1862, market inspectors, and in 1876 district inspectors, were detailed to take samples and to make the preliminary examinations under the direction of district veterinarians. The latter were required to carry out the scientific examinations and to pass judgment on the samples.

By an agreement of hygienists, food chemists, dairy experts and agriculturists, the latest local police measures were inaugurated in 1906, and at the same time the inspection forces were reorganized.

The city was divided into six control districts, and the inspectors assigned to these districts had to carry out the requirements of the authorities and the experts relative to the supervision of the milk traffic. The scientific part of the inspection is conducted in the corresponding divisions of the examining station, that is in the chemical and the veterinary bacteriological divisions. Assistant inspectors are assigned to the inspectors for aiding them in the work and for the transportation of the samples. The inspectors are required to supervise the execution of the regulations and to report any violations of these measures to the milk control station. The supervision should be adapted as far as possible to the hours in which the business, sale and operation of the milk establishments are carried on, but may be carried out at all hours of the day and night, and it should be so regulated that the dairies at no time could feel safe from the restraint of supervision. The duty includes:

1. The control of and supervision over all milk brought into the city, all milk which is in traffic within the city, all transportation containers, all dairies, milk shops and production establishments within the city limits, and the taking of samples.

and production establishments within the city limits, and the taking of samples. 2. The procuring of necessary milk samples for examination, both from stable and salesroom, and of other material necessary for evidence.

3. Conducting research work in individual cases, and making out reports and complaints.

All collected samples of milk, samples of other food substances which are sold in the dairy, milk cans which do not correspond with the regulations, various containers in which the milk is kept, measured and sold should be submitted at the milk control station for opinion. A report should be made on the fittings and condition of the rooms and premises where the milk is stored, and from which it is distributed. The transportation of the milk samples to the official milk control station should be accomplished immediately after the sample is procured. A preliminary examination precedes the taking of samples by the inspectors, which consists in an examination by the senses (appearance, odor), and in the use of the lactodensimeter and thermometer. The transmission of the samples to the scientific division is accompanied by forms on which the results of the preliminary tests are indicated, and they also give the date, hour, place of collection, origin of the sample, name of the dealer or producer, number of the sample, and its relation to a certain case.

The samples are immediately examined in the scientific division, the inspectors are informed of the results as soon as possible, and their subsequent procedure in special cases is indicated by the recommendations of the experts and the director of the station.

If stable control and the taking of samples in a stable outside of the city limits appear necessary, or if such are suggested by the scientific workers of the official milk control station, the inspector obtains these samples after obtaining, through the city authorities, permission to go upon the premises. In taking samples in a stable the principal stress must be laid on the dairy management and therefore preliminary tests of the samples taken in the stable are eliminated. The scientific experts attach their

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opinion to the reports of the inspectors, the full report, with the result of the researches, being transmitted through the official control station to the magistrate, who, depending on the case, transmits the material to the proper courts.

The separation of the laboratories into a chemical and a bacteriological division has already been briefly mentioned. The chemical division examines for simple and combined adulteration by the addition of water, removing of the cream, or both, by establishing the specific gravity, the fat contents, calculating the amount of total solids, and the fat-free solid content, determining the **refraction** index of the milk serum by the nitrate test, testing for the degree of acidity and testing for other chemical adulterations. The bacteriological division examines as to fitness for consumption by establishing the purity, odor, taste, consistence, age and freshness, the procedures of each division are kept separate as much as possible, and this separation of the divisions has proven of splendid advantage. Co-operation between these divisions when the work overlaps, and mutual support aid in the success.

Owing to the activity of the official milk control station it was soon noticed that marked adulterations had become very rare, and that objections and condemnations on account of gross contamination were reduced to a minimum. Considerable objection still exists relative to the transportation cans which are frequently used in a most insanitary condition. The regulations relative to the proper closing of the cans are now almost uniformly observed. The acid content of the milk is only exceptionally increased by fermentation, and the spoiled milk originates usually from milk collecting establishments and cheese factories, whereas individual producers as a rule supply fresh milk. The increased degree of acidity is traced in most instances to improper cooling, dirty transportation cans, mixing of fresh and old milk and adulteration with skimmed milk. Preserving agents scarcely ever come into consideration in Munich.

It is to be regretted, however, that the limits of milk control activities have apparently been attained, in so far as they concern the testing of milk ready for consumption. Nevertheless, attempts have been made by extending the control to the stable and to the producing animal in order to further improve the milk supply. In certain cases good results have been obtained through giving instructions and warnings as to the requirements, or at least in advising the adoption of all precautions which are possible in practice, for instance in the streptococcic mastitis question, which this city was first to take up on practical lines on a large scale. The results attained are by no means to be underestimated, but while there is no doubt that with the hard battles considerable results have been obtained for the moment among a small percentage of the producers, still no one can offer a guarantee that even on the morrow the same conditions will not prevail as formerly, and in this lies the insufficiency of market milk control and of the system of taking samples from time to time. In the future other measures will have to be given consideration in sanitary milk inspection, if it is desired that conditions which are frequently intolerable, and which prevail at present in the milk industry, should be eliminated.

A guarantee of good and harmless quality for market milk

forms an absolute hygienic requirement, and at the same time it is the prerequisite for increasing milk consumption to its full extent. This can be attained only through strict regulation and flawless supervision of the milk from the beginning of its production up to the time of its delivery, taking advantage of the great progress which has been made by science in recent times.

Milk hygiene must commence in the stable. A perceptible step in advance is gained by the introduction of stable supervision. The preliminary requirements for the production of unobjectionable milk are healthy milking animals, healthy udders. healthy milkers and clean utensils and surroundings. In this instance the veterinarian is the proper counsellor, his preliminary training offering the necessary assurance that these requirements for well-managed dairy business will be fulfilled. Besides the examination of the health of the cows, the supervision must be extended to the care of the animals, stable conditions, and the keeping and feeding of the animals. The necessity for the most stringent cleanliness in milking and for the careful preparation of the milk by means of filtration, and cooling must always be impressed upon the dairyman, as well as the necessity for satisfactory transportation.

Some hygienists consider the processes of decomposition brought on by contamination and improper treatment of milk as especially important causes for the rapid spoiling of milk. The author considers that their special significance should be laid, in cases of milk poisoning the same as in meat poisoning, to those disease-producers and their products which prove toxic in the animal body, and which originate in the milk-producing animal. The veterinarians prove their value in sanitary police supervision of milk production, by seeing that the cities are supplied with good milk, suitable as food for infants, and by watching the dairy industrv. This supervision at the site of production produces better results than the most painstaking and well organized inspection of the finished product. The great dangers which threaten man through the causative agents of septic metritis, acute and chronic mastitis, enteritis, etc., are considerably reduced. The control of the milk traffic and milk industry requires especially the cooperation of all factors which come into consideration. Until uniform regulations for sanitary police supervision are established it will remain the duty of veterinarians and physicians to point out the importance of hygienic measures to the producers through continuous education, indicating also the economic advantages which may be gained for their own interests. In northern Bavaria the supervision of stables, dairies and distributing stations has already been inaugurated by the employment of district dairy inspectors.

Through periodical stable inspections considerable advancement could be made at the present time. This supervision should not only include the so-called certified milk or infants' milk, but also the production of all milk consumed, since the largest proportion of the parents of infants and consumers in general cannot purchase certified milk, and the children of this class who cannot afford to buy certified milk are the ones principally exposed to the dangers of infant mortality. Such classifications of milk may be of advantage to the milk trade, but they must not be taken into consideration by sanitary officials who are supervising the milk traffic. We are clear with regard to the ultimate aims which we must bear in mind in the sanitary police supervision; whether these aims will ever be realized is a question of economic and social conditions. At the present time the attainment of the ideal goal of flawless supervision of milk from its production to its consumption is made very difficult by these very conditions. As long as the cheapness of milk as a food product for the masses stands in the foreground in the interest of the people, a place in which it actually must stand, a proper, thoroughly organized control of the production can hardly be inaugurated.

Recommendations for such control have been made by Meinert and others, and recently by Schern. For an effective execution of control over the production of milk, supervision must be established in both city and country. Milk is produced not only in the country but also in the city. All milk produced must be subjected to uniform control. Within these districts of control the milk-producing cities should therefore be included. A veterinarian periodically examines the dairy herds and the milk of each animal, the individual animal in these districts, etc., without previously giving notice to the owner of the animals as to the time of the inspecton. In this inspection the milkers are also observed as to their state of health. The procured milk is examined to see whether it is clean and sufficiently cooled. The stables are examined to determine whether they meet the requirements as to light, ventilation and cleanliness. The control of production is linked with the supervision of transportation in certain milk-collecting places, and finally the inspection of the dealers at the place of consumption follows. Such a complete supervision is not considered possible in practice without considerable increase in the cost of the product, and this should and must be avoided. The sanitary milk officials will have as their most important duty the finding of ways and means for the practical execution of supervision, which may be accomplished without great economic losses of production and efficiency, and without injuring the other factors in the milk industry. Meinert believes in the possibility of supervising the places of production under supervision of the state, by the appointment of physicians, veterinarians and practical agriculturists for this purpose.

For each township the milk producers should select trustworthy men as supervisors, who by means of frequent examinations at the time of milking should convince themselves of the manner in which the requirements of the legislative measures are being observed. The activity of these supervisors should be principally along educational lines. These men should call the attention of the owners to existing deficiencies in the management of the dairy, they should offer remedies to eliminate these deficiencies, and after the lapse of a certain time, they should satisfy themselves that their advice has been carried out. In case of disease of the dairy cattle it should be reported to a veterinarian for consultation and judgment. The supervisors should be instructed as to their duties by the official veterinarians. Creamery corporations could select their own supervisors instead of depending on the local supervisors for the inspection of the dairies producing milk for their plants. Larger establishments could voluntarily subject themselves to direct state control. Besides this practical supervision, the district veterinarian should examine the animals every three months as to their condition of health, and judge the character of their milk. Persons who are connected with the procuring and handling of milk should be placed under the control of an official physician. The producers, supervisors, milkers, and all persons connected with the dairy should be examined as to the possibility of their transmitting human diseases to the consumer through the milk acting as an intermediate host, and the supervisors should report their observations as to any sickness among the attendants to the physician in authority. The entire system is subject to control by the state, which appoints its own officials for larger districts to carry out the supervision of the work.

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It must always be considered that the populace has an interest not only in the procurance of unadulterated and unspoiled milk, but also in having the milk marketed at such a price that it may remain accessible as a product of consumption for the masses, and if possible its consumption should be increased. Accordingly too far-reaching, stringent requirements should be avoided, as well as all requirements that cannot be met by the prevailing condition of production, on account of financial and technical grounds. Healthy dairy cattle and the best possible cleanliness of the stables and surroundings are more important than special feeding regulations, or requirements for stable buildings which the small farmer is not in a position to adopt. In order to prevent too stringent, or onesided requirements altogether, the adoption of a uniform standard would be necessary for the entire country, and each state could establish its own regulations which would conform with the conditions of that particular locality. The introduction of the terms "inferior value" and "conditionally passed" for certain low grades of milk would reduce the economic loss which results from the use of the terms "spoiled," and "injurious to health."

If stable inspection is inaugurated, a thorough organization may conduct a supervision by which the owners of small herds can also comply with the requirements. The present system of milk inspection not infrequently fails to include such small dairies because the supervision of the entire milk traffic is extremely difficult, but with the introduction of stable inspection the enforcement of hygienic requirements that shall include the small producer will be found to be not only practicable, but also very desirable. Dairy associations and contractors with co-operative creameries should regulate fluctuations of deliveries and consumption, so as to provide the best possible utilization of the excess of production, or the milk which has been declared by the city inspectors as unfit for drinking purposes may be conditionally passed if designated as inferior milk.

Such a system has been adopted by the dairy association of Hamburg, in order to meet the economic losses caused by the stringent enforcement of the milk inspection regulations.

The author believes that with legislative regulation of milk inspection, and especially with supervision of the production, it will be possible, even with the newly created conditions, to supply the population with good, clean, wholesome milk at relatively low prices. With good will and co-operative work, as well as with considerate enforcement of the regulations, the desired goal may possibly be reached within a short period of time.

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# Chapter X.

# MILK INSPECTION.

## (a) Taking of Samples.

1. Market Milk. The taking of the sample must take place only after the fat of the cream gathered during transportation has been sufficiently distributed through shaking or stirring. Especial attention is required when separation has occurred through freezing.

2. Stable Samples. The inspector who takes the sample must have his whole attention directed to the production and handling of the milk, as the people suspected of adulteration often display unbelievable slyness in order to deceive the inspector. Particular care should be taken that the mixing tank or vat does not leak, that the milk pails and other vessels do not contain wash water, and that, during the milking, the milk is not adulterated with water from bottles hidden in the clothing of the milkers; it is important that the milking should be complete.

If nitrate has been found in a suspected sample, a water test for nitrates should be made from each well at the place of production.

Only after milking, are inquiries to be made regarding the feeding, keeping, care, and condition of the individual animals, etc. The stable samples must be thoroughly mixed.

3. At least  $\frac{1}{2}$  liter should be taken from each market or stable sample, in order to have sufficient material for all examinations.

4. When the samples have to be carried a long distance to the place of examination, they must be preserved with 0.1% formalin. For chemical examinations 0.1% of potassium bichromate is permissible, which is obtained by the addition of a 1% solution to 100 parts of milk. The addition must always be stated.

For bacteriological examinations, the preservation of the sample is not permitted, except in cases where a microscopical examination only is desired for the determination of inflammations of the udder or the presence of tuberculosis. 5. The sample bottles must be filled up to the neck, in order to prevent the formation of butter during transportation.

6. The bottles should be closed so that an unauthorized opening is excluded.

The transportation to the place of examination must take place as soon as possible after the taking of the samples, and if transported by rail, sufficient packing should be provided to prevent breakage of the bottles.

If samples are desired from individual cows and not market or mixed milk samples, smaller amounts, for instance, 100 gms. or less, are sufficient, provided that only an examination for inflammation of the udder is involved. With samples taken for chemical examination from individual cows, a complete milking is necessary for satisfactory results. If examinations as to changes of milk through diseases are to be made, at least 1/2 liter must be sent in from each milking until the day after recovery. In certain cases, for instance, with emergency inoculation in footand-mouth disease, the taking of samples must begin before the inoculation and continue until complete recovery.

When examinations for inflammation of the udder are desired, the sample of milk may be poured into a reagent glass by means of a dipper, which must be thoroughly cleansed after each sample, or, the samples are taken in such a manner that in pouring the milk from each cow from the milk pail into the cooler, the opening of the reagent glass is kept in a position to fill the glass.

In taking samples from each quarter, it is advisable to milk the secretion of each quarter in a cleanly manner into the reagent glass. The sample is taken from the middle milking, that is after the quarter has been partially milked. All samples have to be accurately marked according to cow and quarter.

In protracted examinations, for instance, for the detection of tubercle bacilli, it is recommended to divide the animals of large herds into groups, and to collect the milk from each five or ten cows into a sample bottle.

For the determination of dirt in milk, which rapidly sinks to the bottom, it is recommended to take an average sample from the well-mixed milk, and allow the sediment to settle in a separate container, which is examined after the milk is poured off.

After arrival at the laboratory, the samples must be examined as soon as possible; however, until the examination, they must be kept in the ice-box or in the cooler.

#### (b) Examination of Milk.

The veterinarian may have to perform the preliminary testing of milk as to adulterations, but he will especially have to consider the changes in milk which are caused by animal diseases, inflammation of the udder, or he has to pass judgment on possible changes caused by certain external or internal influences. A final opinion should never be given, except after a most intimate knowledge of the special conditions.

Milk mixed from many cows has to be judged differently from milk of one or a small number of cows, because in the latter case the fluctuation through internal or external influences may be very marked, whereas the presence of abnormal secretions from one or several cows is either modified or concealed through mixing their milk with that from many healthy cows.

An adulteration should never be established or the degree of adulteration calculated, without making comparative tests of satisfactory samples from the same source. A definite diagnosis of the degree of adulteration based merely on the values of accepted averages, normal or experimental, would be erroneous, of which no scientific milk inspector should be guilty.

Before the beginning of the examination, each sample should be sufficiently mixed by shaking, without having any considerable amount of air shaken into the milk.

The testing of milk is divided into the preliminary examination and the special scientific examination.

Through tests by means of the senses milk is first examined as to the color. Adulterated market milk is often bluish, and secretions from animals with udder diseases often make the milk reddish.

The odor is determined either immediately after emptying the cans or in the laboratory by heating the milk in glass beakers up to the formation of steam. The odor of good and palatable market milk may even vary considerably. If, however, special odors are very conspicuous, the presence of certain milk defects must be considered, possibly as the result of bacterial action.

The consistence of the milk should not be too thin or watery, (suspicion of adulteration), and neither should it be sticky, slimy, greasy or curdling, which changes indicate diseases, particularly udder affections, or the presence of certain bacteria of milk which have propagated since the milk was secreted.

A fine vesicular foam appearing after shaking should rapidly become large bubbles and disappear. The remaining fine vesicular foam is the result of shaking soapy milk.

The taste is to be judged in the same manner as the odor. The milk should be delivered and sold in a cold condition, and therefore in the collection of all samples of milk the *temperature* should be ascertained.

With market milk the positive result of the boiling test, that is when the milk curdles, is a sign of advanced decomposition (10 to 12 degrees of acidity according to Henkel-Soxhlet).

With the fresh milk of individual cows, curdling after boiling indicates inflammation of the udder, the curdling being mostly limited to the milk of individual quarters or to the colostral milk at the beginning or at the end of lactation. The **alcohol test** also curdles market milk which has become spoiled (8 to 9 degrees of acidity).

A positive reaction with fresh samples from individual cows or with samples from separate quarters indicates either a physiological or severe pathological inflammatory condition of the milk glands. The milk is mixed with an equal amount of 68% alcohol.

Recently, the **alizarol test** has been recommended for the determination of spoiled milk. Milk mixed with an equal part of alizarol becomes brownish violet, as long as it is fresh and not spoiled; otherwise the color turns brown and yellow, and the milk curdles, with the formation of thick flakes.

For the control of market milk or for the diagnosis of udder diseases the author found that the alizarol test (milk with 68% alcohol and as much alizarin as is soluble) is without any value in testing samples of individual cows or quarters.

The degree of **acidity of the milk** is established by titration with standard alkali.

1. According to Henkel-Soxhlet, with  $\frac{1}{4}$  normal sodium hydrate solution, fresh market milk has about 6.0 degree of acidity.

2. According to Thörner and Pfeifer, with  $\frac{1}{10}$  normal sodium hydrate solution in 10 c. c. of milk and 20 or 40 c. c. of water, respectively, with 5 drops of a 2% solution of phenolphthalein (the figures thus obtained are multiplied by 10) fresh milk has about 18 degrees of acidity.

According to Henkel-Soxhlet, the acidity is determined by titrating 50 c. c. of milk, to which 2 c. c. of a 2% alcoholic solution of phenolphthalein have been added, to a faint but permanent pink. Each  $\frac{1}{2}$  c. c. of alkali ( $\frac{1}{4}$  normal sodium hydrate) corresponds to a degree of acidity.

Schern utilizes 10 c. c. of milk and titrates drop by drop with 1/40 normal sodium hydrate solution after having added 1 to 2 drops of the solution of phenolphthalein. The titration takes place in a mixing cylinder which is so graduated that the difference of the level of the fluid before and after the titration shows the degree of acidity.

An increased degree of acidity in market milk, for instance, 7 or more after the method of Henkel-Soxhlet, does not always indicate a spoiled condition. This can only be presumed when the increase of acidity in a certain time and at a certain temperature is very rapid, in other words, when the curve of acidity is abrupt. After 12 to 24 hours the degree of acidity is again determined. Fresh milk, kept at 20 degrees C., shows from 10 to 15 to 20 degrees of acidity after 24 hours. Milk at the end of the incubation period, before bacterial multiplication begins, has 25 to 30 degrees of acidity, and old milk 30 to 40 degrees. The increase in degrees of acidity between fresh milk and older milk is caused by the formation of lactic acid. One c. c. of  $\frac{1}{10}$  normal sodium hydrate=9 mg. lactic acid. In spite of this, the degrees of acidity of Henkel-Soxhlet cannot be computed into the degrees of acidity of Pfeifer because through dilution with water the solution of slightly soluble phosphates decreases the degrees of acidity obtained, and, therefore, the degrees of acidity of Thörner and Pfeifer show lower values than those of Henkel-Soxhlet.

Milk from individual cows often have greatly decreased or increased degrees of acidity. The decreased, or, more rarely, increased degree of acidity of a single sample creates the suspicion that the cow is suffering from udder disease. High acidity of all four quarters is present with colostrum and in milk of fresh cows. The milk from cows at later periods of lactation is frequently alkaline and has a lower degree of acidity.

Dropped on litmus paper, market milk shows an amphoteric reaction. Alkaline reactions of single samples must be judged the same as low degrees of acidity. If market milk shows an alkaline reaction, an alkali may have been added for preservation. The reaction of market milk is acid to rosolic acid. On the addition of alkali to milk, the milk, upon adding rosolic acid-alcohol, turns rose red. With fresh single samples of milk the red color after the addition of rosolic acid-alcohol is an indication of the presence of inflammation of the udder; cows in the late periods of lactation may also show red coloring of the milk.

To lacmoid the milk is alkaline, and also to dimethyl orange. The so-called Höyberg test to determine "fibrin and pus" in samples of milk from individual cows is based on the difference in the reaction of the milk.

The test is conducted so that 5 c. c. milk (individual cows or quarters) are mixed with 5.5 c. c. solution of rosolic acid, which is prepared from 0.45 c. c. of a 1% solution 5 c. c. plus alcohol.

A positive reaction to the test creates suspicion while a negative result does not exclude it.

As the reaction of milk from diseased quarters frequently is perfectly normal or acid, the test does not compare with the Trommsdorff test, and especially the microscopical examination. Besides, it is very difficult to distinguish the fine differences in the color shades.

The determination of the alkalinity of milk with  $\frac{1}{10}$  normal acid has so far not been adopted in practice.

The dirt content of milk should not be weighed, as recommended by Renk, since the amount of visible foreign material should not determine the disposition, but rather its quality should be considered. Besides, much dirt is dissolved in the milk, which neither can be determined through filtration nor through weighing the filters. The amount of dirt is estimated in degrees, through the sedimentation method or through filtration, and the quality is thus determined; as a rule it represents remnants of feed, feed dust, portions of litter, manure of cows, cow hairs, etc. From the uniformly fine or coarse particles of dirt in milk, or from the presence of cow hair in large amounts and larger particles of dirt, it may be determined whether the dirty milk has been strained after the milking.

The filtration methods in which disks of cotton are used as filters have an advantage in that they indicate more distinctly the actual content of dirt than the sedimentation methods, where a considerable proportion of the dirt is drawn up into the cream during the separation. The author uses an apparatus in which a disk of cotton is held in a simple plate-shaped filter, over the vessel into which the milk is to be poured. The cotton disk is pressed into the filter by a glass cylinder, as is the case with the apparatus of Fliegel and Bernstein. At the present time such dirt testing apparatus may be purchased from nearly all dealers. For the household and for small amounts of milk certain filters are recommended like those in which the filling funnel represents a bottomless bottle, to the mouth of which a ring and a wire strainer containing a disk of cotton are attached, by means of a wire fastener.

Henkel's control filter is also based on the principle of filtration through cotton by which an angle-shaped segment of the filtering disk remains free from dirt in order to control the purity of the milk. The same result is attained with other methods where the border of the filtering disk remains free from dirt.

At the places of official examination of milk distinction is made between slight, moderate, strong, very strong, and exceptionally strong pollution, and the milk accordingly is judged as either clean or spoiled or even injurious to health.

Market milk may be tested at receiving stations either by drawing up samples from the bottom of the cans with the aid of long pipettes, or, as is customary in Munich, by pouring the milk from the original can into another vessel. The residue of the milk in the first case is taken as a sediment sample, while an average sample is taken from the mixed milk of the second container in order to make a quantitative estimation.

Trommsdorff's test is splendidly adapted to the detection of finely divided particles of dirt, the heavy particles being collected in a capillary tube.

The methylene blue reductase test gives very good information relative to bacterial multiplication. A solution of methylene blue in water, serves as a reagent, consisting of 195 parts  $\dot{H}_2O$  and 5 parts saturated alcoholic methylene blue solution. The test is conducted by placing 20 c. c. of milk and 1 c. c. of methylene blue solution in a reagent glass at 40° C. and the time is determined in which the sky blue mixture becomes completely white. Milk which becomes white in less than 3 hours is already old. The age, however, does not refer to the hours since its production, but means that the milk has "aged." Milk which is obtained in a dirty condition, has not been cooled, and has been transported in poorly cleaned cans, ages more rapidly than milk which has been properly treated.

In making the test it is not necessary to cover the sample in the reagent glass with boiled oil or kerosene since there is no advantage in such a procedure. In the same way the "reductase" which is recommended by commercial firms is of no advantage.

Fresh milk from individual cows may be rapidly reduced owing to the large content of cells.

Very valuable results are obtained with the reductase test when conducted in connection with the microscopic examination of the sediment.

Frequently, in the testing of the centrifugal sediment, large numbers of bacteria are found not infrequently agglutinated in colonies, and the milk, in spite of the apparently high content of bacteria, has very little reducing power. This is an indication that the milk has been transported in uncleaned cans, but does not in itself prove decomposition.

Microscopically in the residue of milk in the can are found milk souring bacteria, diplococci, streptococci, sarcines, besides oidia, coli, and rods.

Such is also the case when the milk is obtained under dirty conditions, but is promptly delivered.

The author determines the reduction property of the milk in the following manner:

For each sample of milk 10 small tubes are used containing 1, 2, 3, 4, up to 10 drops of methylene blue solution, respectively. Into each tube 5 c. c. of milk is added. After  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1, 2 hours, etc., observations are made as to what extent the milk has been reduced. This method, in spite of its apparently greater technique is nevertheless quite simple, as the constant watching of the samples in order to determine the time is unnecessary. Both time and degree are determined. If, for instance, a milk is reduced in three hours to tube 8, then the formula will be: R3=8. Good milk reduces the first 2 tubes only after 2 to 3 hours; fresh milk only after 10 to 12 hours.

The Schardinger reduction of formalin methylene blue is of no value for the examination of market milk, as it also gives positive results with fresh, raw, and boiled spoiled milk. In the same way it is not suited for examination of milk from individual cows. Milk, which does not decolorize in a few minutes at  $60^{\circ}$  C. by the Schardinger reagent, consisting of 190 parts water, 5 parts formalin and 5 parts saturated alcoholic methylene blue solution, may be from fresh cows, if the individual sample is taken from the total amount of milk of the cow (the first portion of milk at the milking does not reduce).

The "Catalase" test is conducted by mixing together 15 c. c. of milk and 5 c. c. of 1% peroxide of hydrogen. The mixture is placed in a fermentation tube such as is used in the examination of wine for the determination of sugar, and kept for 2 hours in an incubator, and after this period the amount of oxygen formed during this time is measured. It should not exceed more than about 1 c. c.

Apparatuses which indicate the total amount of gas formed are more suitable; for instance, the "Catalaser" constructed by Henkel, or still better the one by Lobeck, in which a gas collecting and measuring tube is so attached above the bulb containing the milk that a fine gas tube leading from the bulb to the upper part of the gas measuring apparatus allows the exit of the oxygen at the 0 point. At the bottom of the gas collecting tube another tube opens, through which the water contained in the measuring tube is displaced by the liberated oxygen. Faitelowitz has constructed a shaking apparatus and a special "Catalaser"; the shaking is supposed to expedite the liberation of the oxygen.

Fresh milk evolves 1 to 2 c. c. of oxygen. Raw or pasteurized milk, spoiled through invasion of bacteria, produces considerably larger amounts of oxygen; likewise milk rich in cellular elements as a result of physiological or pathological irritations of the udder, or mixed milk polluted by such secretions. The test is useless for the examination of market milk as to the presence of inflammations of the udder.

High oxygen values of the catalase test in connection with low reductase values against the watery methylene blue, create a suspicion of mastitis.

High values by both methods indicate principally a spoiled condition, without differential diagnostic value relative to inflammation of the udder or bacterial decomposition. If the testing of the milk indicates that the product was pasteurized, sterilized or otherwise heated, and the catalase test is positive, the generation of oxygen proves the spoiled condition of the milk as a result of bacterial decomposition.

With fresh individual samples and samples from individual quarters the increased value of catalase proves the presence of mastitis, provided that no severe general diseases are present, and provided physiological irritating conditions are excluded. If all samples from the 4 quarters show increased catalase values, mastitis may be present in all 4 quarters, or there may exist a general disease, as for instance tuberculosis or peritonitis.

The test for **amylase** is only applicable to raw milk. Into each of ten test tubes are placed 10 c. c. of milk and 0.1, 0.2 up to 1 c. c. of a 1% solution of soluble starch which is dissolved through heating. The series of tubes are placed for half an hour in the incubator. Then they are rapidly cooled, and to each is added 1 c. c. of a solution of iodine and iodide of potassium (1 of iodine, 2 of iodide of potassium, 300 of water). If the total amount of starch has been converted into sugar, the color of the mixture will be yellow. A grayish-yellow with a grayish-blue tinge indicates unchanged residues of starch. Generally only tubes 1 and 2 but sometimes tube 3 become yellow.

In the presence of large amounts of amylase, which usually runs parallel with the cellular contents, even the other tubes will appear yellow. An increased amount of amylase indicates physiological or pathological irritation of the udder. The raw condition of the milk is tested by the determination of peroxydase. There are used either guaiac tinctures, the efficacy of which has been tested, or still better mixtures of guaiac-guaiacol with peroxide of hydrogen, for instance, resina guaiaci 10.0, guaiacol 10.0, 3% perhydrol quantum satis, absolute alcohol 80.0 (Schern).

Raw milk becomes blue, heated milk turns yellow. The Rothenfuss reagent is very reliable and is also recommended on account of its keeping qualities.

First solution: 1 gm. paraphenylendiamin hydrochloride, 15 c. c. water.

Second solution: 2 gm. crystallized guaiacol, 135 c. c. 96% alcohol.

After dissolving, both are mixed together which results in a white or whitish-yellow reagent. For the execution of the test a 0.2% solution of peroxide of hydrogen is also essential. The milk to be tested is mixed with a few drops of a solution of peroxide of hydrogen, and then the reagent is added. Raw milk at once becomes intensely violet, while milk heated to over  $80^{\circ}$  C. remains white.

The reaction is prettier and more distinct when instead of milk, milk serum is used, which is prepared in the following manner:

100 c. c. of milk is mixed with 6 to 12 c. c. of lead acetate solution, strongly shaken, and filtered through a folded filter. At the plane of contact of the serum with the reagent, a violet ring appears if the milk is raw.

If the reaction does not appear, and the lead-acetate-serum becomes turbid through boiling, the milk has been heated above 80° C. and probably below the boiling temperature, which however was surely reached when on boiling of the serum no more albumen is precipitated.

The fermentation test has less importance for the examination of milk to be consumed than of milk to be utilized for the manufacture of cheese.

The milk is filled into tall, wide test tubes and the latter are placed for 24 hours in the incubator at 38-40° C.

Fresh milk does not curdle after 12 hours; curdled milk should have a pure sour odor and taste, and at the same time a porcelainlike, scaly, coagulum with only a few gas bubbles. Many gas bubbles and fissures in the coagulum indicate the presence of aerogenes-coli and other bacteria which split up the milk sugar with the formation of gas. A cheese-like curd develops as a result of the presence of rennet producing species of bacteria, which are peptonizing bacteria, the presence of which is undesirable in drinking milk. Not infrequently the best milk produces imperfect curds.

Milk from diseased animals curdles more poorly, with the formation of an abnormal curd.

Still less important than the fermentation test is the rennet fermentation test which is used in cheese factories, where the milk, before being placed in the incubator is mixed with a solution of rennet. The resulting curd should be elongated and wormshaped, contain few gas bubbles, and should not look twisted or pressed flat or swollen.

The rennet inhibitory test recently recommended by Schern accomplishes other purposes than the rennet fermentation test. It tests the power of resistance of the milk against the effect of the rennet. For the test the following are necessary:

1. A number of test tubes.

2. Measuring pipettes of 10 c. c. capacity with  $\frac{1}{2}$  c. c. graduation and 1 c. c. pipettes divided in tenths and hundredths of c. c. graduations.

3. A water bath or an incubator with a number of perforated racks.

4. An icebox.

5. 0.85% solution of common salt.

6. Solutions of rennet, the values of which are known and which remain constant (standard solutions of a known titer).

In the performance of the test it is desired to ascertain:

1. Whether the titer of the solution of rennet still persists with sound milk.

2. Whether the milk to be tested by means of the rennet titer does not curdle, or how much more rennet is necessary to make the milk curdle.

The samples are placed for 1 hour in the icebox, and then for 2 hours in the incubator, whereupon through pouring, a test is made as to which dilution of rennet has curdled the milk or whether the milk curdles at all up to the limit of titration.

The test is not applicable to market milk, but only for fresh individual samples or samples of milk from individual quarters.

Milk which utilizes considerably more of the rennet solution than the amount which corresponds with its titer is suspected of not being normal.

The test is too laborious for practical control work and does not offer any advantages for the recognition of inflammation of the udder over the microscopical examination of the centrifugal sediment.

The methods by which the milk is examined for the content of complement or amboceptor have the same shortcomings. They are of no importance in control work.

The test for complement is as follows:

1. 5% suspension of washed blood corpuscles from guinea pigs or rabbits in 0.85% salt solution.

2. Hemolytic amboceptor of normal blood from cattle or goats heated to 56° C.

3. Milk.

The milk is placed in tubes arranged in 2 rows of 5 tubes each in quantities of 1.0, 0.5, 0.25, 0.1 and 0.0 c. c. respectively. One row is inactivated by heating to 56° C.; then in all the tubes the contents are brought up to 1 c. c. by adding salt solution. Further, to each tube are added 0.2 c. c. of the inactivated cattle or goat serum and 0.5 or 1 c. c. of the blood-cell suspension. The rack is then placed for 2 hours in the incubator (shaken frequently) and placed over night in the icebox.

Hemolysis occurs in physiological and pathological irritations of the udder.

The test for amboceptors is carried out in a similar manner, with the exception that the milk in all the tubes is inactivated, and into the tubes of one row complement is added in quantities determined by titration.

In the test for amboceptor and complement the various substances which enter into the test should be controlled for possible errors.

Trommsdorff's method is best adapted to determining the quantity of centrifugal sediment in milk.

The tubes which terminate at the bottom in a graduated capillary tube (Trommsdorff's tubes) are filled with 10 c. c. of milk and centrifugalized for several minutes in a centrifuge at about 1500 to 2000 revolutions per minute. All elements having the greatest specific gravity collect in the capillary tube.

All sediment of a yellow, clay or reddish color which does not consist of cow manure and which is sharply separated from the layer of skimmed milk irrespective of its quantity, should be suspected as being due to an inflammation of the udder, since larger quantities of tissue cells are thrown off only in pathological or physiological irritations of the udder. In market milk this test gives uncertain results, but in individual samples and in samples of individual quarters the results may be well utilized. If the centrifugalized sediment is not distinctly separated from the skimmed milk, and the scale is therefore not readable, then the tube is filled with clear, cool water and the capillary end is turned upward. The water having a lower specific gravity, penetrates into the capillary tube up to the border of the sediment.

For testing of individual quarters the author recommends the sedimentation test in tubes with chisel shaped ends and with funnel shaped mouths. The milk is drawn directly into these tubes from the quarter, after discarding the first milk. The four samples from a cow are allowed to stand for about 8 hours and then examined for the presence of sediment. Sediment, which apparently does not consist of cow manure, indicates an inflammation of the udder, provided the colostral stage has passed and the animal is not close to the end of its lactation period.

The sedimentation test may be and should be undertaken by every dairyman. Its application is easy. Milk which separates recognizable quantities of sediment should not be sold or used as food for man.

In the scientific examination of market milk and of individual samples, the microscopical study of the milk can no longer be neglected. In Munich the sediment of the milk is examined microscopically. A platinum-wire loop (the wire must be completely folded) is passed into the depth of the capillary tubes and without turning, the sediment is lifted out by pressing the wire against one side and drawing out a loopful. In the procedure the contact of the wire with the milk or cream of the tube should be prevented. The smear is made in the usual manner; the best way is to place the loop of the wire on the slide and by lifting it up a somewhat thicker droplet remains at the place of contact. This may be spread over the slide and is especially well adapted for microscopical examination.

The smear is dried in the air, fixed by heat or alcohol and stained by the ordinary methods. For staining, the author recommends a thionin solution which consists of  $\frac{1}{3}$  concentrated alcoholic thionin solution and  $\frac{2}{3}$  distilled water. In market milk are observed many plant fibers and plant cells, plant hairs, staphylococci united into colonies, colon varieties, acid fast rods, sarcina, blackleg and anthrax-like bacteria with or without spores, if the milk was produced under dirty conditions. If such a milk sample is older, not infrequently mycelial threads germinate from the mould spores. If the milk is bad and was transported in dirty cans, besides the colon-like bacteria, staphylococci, diplococci, streptococci, sarcina, oidia and cheese bacteria may be seen.

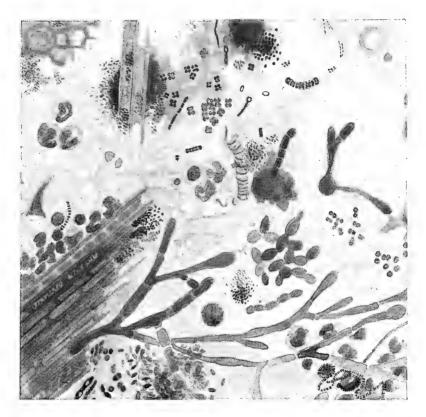
If the milk is at the point of decomposition, diplococci and streptococci dominate the field.

A diagnosis that milk is mixed with the secretion of a cow affected with streptococcic mastitis is only permissible, when besides the cells from the animal body (leucocytes, epithelia undergoing fatty degeneration, erythocytes, etc.), the animal forms of streptococci are demonstrated.

The positive finding is decisive but the negative does not exclude. Market milk which, besides numerous leucocytes, shows no animal streptococci, only creates a suspicion that the secretion is from cows with affected udders.

The increased amount of horny epithelia of the teats in samples of individual cows indicates the fresh milking period of the animal.

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Sediment of dirty market milk which contains the secretions of cows with affected udders, and which has been transported in filthy cans. Thionin stain.  $1\times1000.$ 



The presence of epithelial nests from the cistern suggests a catarrh of the cistern.

An increased number of leucocytes and colostral cells indicates an irritation of the parenchyma. The causes of the irritations are often manifested by the presence of diplococci in phagocytes or isolated diplococci and frequently atypical short forms, or the typical animal forms of the *Streptococcus longus* or *S. brevis* are recognized.

Even atypical forms of diplococci and streptococci, when present in such forms in milk only a few hours old, are an indication of streptococcic mastitis (care must be exercised in the absence of experience and if the evidence is to be used in court).

In pyobacillosis the typical, short, slender bacilli are found which simulate in their morphology the bacilli which frequently cover the horny cells of the outside skin of the teats.

In samples from individual cows, the microscopical examination may establish the diagnosis of "tuberculosis of the udder," when the specific organisms are found enclosed in leucocytes or curds. The staining is carried out with hot carbol-fuchsin solution (1 part fuchsin to 10 parts of absolute alcohol, and 90 parts of a 5% carbolic acid solution). By decolorizing with 33% aqueous nitric acid and subsequent washing with alcohol the non-acid fast rods and the body cells are decolorized. The decolorized elements may be given a blue contrast stain, against the red tubercle bacilli by subsequent staining of the preparation with aqueous methylene blue or methylene blue anilin water.

In order to avoid the dragging of too many tubercle bacilli into the cream by the cells and fat globules, for the microscopical examinations, the milk should be first homogenized. The following methods are the simplest:

a. Knut Arnell recommends mixing 25 c. c. of milk with 2 c. c. of concentrated ammonia and 100 c. c. of a mixture of equal parts of ether and petroleum ether in a sedimentation cylinder, which runs to a point at the bottom and at its lower part is supplied with a stopcock. This is frequently shaken and then allowed to stand for the separation. The ammonia-case solution is then drawn off, the remaining content is centrifugalized and the sediment examined.

b. Thörner recommends mixing 20 c. c. of milk with 1 c. c. of 50% potassium hydrate. This is heated in boiling water until in complete solution and then centrifugalized.

c. Biedert recommends adding 10 c. c. of milk to 1000 c. c. of water containing 4 to 8 drops of sodium hydrate solution. This is shaken, boiled and then set away for sedimentation.

If after staining, the slender rods which remain red are present only in small numbers, or if the sediment shows no cells which indicate an inflammation of the udder, or if the examined milk proves to be a dirty market milk, then the diagnosis must be established by incubation, since the milk may contain from the feed, etc., only harmless, acid fast rods.

The inoculation is made into guinea pigs by injecting them either subcutaneously or intramuscularly in the hind leg with either 1 c. c. of the full milk, or better, with the centrifugal sediment mixed with a small amount of the skimmed milk with or without cream. The sediment should be obtained by using rapidly revolving electric centrifuges.

If the samples have to be transported long distances, they should be mixed before transportation, and if possible immediately after drawing the milk, with 1:2000 to 1:3000 of formalin (boric acid 1:50 or 1:100 is also satisfactory). The tubercle bacilli which are protected by a waxy capsule from the effects of the preserving agents, are not harmed by such preservation to such an extent that they could no longer be demonstrated by inoculations.

For each milk injection at least two guinea pigs should be used, since occasionally the inoculated animals die as a result of some other intercurrent disease.

In the presence of tubercle bacilli the regional lymph glands swell after several days or a few weeks. Such animals, if they do not die before, should be killed on the appearance of these swellings, and they as well as those which died should be examined for the presence of tuberculosis. The surviving guinea pigs should be kept under observation for several months.

In examining entire herds, it is advisable to group the cows; for instance, five animals may form one group and the mixed milk of this group should be separately inoculated.

The counting of bacteria is carried out either by the ordinary method of plating which is made with certain dilutions of milk on agar or gelatin or also by direct counting in smears which should be prepared according to Olav Skar.

The method consists in mixing in a reagent glass 4/10 c. c. of a 2% solution of carbol-methylene blue (for animal cells and bacteria) and 3.5 c. c. carbol-methylene blue, with 0.5 c. c. of a 3% sodium hydrate solution (for bacteria alone). Then 10 c. c. of milk is added to the stain with a pipette and heated for about 10 minutes at 70° C. Of the mixture, 1/50 of a c. c. is uniformly smeared upon a certain sized field ( $24 \times 20$  m.m.) of a special slide, and dried in the air. Without any further fixation or other treatment, a number of fields in the smear are counted in their entire length and width, and with the aid of the ocular micrometer the number of bacteria in the counted fields is calculated to the c. c. of milk, according to the standard given below. When chains and clumps are encountered each bacillus must be counted. The ocular micrometer of Zeiss in Jena as applied by Skar has a determined field capacity so that one bacterium, with the above technique and with a certain tube length of the microscope, viewed with a 1/12 oil immersion

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objective, indicates the following number of bacteria for the various fields which are designated by letters:

In  $\frac{1}{4}$  of square a = 40,000,000 per c. c. of milk In  $\frac{1}{2}$  of square a = 20,000,000 per c. c. of milk In total square a = 10,000,000 per c. c. of milk In  $\frac{1}{4}$  of square b = 8,000,000 per c. c. of milk In  $\frac{1}{2}$  of square b = 4,000,000 per c. c. of milk In total square b = 2,000,000 per c. c. of milk In circle ..... c = 1,000,000 per c. c. of milk In total field of observation = 800,000 per c. c. of milk

The total number of bacteria found in all the counted fields is multiplied by the relative number corresponding to the field of the size that was counted and divided by the number of counted fields. If, for instance, in 20 ocular fields of size "a" Skar found the number to be 150 bacteria, then these figures give:

 $\frac{150\times10,000,000}{20} = 150\times500,000 = 75,000,000 \text{ per c. c. of milk.}$ 

Skar always found many more bacteria by this method than were found by the plate method (2 to 70 times as many).

The direct counting is more rapid and more accurate than the plate counting method.

As already mentioned, for practical control work the counting of bacteria may be omitted. In this work the reductase test offers a quicker determination of the spoiled condition of the milk.

For special examinations the following methods are recommended: For determining age and decomposition the reductase test and periodically repeated acid tests, besides the microscopical examination of the sediment, should be applied.

For determining decomposition of pasteurized milk, the peroxydase test, in combination with the reductase test, and, at times also the catalase test and microscopic examination of the sediment, should be used.

For the judgment of dirty milk, the determination and estimation of the dirt content, the reductase test, periodical acid test and microscopic examination of the sediment should be made.

## Inflammation of the Udder.

(a) Market milk:

- 1. Determination of the quantity and appearance of the centrifugal slime.
  - 2. Microscopic examination of the sediment for parenchyma cells and the presence of animal forms of streptococci.
- 3. In tuberculosis: Inoculate.

(b) Individual samples:

1. Trommsdorff test.

N # * 1 1		
Milk	Inspe	etion.

- 2. Microscopical test of the sediment and examination as to the presence of parenchyma cells and diplococci, streptococci of the short and long forms, particularly the animal types or the pyogenes or tuberculosis bacillus.
- 3. If necessary the catalase test or examination for amylase.
- (e) Samples from individual quarters:
  - 1. Trommsdorff or sediment tests.
  - 2. Microscopy and, if necessary, inoculation.
  - 3. Catalase or amylase test.

In examinations for milk defects, the following tests are recommended:

- 1. Tests with the senses.
- 2. Shaking test (soapy milk).
- 3. Reductase test (frequently the reduction appears very slowly, for instance, with tallowy and soapy milk).
- 4. Acid tests.
- 5. Historical consideration of the conditions of stables, pasturage, litter, feed and water, preparation of milk.
- 6. Fermentation test.
- 7. Cultivation of bacteria from the milk, feed, pastures, etc., at temperatures at which the defects in the milk appeared.
- 8. Examination of cultures in sterilized milk.

(a) Pure cultures,

(b) Special mixtures of colonies.

The veterinarian should also be able to conduct the routine examination methods usually conducted in the milk laboratory and the preliminary chemical tests for adulteration, provided food chemists are not available in such localities. It would lead too far to mention at this time all the methods which may be applied in suspected adulterations. Only a few methods will be described, particularly those which are employed at the official milk laboratory in Munich.

Determination of the specific gravity of milk. It is best to test the milk for its specific gravity after heating it to 15° C. or in the neighborhood of this temperature. The milk is shaken up and it is advisable to make the test in suspended cylinders with the aid of an aerometer and a thermometer. It is not advisable to use aerometers into which thermometers are fused. The lactodensimeter used for milk must be officially tested. The specific gravity of milk varies in accordance with its contents in dissolved, suspended and emulsified ingredients.

The lactodensimeter is slowly immersed in the milk and should not touch the walls of the cylinder. After the instrument has come to a rest, the place at which the level of the fluid touches the lactodensimeter is read. The numbers on the lactodensimeter indicate the second, third and fourth decimals of the specific gravity. For each additional degree above  $15^{\circ}$  C., 0.2 should be added to the reading of the lactodensimeter, and for each degree below  $15^{\circ}$  C., 0.2 should be deducted. In this way the corrected readings of the lactodensimeter are obtained, before which must be placed 1.0 in order to obtain the specific gravity. The Munich lactodensimeter which is adjusted to a temperature of  $15^{\circ}$  C. is recommended for general use.

The specific gravity of the milk may also be determined by the so-called pyknometer. This method is suitable for small quantities of milk. A third method of determination of the specific gravity is Westphal's modification of Mohr's balance. This is so constructed that the lever arm of the balance from its zero point (the axis of the balance) is provided with 9 notches at such distances, that a rider suspended on it indicates from 1 to 10 times its weight, depending upon whether it is pushed towards the end of the arm or towards its axis. Point 10 at the end of the arm is provided with a loop. A weight A, suspended in the loop of point 1, acts on point 1 only as a weight of A/10 at point 10.

The weights A,  $A_1$ , and  $A_2$ , may be mutually interchanged and indicate the integer and the first decimal figure of the specific gravity, depending upon whether they are suspended in the loop or in the notches. The weight B=1/10 of A, and indicates when in the notches 1/100, while C represents 1/10 of B and when in the notches indicates the 1/1000 of the specific gravity.

By shifting the weight C between two notches the fourth decimal point may also be approximately established.

Hydrometers made of glass and the use of separate thermometers are recommended.

The specific gravity of market milk varies between 1,029 to about 1,033.

The increased or decreased specific gravity, as compared with the average specific gravity of the milk of the respective locality, can at the most only be suspected as being caused by dilution with water or by removal of the cream. In case of double adulteration the specific gravity may remain normal.

After the specific gravity has been determined, the fat content is established by one of the ordinary empiric methods. Gerber's acidbutyrometric method is employed at the milk control station of Munich.

The following apparatus is needed for this method.

1. Centrifuge.

2. Butyrometer (round butyrometer, flat butyrometer, "optical" butyrometer) which is a milk receptacle, ending in a graduated tube into which are poured sulphuric acid, amyl alcohol and milk, and which is closed by means of a rubber stopper.

3. A 10 c. c. pipette or an automatic measure adjusted for

10 c. c., for measuring the sulphuric acid, an 11 c. c. pipette for milk and a 1 c. c. pipette or a corresponding automatic measure for amyl alcohol.

4. Commercial sulphuric acid of a specific gravity of 1,820 to 1.825 (at  $15^{\circ}$  C.).

5. Amyl alcohol with a specific gravity of about 0.815 (at 15° C.) and a boiling point of 128 to 130° C.

6. Shaking apparatus with a protective cover.

7. Water bath.

First sulphuric acid (10 c. c.) is poured into the butyrometer, then 11 c. c. of milk, and finally 1 c. c. of amyl alcohol. The tube is closed with a rubber stopper and then roughly shaken. Through mixing, the contents become greatly heated. After the disappearance of all flakes and after the tube has been held for several minutes at  $65^{\circ}$  C., it is centrifugalized. Following the centrifugalization it is again heated at  $65^{\circ}$  C. and then quickly read.

In order to avoid the more or less dangerous handling of sulphuric acid, Sichler's "sinacid" and Gerber's "sal" methods have recently been inaugurated, in which alkaline salt solutions are employed instead of the sulphuric acid. The results are approximately the same as in the acid butyrometers.

[In the United States the most popular method for determining the amount of fat in milk is by the Babcock test. If carefully applied the results can be relied upon without question.

In the application of this test sulphuric acid is used to free the fat globules by dissolving the casein. Then by proper centrifuging the fat is collected in such a manner that it may be readily measured.

For the execution of the test special test bottles are provided. A definite amount of milk (17.6 c. c.) is placed into a test-bottle to which 17.5 c. c. of commercial sulphuric acid of a specific gravity of 1.82 to 1.85 is added by means of a pipette, burette or other measuring apparatus. The contents are then thoroughly and carefully mixed, as a result of which the fluid turns brown and becomes somewhat heated. The bottles are then placed into a centrifuge which is specially constructed for this purpose, and centrifuged for 5 minutes at 900 to 1,200 revolutions per minute. After removing the bottles from the centrifuge they are filled with hot water up to the lower part of the neck and they are again centrifuged for two minutes. A sufficient amount of hot water is now added to float the melted fat into the neck of the bottle which is provided with a graduated scale, and the centrifuging is repeated for one minute. The volume of fat can be easily read from the graduated portion of the bottle, and this reading should be done while the neck of the bottle is still hot.—Trans.]

The fat content of the milk fluctuates between wide limits. The total solids and the fat-free solids may be established with the aid of the specific gravity and the obtained fat content. d=percentage of solids= $\frac{1}{2} \times f + 2.665 \times \frac{100 \text{ s}-100}{\text{ s}}$ . In this equation f=fat content and s=specific gravity.

Fleischmann has prepared two tables for the values of  $\frac{1}{2}\times f$ and for the value of  $2,665 \times \frac{100 \text{ s}-100}{\text{ s}}$ , for the specific gravity from 1,028 to 1,0369 and for 2.5% to 5.49% of fat, so that through

simple addition of the determined values, the respective content of solids can be established.

Still simpler is the calculation of the solids by Ackermann's "slide-ruler," which consists of two disks united at the turning point and which slide against each other. The larger of these contains the numbers for the solids and fat contents, while the smaller has the numbers for the specific gravity. By turning the small disk the established specific gravity number and the fat content number of the tested sample are placed opposite each other. An indicator fastened to the inside disk points to the amount of the total solids.

The amount of fat-free solids is obtained by subtracting the percentage of fat from the percentage of total solids. This is an important factor in suspected cases of adulteration. Marked differences are the result of artificial influences but in individual samples the difference may be due to natural fluctuations.

Milk with a high fat content has as a rule more fat-free solids. The milk of cows of the highlands has more fat-free solids than the milk of cows from the lowlands.

According to Fleischmann, it should be noted that the fluctuations of the different values indicated herein, vary in the different milking periods as compared with the annual average. These fluctuations may amount to:

10% in the lactodensimeter reading (specific gravity).

30% in the fat content.

14% in the total solids.

10% in the fat-free solids.

According to Fleischmann, the fat-free solids do not fall below 7.9%, and the specific gravity of the total solid matter (m) which may be determined by the following formula:

$$m = \frac{s \times d}{s \times d - 100 s + 100}$$

exceeds only exceptionally 1.4 and is usually 1.31 to 1.36.

Excepting the milk of individual cows or that of a few cows, according to the agreement of German food chemists, the presence of adulteration is established as follows:

1. Adulteration with water when the specific gravity of the

milk is below 1.028, that of the serum below 1.026 and the content of fat-free solids falls considerably below 8%.

2. Cream has been removed from the milk when in the presence of an increased specific gravity of the milk and normal specific gravity of the serum or normal content of fat-free solids, the percentage of fat content of the milk solids falls considerably below 20%; that is, the specific gravity of the latter is increased considerably above 1.4.

3. Adulteration with water together with removal of some of the cream may be suspected when with a normal specific gravity of the milk, that of the serum falls below 1.026, and with a diminished amount of all the ingredients of the milk the fat content of the solids falls considerably below 20%; that is, the specific gravity of the latter is increased considerably over 1.4. The percentage of fat contents of the solids is obtained from the following formula:

$$\frac{f \times 100.}{d}$$

At the same time it must be remembered that no definite figures of limitation should exist and that only comparative figures are convincing. The sample for comparative tests may be obtained by informing the manager of the dairy or the responsible producer that the milk is suspected of being watered, and he is directed to devote special attention to the milk production and its subsequent handling. If following this procedure the milk becomes notably changed the test may be considered as comparative, provided that it is not preferred to undertake immediately an official comparative test. This must be done as quickly as possible, and not later than the third day after the suspicion has been established. According to Herz the following formulas apply in the calculation of adulterations:

w = the added water contained in 100 parts of milk.

- v = the added water to 100 parts of milk.
- p = the fat removed from 100 parts of milk.
- $\mathbf{r}_1 =$  the fat-free solids of the stable sample.
- $\mathbf{r}_2 =$  the fat-free solids of the suspected market sample.
- $f_1 = fat$  contents of the stable sample.
- $f_2 = fat$  contents of the suspected market sample.
- M = 100 w, the amount of original undiluted milk contained in 100 parts of watered milk.

$$w = \frac{100 \times (r_1 - r_2)}{r_1}$$
$$v = \frac{100 \times (r_1 - r_2)}{r_2}$$
$$p = f_1 - f_2 + \frac{f_2 \times (f_1 - f_2)}{100}$$

Finally in combined adulteration we have:

$$\mathbf{p} = \mathbf{f_1} - \frac{\left[100 - \frac{\mathbf{M} \times \mathbf{f_1} - 100 \mathbf{f_2}}{\mathbf{M}}\right] \times \left[\frac{\mathbf{f_1} - \mathbf{M} \times \mathbf{f_1} - 100 \mathbf{f_2}}{\mathbf{M}}\right]}{100}$$

Other formulas according to Vogel are: The addition of water in per cent

$$= \frac{f_1}{f_2} \times 100 - 100$$

The removed amount of fat in per cent

$$=100 imesrac{\mathrm{f_1}-\mathrm{f_2}}{\mathrm{f_1}}$$

or according to Böhmländer:

 $v = \frac{r_1}{r_2} \times w - W$ , in which w = the contents of water in the suspected sample and W = the contents of water of the unsuspected sample, and

$$E = 100 \left(1 - \frac{f_2 \times r_1}{f_1 - r_2}\right)$$

(E = removed fat in % of the fat content)

The establishment of the suspicion and the establishment of the amount of adulteration are greatly supported by the examination of the milk serum free from proteins, which contains the substances dissolved in the milk, the content of which is subject to only the slightest variations. The examination is carried out

1. Through the specific gravity, or

2. Through refractometric observations.

It is best to prepare the serum according to Ackermann by the addition of 0.25 c. c. of a solution of calcium chloride of a specific gravity of 1.1375, to 30 c. c. of milk, which mixture is thoroughly shaken in a reagent tube. The tube is closed with a rubber stopper through which a glass tube is inserted as a reflux condenser, and it is then heated to boiling for 15 minutes. The tubes are then rinsed in cold water and the condensation water of the cooling tube is united with the serum by slow, repeated and careful shaking. With milk which is in a state of decomposition, the milk serum turns cloudy and the values of refraction are also changed. Milk from affected udders which is considerably changed, frequently curdles only following the addition of double quantities of calcium chloride solution. Differences in the refraction values of 2.4 to 2.50 indicate about a 10% adulteration. The removal of cream does not change the refraction value of the milk.

For refraction the serum prepared according to Ackermann does not have to be filtered, but this should be done for the determination of the specific gravity, which is usually 1.026 to 1.027. In applying this method it may become necessary to use larger amounts of serum. Differences of about 0.0025 in the specific gravity indicate mixing with about 10% of water.

The positive demonstration of nitrates in milk is always a proof that the milk has been watered and with water that should be considered as objectionable as drinking water, or that has been used for the rinsing of the milk containers, and which according to its quality must be considered as impure. Milk which gives the nitrate reaction should therefore be considered not only as adulterated but also as being spoiled in the sense of the pure food law and even as injurious to health. The nitrate test may become of great importance for the conviction of certain persons, when for instance the water of the well at the barn contains nitrates, while the one at the farmhouse has no nitrates and vice versa. According to Rothenfusser the nitrate test is a substantiating proof of great importance. Milk samples contaminated with manure and litter do not give the nitrate test.

Nitrates and nitrites do not occur in milk of animals fed and cared for in the usual manner on the farm. Rothenfusser ascertained that the unavoidable residue of water used in rinsing the milk containers represents only a tenth or a twentieth part of the amount of water, which is necessary to make a 1% addition and that the residue of water retained in the rinsed vessel must possess the qualities of ditch water in order to be capable of adding to the milk a demonstrable amount of nitrates. According to Rothenfusser the test may be carried out to the best advantage, by placing into small, flat, white porcelain vessels 2 c. c. of pure sulphuric acid of a specific gravity of 1.84, over which a small amount of crystallized diphenylamin is sprinkled from a sprinkling cylinder (a reagent tube with a perforated cork stopper, into which a short glass tube of about 3 mm. inside diameter is inserted).

Then a small quantity of chloride of calcium serum of the milk to be examined is allowed to flow in from the edges of the vessel. The appearance of the grayish-blue clouds and stripes in the fluid indicates the presence of nitric acid in the milk.

# CHAPTER XI.

# FUNDAMENTAL PRINCIPLES OF LEGISLATIVE MILK CONTROL.

The practical inauguration of milk hygiene is, of course, only possible where proper laws and ordinances are at our command for the accomplishment of the result. The laws, regulations and ordinances of the various states and municipalities promulgated for the purpose of controlling the milk supply lack uniformity in many of their essential requirements, and it is apparent that these measures were drafted to meet the conditions prevailing in the different localities. Furthermore, a general federal control of the milk supply cannot be considered practicable, except possibly in so far as interstate shipments of milk may be involved.

The individual states may foster the interests of public health by the inauguration of such legislative measures as will assure a wholesome and clean milk supply for the different municipalities within the state, particularly when the necessity prevails for the shipment of milk from long distances to a city. For this purpose the state might well be divided into districts, supervised by competent inspectors who would primarily inspect the cattle and stables, the methods of producing the milk and its transportation. Such inspectors could also be of splendid service in the control and possible eradication of contagious diseases which not only may have an effect on milk production, but which are also of importance to animal industry in general.

The largest proportion of actual control work, however, will have to be carried out by the different municipalities, where, with proper ordinances and with competent inspectors and laboratory officials, an efficient control could be maintained which would assure a wholesome milk supply to the consumers. It is apparent that such ordinances must be drafted to meet the local conditions, which would depend upon the dairying industry in that section, the number of the population and the feelings of the people.

While there exists no uniformity in the regulations governing milk inspection and milk hygiene in general in the different parts of the United States, the necessity for at least a uniform standard has been met in a most satisfactory manner. After considerable agitation medical milk commissions were organized in various cities of the United States for the purpose of establishing milk standards and also of obtaining such legislation as would assure clean and wholesome milk to the communities. The organization of milk commissions in this country was undoubtedly an important step towards the improvement of the quality of milk, and only by the concerted work of these and similar organizations can the country at large be assured of a proper milk supply. It is regrettable that milk, the most important food, is not considered by the laity of sufficient importance to be subjected to the most rigid control, especially since it constitutes the principal, and in early life the only food of children.

The second report of the Commission on Milk Standards, appointed by the New York Milk Committee, embodies the principles for a wholesome milk supply and would serve well as a basis for the formulation of effective ordinances. Therefore it is deemed advisable to reproduce these principles from the Public Health Reports of August 22, 1913, United States Public Health Service.

## Need of Milk Control.

Proper milk standards, while they are essential to efficient milk control by public health authorities and have as their object the protection of the milk consumer, are also necessary for the ultimate well-being of the milk industry itself. Public confidence is an asset of the highest value in the milk business. The milk producer is interested in proper standards for milk, since these contribute to the control of bovine tuberculosis and other cattle diseases and distinguish between the good producer and the bad producer. The milk dealer is immediately classified by milk standards, either into a seller of first-class milk or a seller of second-class milk, and such distinction gives to the seller of firstclass milk the commercial rewards which he deserves, while it inflicts just penalties on the seller of second-class milk. For milk consumers, the setting of definite standards accompanied by proper labeling makes it possible to know the character of the milk which is purchased and to distinguish good milk from bad milk. In the matter of public health administration, standards are absolutely necessary to furnish definitions around which the rules and regulations of city health departments can be drawn, and the milk supply efficiently controlled.

#### Public Health Authorities.

While public health authorities must necessarily see that the source of supply and the chemical composition should correspond with established definitions of milk as a food, their most important duty is to prevent the transmission of disease through milk. This means the control of infantile diarrhea, typhoid fever, tuberculosis,

diphtheria, scarlet fever, septic throat infections, and other infectious diseases in so far as they are carried by milk.

#### Septic Sore Throat.

Septic sore throat deserves special mention because of the frequency in recent years with which outbreaks of this disease have been traced to milk supplies. The suggestion has been made that the infection of the milk is due to udder infection of the cow and on the other hand it has been suggested that it is due to contact with infected persons. The uncertainty can not be dispelled until cases of septic sore throat are regularly reported and tabulated by public health authorities. The commission therefore recommends that public health authorities make septic sore throat a reportable disease.

# Economic Problem.

The commission recognizes the magnitude of the milk industry, and that the improvement of milk supplies is primarily an economic problem. The success achieved by the experiment in milk production, which has been carried out on a very large scale by the New York Dairy Demonstration Co., is an illustration of the fact that an extra price or premium paid to the producer for cleanliness and care will bring results far more quickly and certainly than instructions or official inspection. But while the basic problem is economic, and must eventually be solved by commerce, public health authorities must show the way and must establish standards and regulations in the interest of consumers, the value of which even the consumers themselves often fail to appreciate.

# Legal Requirements.

A prime requisite of effectiveness is that local milk laws shall not exceed sanitary limitations. The commission has not entered into a discussion of fundamental state laws, but it recommends that state laws be amended wherever necessary in order that every municipality may have the legal right to adopt whatever ordinances it sees fit for the improvement of the milk supply. The commission advocates that local health laws be carefully drawn with regard to their legality under the general laws of the localities to which they apply, since a decision against a milk law in one locality is liable to be used as a precedent against milk laws elsewhere.

# Standard Rules and Regulations.

The commission has drawn up a set of standard rules and regulations for the control of milk. These are the result of a study of the printed rules and regulations of the cities of the United States and of foreign countries and represent an immense amount of work on the part of the special committee of the commission to which the task was assigned. Some communities are in a position to adopt all of these rules and regulations at the present time, while other communities will be obliged to adopt a few rules at a time as public sentiment and local conditions warrant. It is realized that some of the rules may have to be modified to meet local conditions. It seems wise to the commission to divide the regulations into two parts: First, requirements, under which head are set down those provisions which are so fundamentally necessary that no community is justified in compromising on them; second, recommendations, under which head are set down provisions which are necessary for a good milk supply, but on which there can be a certain amount of latitude for compromise by those communities in which public sentiment is not ready to support more than a moderate degree of protection of human life.

#### Administrative Equipment.

Another prime requisite is that the administrative departments shall be adequately equipped with men, money, and laboratory facilities. In smaller communities cooperation between local boards of health to the extent of exchanging reports would eliminate much duplication. Where a community can not maintain a laboratory it can enter into laboratory arrangements with other communities, and several can combine in the use of a common laboratory. Much of the expense of tuberculin testing can be borne by the national and state governments. The commission is of the opinion that results can not be expected from laws where there is not sufficient appropriation and where there is no machinery for their enforcement. On this subject the commission passed a resolution as follows:

Whereas the appropriations generally made for the purposes of carrying on laboratory analyses of milk are now in most cases entirely inadequate: Therefore be it

*Resolved*. That this commission recommends for the consideration of the authorities concerned an appropriation of funds commensurate with the importance of laboratory methods, which are of paramount importance in the hygienic control of the milk supply.

# Grading of Milk.

There is no escape from the conclusion that milk must be graded and sold on grade, just as wheat, corn, cotton, beef, and other products are graded. The milk merchant must judge of the food value and also of the sanitary character of the commodity in which he deals. The high-grade product must get a better price than at present. The low-grade product must bring less. In separating milk into grades and classes the commission has endeavored to make its classification as simple as possible and at the same time to distinguish between milks which are essentially different in sanitary character.

In general two great classes of milk are recognized, namely, raw milk and pasteurized milk. Under these general classes there are different grades, as indicated in the report of the committee on classification.

# Pasteurization.

While the process of pasteurization is a matter which has attracted a great deal of attention in recent years, the commission has not entered into any discussion of its merits or demerits, but has given it recognition in its classification as a process necessary for the treatment of milk which is not otherwise protected against infection.

The commission thinks that pasteurization is necessary for all milk at all times, excepting grade A, raw milk. The majority of the commissioners voted in favor of the pasteurization of all milk, including grade A, raw milk. Since this was not unanimous the commission recommends that the pasteurization of grade A, raw milk, be optional.

The process of pasteurization should be under official supervision. The supervision should consist of a personal inspection by the milk inspector. The inspections shall be as frequent as possible. Automatic temperature regulators and recording thermometers should be required and the efficiency of the process frequently determined by laboratory testing.

## Pasteurizing Temperatures.

The destruction of the chemical constituents of milk by heat occurs at higher temperatures than those necessary for the destruction of the bacteria of infectious diseases transmissible by milk.

The commission passed a resolution regarding the temperature of pasteurization as follows:

That pasteurization of milk should be between the limits of 140° F. and 155° F. At 140° F. the minimum exposure should be 20 minutes. For every degree above 140° F. the time may be reduced by 1 minute. In no case should the exposure be for less than 5 minutes.

In order to allow a margin of safety under commercial conditions the commission recommends that the minimum temperature during the period of holding should be made 145° F. and the holding time 30 minutes. Pasteurization in bulk when properly carried out has proven satisfactory, but pasteurization in the final container is preferable.

It is the sense of the commission that pasteurization in the final container should be encouraged.

## Labeling and Dating of Milk.

The commission voted that all milk should be labeled and marked with the grade in which it is to be sold. In dating milk uniform methods should be adopted for all grades of both raw milk and pasteurized milk, both using the day of the week or both using the day of the month. All milk should be dated uniformly with the date of delivery to the consumer. Raw milk should not be dated with the date of production, while pasteurized milk is dated with the date of pasteurization, since this places certified milk at a disadvantage by making it possible for pasteurized milk of a lower grade to carry a later date. The stamping on the label of the day of the week is sufficient for dating.

# Bacteria.

The subject of bacteria in milk received more attention than any other matter brought before the commission. The commission recognizes that bacteria in milk in the majority of instances indicate dirt, or lack of refrigeration, or age, while in the minority of instances the bacteria of disease may be present. The routine laboratory methods for examining milk have as their purpose only the control over dirt, refrigeration, and age, and it is a rare thing for a laboratory to undertake the examination of milk for the bacteria of disease because of the extreme difficulties in detecting The more efficacious method of protecting milk from infecthem. tion by the bacteria of human contagion is by medical, veterinary, and sanitary inspection, and by pasteurization. Milk with a high bacteria count is not necessarily harmful, but when used as a food, particularly for children, is a hazard too great to be warranted. Milk with a high bacteria count, therefore, should be condemned. Milks with small numbers of bacteria are presumed to be wholesome, unless there is reasonable ground for suspecting that they have been exposed to contagion.

#### Bacterial Standards.

The commission recognizes the difficulty in interpreting bacteria counts. At times misleading conclusions have been drawn from such counts. In establishing the bacterial standards for a city it is always necessary to take into consideration the necessary age of the milk and in lesser measure the distance hauled and methods employed in its hauling. It will always be possible for a community which consumes milk produced on its own premises, or within 12 hours of its production, to insist upon and maintain a lower bacterial standard than can one where the milk is hauled many miles into town in a wagon, to be consumed within 24 hours after it is produced. In like manner this second type of city can always maintain a lower bacterial standard than a city where the general milk supply is hauled by railroad long distances and is several days old when consumed. In drawing conclusions as to the relative efficacy of milk control in cities comparisons must be made between cities of the same class.

The commission deems it of the utmost importance that some standard method should be adopted for estimating and comparing the bacterial character of milks, since by this means only is it possible to grade and classify milks and to enforce bacterial There is much diversity of opinion as to the best standards. method of valuing bacteria counts. The average of a series gives results which are misleading about as frequently as otherwise. In the average a single high figure may unduly overbalance a large number of exceedingly low counts. There are objections to the use of the "median" or middle number when the counts are arranged in order of size, for the reason that the middle figure does not distinguish between two groups in one of which there may be some very high counts above the median and in the other of which there are none. The method of dividing results into groups as recommended by the American Public Health Association, while a step in the right direction, is cumbersome and does not clearly indicate whether or not a milk conforms to a given bacterial standard.

The commission passed a resolution at its last meeting regarding the number of bacterial tests necessary to determine the grade into which a milk falls, as follows:

That the grade into which a milk falls shall be determined bacteriologically by at least five consecutive bacteria counts taken over a period of not less than one week nor more than one month, and at least 80 per cent (four out of five) must fall below the limit set for the grade for which the classification is desired.

# Laboratory Examinations for Bacteria.

On the subject of laboratory examinations of milk for bacteria the commission passed the following resolutions:

1. That the interests of public health demand that the control of milk supplies, both as to production and distribution, shall include regular laboratory examinations of milk by bacteriological methods.

2. That among present available routine laboratory methods for determining the sanitary quality of milk the bacteria count occupies first place.

3. That bacteriological standards should be a factor in classifying or grading milks of different degrees of excellence.

4. That in determining the grade or class of a raw milk the specimen taken for bacteriological examination should be of milk as offered for sale.

5. That there should be bacteriological standards for pasteurized milk which should require laboratory examination of samples immediately before pasteurization as well as of milk offered for sale.

6. That the bacteria count of milk indicates its quality and history as it is modified by contamination, handling, dirt, temperature, or age. A high count indicates the necessity of investigation and inspection. 7. That there be adopted as standards for making the bacteria count the standard methods of the American Public Health Association, laboratory section, recommending, however, the following amendments:

A. That the culture medium used for testing milk be identical in its composition and reaction with the culture medium used for the testing of water provided in the standard methods of water analyses of the American Public Health Association.

B. That incubation of plate cultures be made at 37° C. for 48 hours.

The bacterial standards given in the report are the work of a special committee of bacteriologists who considered all of the bacterial standards now in use. It is believed that the standards suggested are fair and wise and give full consideration to the state of the industry and of public health control. The commission believes that the adoption and enforcement of these bacterial standards will be more effective than any other one thing in improving the sanitary character of public milk supplies. The enforcement of these standards can be carried out only by the regular and frequent laboratory examinations of milks for the numbers of bacteria they may contain.

# Chemical Standards.

The chemical standards suggested are the work of a special committee, composed of chemists, which has carefully considered the natural composition of milk and the Federal and State standards already established. The standard of 3.25 per cent fat and 8.5 per cent solids, not fat, here proposed is in accordance with the recommendations of the Association of Official Agricultural Chemists and has been adopted by the United States Department of Agriculture and by a larger number of States than has any other standard. The simplification of the Babcock test makes the determination of fats and solids not fat an easy procedure quickly applied. Such chemical examinations of milk can be readily adopted and executed by any health-board laboratory at a very moderate expense. It is believed that such chemical standards as are suggested will inflict no real hardship on the milk producers of this country and that the provision regarding substandard milks is a liberal one.

#### Microscopic Examination of Milk.

Because of studies which have been made during the past year the commission thinks it wise to omit temporarily any definite statement on the subject of microscopical examination of milk, and the determination of pus and bacteria by sedimentation methods, until further studies have been made. A special subcommittee has been appointed for this purpose which will make studies during the present year and the commission will take action on this matter at one of its later meetings.

## Mislabeling.

The commission resolved that the sale of milk which is mislabeled or misbranded shall be punished by suitable penalties.

# Publicity.

The commission fully considered the matter of the publication of laboratory examinations of milk by city and town health authorities. When proper standards and regulations are established and adequate facilities furnished for laboratory work, it is believed that the laboratory tests will give an index of the character of the milk delivered to the public by milk sellers which is entirely fair and impartial. There can be no objection to publicity under such circumstances. It is an advantage to the seller of high-grade milk. It is an advantage to the consumer who desires to select a high-grade milk. It has much educational value both to producer and consumer. Therefore the commission recommends "that the reports of laboratory analyses of milk made by departments of health be regularly published."

#### Medical Inspection.

It is the sense of the commission that the medical inspection of dairy employees should be emphasized in all ways possible.

# Milk Dealer's License.

The commission resolved that a dealer shall be required to have a permit or license to sell any grade or class of milk and to use a label for such class or grade. Such permit or license shall be revoked and the use of the label forbidden when the local health authorities shall determine that the milk is not in the class or grade designated.

# Designation of Grade.

The commission resolved that the grade of milk shall be designated by letter. It is the sense of the commission that the essential part is the lettering and that all other words on the label are explanatory.

In addition to the letters of the alphabet, used on caps or labels, the use of other terms may be permitted so long as such terms are not the cause of deception.

Caps and labels shall state whether milk is raw or pasteurized. The letter designating the grade to which milk belongs shall be conspicuously displayed on the caps of bottles or the labels on cans.

# Classification of Milk.

It was resolved that the classification of milk contained in the first report of the commission be amended as follows:

Milk shall be divided into three grades, which shall be the

same for both large and small cities and towns, and which shall be designated by the first three letters of the alphabet. The requirements shall be as follows:

# Grade A.

Raw milk.—Milk of this class shall come from cows free from disease as determined by tuberculin tests and physical examinations by a qualified veterinarian, and shall be produced and handled by employees free from disease as determined by medical inspection of a qualified physician, under sanitary conditions such that the bacteria count shall not exceed 100,000 per cubic centimeter at the time of delivery to the consumer. It is recommended that dairies from which this supply is obtained shall score at least 80 on the United States Bureau of Animal Industry score card.

Pasteurized milk.—Milk of this class shall come from cows free from disease as determined by physical examinations by a qualified veterinarian and shall be produced and handled under sanitary conditions such that the bacteria count at no time exceeds 200,000 per cubic centimeter. All milk of this class shall be pasteurized under official supervision, and the bacteria count shall not exceed 10,000 per cubic centimeter at the time of delivery to the consumer. It is recommended that dairies from which this supply is obtained should score 65 on the United States Bureau of Animal Industry score card.

The above represents only the minimum standards under which milk may be classified in grade A. The commission recognizes, however, that there are grades of milk which are produced under unusually good conditions, in especially sanitary dairies, many of which are operated under the supervision of medical associations. Such milks clearly stand at the head of this grade.

#### Grade B.

Milk of this class shall come from cows free from disease as determined by physical examinations, of which one each year shall be by a qualified veterinarian, and shall be produced and handled under sanitary conditions such that the bacteria count at no time exceeds 1,000,000 per cubic centimeter. All milk of this class shall be pasteurized under official supervision, and the bacteria count shall not exceed 50,000 per cubic centimeter when delivered to the consumer.

It is recommended that dairies producing grade B milk should be scored and that the health departments or the controlling departments, whatever they may be, strive to bring these scores up as rapidly as possible.

#### Grade C.

Milk of this class shall come from cows free from disease as determined by physical examinations and shall include all milk that is produced under conditions such that the bacteria count is in excess of 1,000,000 per cubic centimeter.

All milk of this class shall be pasteurized, or heated to a higher temperature, and shall contain less than 50,000 bacteria per cubic centimeter when delivered to the customer. It is recommended that this milk be used for cooking or manufacturing purposes only.

Whenever any large city or community finds it necessary, on account of the length of haul or other peculiar conditions, to allow the sale of grade C milk, its sale shall be surrounded by safeguards such as to insure the restriction of its use to cooking and manufacturing purposes.

# Classification of Cream.

Cream should be classified in the same grades as milk, in accordance with the requirements for the grades of milk, excepting the bacterial standards which in 20 per cent cream shall not exceed five times the bacterial standard allowed in the grade of milk.

Cream containing other percentages of fat shall be allowed a modification of this required bacterial standard in proportion to the change in fat.

# Chemical Standards.

*Cow's milk.*—Standard milk should contain not less than 8.5 per cent of milk solids not fat and not less than 3.25 per cent of milk fat.

Skim milk.—Standard skim milk should contain not less than 8.75 per cent of milk solids.

 $\hat{C}$  ream.—Standard cream contains not less than 18 per cent of milk fat and is free from all constituents foreign to normal milk. The percentage of milk fat in cream over or under that standard should be stated on the label.

Buttermilk.—Buttermilk is the product that remains when fat is removed from milk or cream, sweet or sour, in the process of churning. Standard buttermilk contains not less than 8.5 per cent of milk solids. When milk is skimmed, soured, or treated so as to resemble buttermilk, it should be known by some distinctive name.

# Homogenized Milk or Cream.

The commission is of the opinion that in the compounding of milk no fats other than milk fats from the milk in process should be used and that no substance foreign to milk should be added to it. The commission is opposed to the use of condensed milk or other materials for the thickening of cream unless the facts are clearly set forth on the label of the retail package. Regarding the process of homogenizing, the commission resolved as follows:

That homogenized milk or cream should be so marked, stating the percentage of fat that it contains.

# Adjusted Milks.

On the question of milks and creams in which the ratio of the fats to the solids not fat has been changed by the addition to or subtraction of cream or milk fat the commission has hesitated to take a position. On the one hand they are in favor of every procedure which will increase the market for good milk and make the most profitable use of every portion of it. On the other, they recognize the sensitiveness of milk, the ease with which it is contaminated, and the difficulty of controlling, standardizing, skimming, homogenizing, souring, etc., so that contaminations do not occur and inferior materials are not used. On this subject the commission passed a resolution presented by a special committee as follows:

Milk in which the ratio of the fats to the solids not fat has been changed by the addition to or subtraction of cream should be labeled "adjusted milk"; the label should show the minimum guaranteed percentage of fat and should comply with the same sanitary or chemical requirements as for milk not so standardized or modified.

# Regulation of Market Milk on Basis of Guaranteed Percentage Composition.

1. Sellers of milk should be permitted choice of one of two systems in handling market milk. Milk can be sold, first, under the regular standard, or, second, under a guaranteed statement of composition.

2. Any normal milk may be sold if its per cent of fat is stated. In case the per cent of fat is not stated, the sale will be regarded as a violation unless the milk contains at least 3.25 per cent of milk fat.

3. As a further protection to consumers, it is desirable that when the guaranty system is used there be also a minimum guaranty of milk solids not fat of not less than 8.5 per cent.

4. Dealers electing to sell milk under the guaranty system should be required to state conspicuously the guaranty on all containers in which such milk is handled by the dealer or delivered to the consumer.

5. The sale of milk on a guaranty system should be by special permission obtained from some proper local authority.

# Penalty.

Every milk ordinance should contain a penalty clause.

## Extension Work.

The commission indorsed the efforts of the New York Milk Committee to obtain funds for the formation of a bureau of extension work, such bureau to act as a collecting station for information regarding standards and regulations as to milk adopted by cities and towns in the United States. The bureau should also furnish information to such cities and towns as appeal for aid in the adoption of milk standards and should conduct a constructive program by placing in the field a man who would visit communities interested in establishing milk standards; and it may use the members of the commission on milk standards for carrying on the work of the bureau so far as possible in their own localities.

The commission has confined its report rather closely to the standard requirements for milk. These requirements can not be met unless proper measures are taken. For instance: The milk must be produced from healthy cows in clean surroundings, and must then be promptly chilled and kept cool thereafter. The handling at all points must be done by healthy employees—employees who are not carriers of contagion.

The reports of the subcommittees on the methods of production, handling, and distribution, while not properly a part of the report itself, are set forth in the following pages.

# STANDARD RULES FOR THE PRODUCTION, HAN-DLING. AND DISTRIBUTION OF MILK.

As a basis for the promulgation of rules and recommendations governing the production, handling, and distribution of milk, it is recognized that we have to deal with two kinds of milk, raw and pasteurized, although there may be several grades of each of these two kinds. In order for any grade to be safe, it is recommended that the regulations herein set forth under the heading "Requirements' should be enforced. The regulations herein set forth under the heading "Recommendations" should be adopted wherever practicable as a means of improving the milk supply above the actual point of safety. (The term "milk" shall be construed to include the fluid derivatives of milk wherever such construction of the term is applicable.)

#### LICENSES.

#### Requirements.

No person shall engage in the sale, handling, or distribution of milk in ----- until he has obtained a license therefor from the health authorities. This license shall be renewed on or before the 1st day of —— of each year and may be suspended or revoked at any time for cause.

# Recommendations.

The application for the license shall include the following statements:

Kind of milk to be handled or sold. (1)

Names of producers with their addresses and permit numbers. (2)

Names of middlemen with their addresses. (3)

(4) Names and addresses of all stores, hotels, factories, and restaurants at which milk is delivered.

(5) A statement of the approximate number of quarts of milk, cream, buttermilk, and skim milk sold per day.

Source of water supply at farms and bottling plants. (6)

Permission to inspect all local and out-of-town premises on which milk is (7)produced and handled.

(8) Agreement to abide by all the provisions of State and local regulations.

#### PERMITS.

#### Requirements.

No person shall engage in the production of milk for sale in -, nor shall any person engage in the handling of milk for shipment into —— until he has obtained a permit therefor from the health authorities. This permit shall be renewed on or before the 1st day of ----- of each year and may be suspended or revoked at any time for cause.

# PRODUCTION OF RAW MILK.

# Cow Stables.

# Requirements.

1. They shall be used for no other purpose than for the keeping of cows, and shall be light, well ventilated, and clean.

2. They shall be ceiled overhead if there is a loft above.

3. The floors shall be tight and sound.

4. The gutters shall be water-tight.

#### Recommendations.

1. The window area shall be at least 2 square feet per 500 cubic feet of air space and shall be uniformly distributed, if possible. If uniform distribution is impossible, sufficient additional window area must be provided so that all portions of the barn shall be adequately lighted.

2. The amount of air space shall be at least 500 cubic feet per cow, and adequate ventilation besides windows shall be provided.

3. The walls and ceilings shall be whitewashed at least once every six months, unless the construction renders it unnecessary, and shall be kept free from cobwebs and dirt.

4. All manure shall be removed at least twice daily, and disposed of so as not to be a source of danger to the milk either as furnishing a breeding place for flies or otherwise.

5. Horse manure shall not be used in the cow stable for any purpose.

#### Milk Room.

#### Requirements.

Every milk farm shall be provided with a milk room that is clean, light, and well screened. It shall be used for no other purpose than for the cooling, bottling, and storage of milk and the operations incident thereto.

# Recommendations.

1. It shall have no direct connection with any stable or dwelling.

2. The floors shall be of cement or other impervious material, properly graded and drained.

3. It shall be provided with a sterilizer unless the milk is sent to a bottling plant, in which case the cans shall be sterilized at the plant.

4. Cooling and storage tanks shall be drained and cleaned at least twice each week.

5. All drains shall discharge at least 100 feet from any milk house or cow stable.

#### Cows.

#### Requirements.

1. A physical examination of all cows shall be made at least once every six months by a veterinarian approved by the health authorities.

2. Every diseased cow shall be removed from the herd at once and no milk from such cows shall be offered for sale.

3. The tuberculin test shall be applied at least once a year by a veterinarian approved by the health authorities.

4. All cows which react shall be removed from the herd at once, and no milk from such cows shall be sold as raw milk.

5. No new cows shall be added to a herd until they have passed a physical examination and the tuberculin test.

6. Cows, especially the udders, shall be clean at the time of milking.

No milk that is obtained from a cow within 15 days before 7. or 5 days after parturition, nor any milk that has an unnatural odor or appearance, shall be sold. 8. No unwholesome food shall be used.

# Recommendations.

1. Every producer shall allow a veterinarian employed by the health authorities

to examine his herd at any time under the penalty of having his supply excluded. 2. Certificates showing the results of all examinations shall be filed with the health authorities within 10 days of such examinations.

3. The tuberculin tests shall be applied at least once every six months by a veterinarian approved by the health authorities, unless on the last previous test no tubercu-losis was present in the herd or in the herds from which new cows were obtained, in which event the test may be postponed an additional six months.

4. Charts showing the results of all tuberculin tests shall be filed with the health authorities within 10 days of the date of such test.

5. The udders shall be washed and wiped before milking.

#### Employees.

#### Requirements.

1. All employees connected in any way with the production and handling of milk shall be personally clean and shall wear clean outer garments.

The health authorities shall be notified at once of any 2. communicable disease in any person that is in any way connected with the production or handling of milk, or of the exposure of such person to any communicable disease.

Milking shall be done only with dry hands. 3.

#### Recommendations.

 $\frac{1}{2}$ 

Clean suits shall be put on immediately before milking. The hands shall be washed immediately before milking each cow, in order to avoid conveyance of infection to the milk.

#### Utensils.

#### Requirements.

1. All utensils and apparatus with which milk comes in contact shall be thoroughly washed and sterilized, and no milk utensils or apparatus shall be used for any other purpose than that for which it was designed.

2. The owner's name, license number, or other identification mark, the nature of which shall be made known to the health

authorities, shall appear in a conspicuous place on every milk container.

3. No bottle or can shall be removed from a house in which there is, or in which there has recently been, a case of communicable disease until permission in writing has been granted by the health authorities.

4. All metal containers and piping shall be in good condition at all times. All piping shall be sanitary milk piping, in couples short enough to be taken apart and cleaned with a brush.

5. Small-top milking pails shall be used.

#### Recommendations.

1. All cans and bottles shall be cleaned as soon as possible after being emptied. 2. Every conveyance used for transportation or delivery of milk, public carriers excepted, shall bear the owner's name, milk-license number, and business address in uncondensed gothic characters at least 2 inches in height.

#### Handling of Milk.

# Requirements.

1. It shall not be strained in the cow stable, but shall be removed to the milk room as soon as it is drawn from the cow.

2. It shall be cooled to  $50^{\circ}$  F. or below within two hours after it is drawn from the cow and it shall be kept cold until it is delivered to the consumer.

3. It shall not be adulterated by the addition to or the subtraction of any substance or compound, except for the production of the fluid derivatives allowed by law.

4. It shall not be tested by taste at any bottling plant, milk house, or other place in any way that may render it liable to contamination.

5. It shall be bottled only in a milk room or bottling plant for which a license or permit has been issued.

6. It shall be delivered in bottles, or single service containers, with the exception that 20 quarts or more may be delivered in bulk in the following cases:

(a) To establishments in which milk is to be consumed or used on the premises.

(b) To infant-feeding stations that are under competent medical supervision.

7. It shall not be stored in or sold from a living room or from any other place which might render it liable to contamination.

#### Recommendations.

1. It shall be cooled to  $50^{\circ}$  F. or below immediately after milking and shall be kept at or below that temperature until it is delivered to the consumer.

2. It shall contain no visible foreign material.

3. It shall be labeled with the date of production.

# Receiving Stations and Bottling Plants.

# Requirements.

1. They shall be clean, well screened, and lighted, and shall be used for no other purpose than the proper handling of milk and the operations incident thereto, and shall be open to inspection by the health authorities at any time.

2. They shall have smooth, impervious floors, properly graded and drained.

3. They shall be equipped with hot and cold water and steam.

4. Ample provision shall be made for steam sterilization of all utensils, and no empty milk containers shall be sent out until after such sterilization.

5. All utensils, piping, and tanks shall be kept clean and shall be sterilized daily.

#### Recommendations.

1. Containers and utensils shall not be washed in the same room in which milk is handled.

#### Stores.

# Requirements.

1. All stores in which milk is handled shall be provided with a suitable room or compartment in which the milk shall be kept. Said compartment shall be clean and shall be so arranged that the milk will not be liable to contamination of any kind.

2. Milk shall be kept at a temperature not exceeding 50° F.

# Recommendations.

1. Milk to be consumed off the premises may be sold from stores only in the original unopened package.

# General Regulations.

# Requirements.

1. The United States Bureau of Animal Industry score card shall be used, and it is recommended that dairies from which milk is to be sold in a raw state shall score at least 80 points.

2. Every place where milk is produced or handled and every conveyance used for the transportation of milk shall be clean.

3. All water supplies shall be from uncontaminated sources and from sources not liable to become contaminated.

4. The license or permit shall be kept posted in a conspicuous place in every establishment for the operation of which a milk license or permit is required.

5. No milk license or permit shall at any time be used by any person other than the one to whom it was granted.

6. No place for the operation of which a license or permit is granted shall be located within 100 feet of a privy or other possible source of contamination, nor shall it contain or open into a room which contains a water-closet.

7. No skim milk or buttermilk shall be stored in or sold from cans or other containers unless such containers are of a distinctive color and permanently and conspicuously labeled "skim milk" or "buttermilk," as the case may be.

8. No container shall be used for any other purpose than that for which it is labeled.

#### Recommendations.

Ice used for cooling purposes shall be clean and uncontaminated.
 No person whose presence is not required shall be permitted to remain in any cow stable, milk house, or bottling room.

# SUBNORMAL MILK.

#### Requirements.

1. Natural milk that contains less than 3.25 per cent, but more than 2.5 per cent milk fat, and that complies in all other respects with the requirements above set forth, may be sold, provided the percentage of fat does not fall below a definite percentage that is stated in a conspicuous manner on the container; and further provided that such container is conspicuously marked "substandard milk."

# PRODUCTION OF CREAM.

Requirements and Recommendations.

1. It shall be obtained from milk that is produced and handled in accordance with the provisions hereinbefore set forth for the production and handling of milk.

# LABORATORY STANDARDS FOR MILK.

Requirements.

1. It shall not contain more than 100,000 bacteria per cubic centimeter.

2. It shall contain not less than 3.25 per cent milk fat.

3. It shall contain not less than 8.5 per cent solids not fat.

#### Recommendations.

1. The bacterial limit shall be lowered if possible.

#### LABORATORY STANDARDS FOR CREAM.

#### Requirements.

There shall be a bacterial standard for cream corresponding to the grade of milk from which it is made and to its butter-fat content.

2.It shall contain not less than 18 per cent. milk fat,

Recommendations.

Same as above for milk.

# SANITARY INSPECTION OF CITY MILK PLANTS

SCORE CARD.

Owner or manager: \_\_\_\_\_. Street and No.: \_\_\_\_\_. City: \_\_\_\_\_. State: \_\_\_\_\_; Trade name: \_\_\_\_\_. Number of wagons: \_\_\_\_\_. Gallons sold daily \_\_\_\_\_. Cream: \_\_\_\_\_. Permit or License No.: \_\_\_\_\_. Date of Inspection: \_\_\_\_\_, 191...... Remarks: \_\_\_\_\_.

Equipment.	Score.		Mothod-	Score.	
	Perfect	Alloweč	Methods.	Perfect	Allowed
Building: Location: Free from contami- nating surroundings Arrangement	2 7		Building         Cleanliness:         Floors       3         Walls       2         Ceilings       2         Doors and windows       1         Shafting, pulleys, pipes, etc. 1         Freedom from odors       2         Freedom from files       3	14	
Separate refrigerator room. 1 Construction	12	••••	Apparatus	. 7	
Minimum of shafting, pulleys, hangers, exposed         pipes, etc.         Apparatus         Boiler         2         (Water heater, 1.)         Appliances for cleansing utensils and bottles         2         Sterilizers for bottles, etc.         2         Bottling machine         1         Capping machine         1         Wash bowl, soap, and towel         in handling moom         Milk-handling machinery 3	15		Bottles       1         Thoroughly washed and       3         rinsed       3         Sterilized with steam 15 minutes       3         Inverted in clean place       1         Handling milk       3         Received below 50° F       3         (50° to 55°, 2); (55° to 60°, 1.)       8         Rapidity of handling       2         Freedom from undue exposure to air       2         Cooling       5         Promptness       2         Below 45° F       3	7	1 1 1
Pipes, couplings, and pumps	22	• • • • • • • •	(45° to 50°, 1.) Capping bottles by machine. 2 Bottle top protected by cover 1 Storage; below 45°F4 (45° to 50°, 3; 50° to 55°, 1.) Protection during delivery. 2 (Iced in summer.) Bottle caps sterilized1 Inspection	6	)
Total			Miscellaneous Cleanliness of attendants 2 Personal cleanliness 1 Clean, washable clothing 1 Cleanliness of delivery outfit 2 Total	4	·····

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- ----, Inspector.

# SANITARY INSPECTION OF DAIRY FARMS

SCORE CARD,

Indorsed by the Official Dairy Instructor's Association.

Owner or lessee of farm: \_\_\_\_\_. P. O. address: \_\_\_\_\_. State: \_\_\_\_\_. Total number of cows: \_\_\_\_\_. Number milking: \_\_\_\_\_. Gallons of milk produced daily: \_\_\_\_\_. Product is sold by producer in families; hotels, restaurants, stores, to \_\_\_\_\_\_dealer. For milk supply of \_\_\_\_\_. Permit No. \_\_\_\_\_. Date of inspection. (Signed) ———. Remarks: — ------. Inspector.

			(signed)	-, Insp	
Equipment	Score.		Methods.	Score.	
	Perfect	Allowed	Methods.	Perfect	Allowed
Cows. Health Apparently in good health1 If tested with tuberculin within a year and no tuber-	6		Cows. Clean	8	
within a year and no to tobe of culosis is found, or if tested within six months and all reacting animals removed. 5 (If tested within a year and reacting animals are found and removed, 3.)			Stables.       Cleanliness of stables.       Floor     2       Walls     1       Ceiling and ledges     1	6	
Food (clean and wholesome) Water (clean and fresh) Stables.	1 1		Mangers and partitions 1 Windows	5	
Location of stable 1 Well drained 1 Free from contaminating sur-	2		Freedom from odors 2 Cleanliness of bedding Barnyard	$\frac{1}{2}$	
roundings 1 Construction of stable Tight, sound floor and proper gutter	4		Clean 1 Well drained 1 Removal of manure daily to 50 feet from stable	2	
Smooth, tight walls and ceil- ing			Milk Room or Milk House. Cleanliness of milk room	3	
Provision for light: Four sq. ft., of glass per cow (Three sq. ft., 3; 2 sq. ft., 2; 1 sq. ft., 1. Deduct for un- even distribution.)	4		Utensils and Milking. Care and cleanliness of utensils Thoroughly wasned 2 Sterlized in steam for 15	8	
<ul> <li>Bedding</li></ul>	17		minutes	9	
Provision for controlling temperature	1 1 5 1 1		Handling the Milk. Cleanliness of attendants in milk room. Milk removed immediately from stable without pouring from pail Cooled inmediately after milk- ing each cow (51° to 55°, 4: 56° to 60°, 1.) Stored below 50° F (51° to 55°, 2: 56° to 60°, 1.) Transportation below 50° F	2	
Milk Room or Milk House. Location: Free from contaminat- ing surroundings Construction of milk room Floor, walls, and ceiling 1 Light, ventilation, screens	$\frac{1}{2}$		$(51^{\circ}$ to $55^{\circ}$ , 2: $56^{\circ}$ to $60^{\circ}$ , 1.) Transportation below $50^{\circ}$ F $(51^{\circ}$ to $55^{\circ}$ , $1.5; 56^{\circ}$ to $60^{\circ}$ , 1.) (If delivered twice a day, al- low perfect score for storage and transportation.)	51	
Separate rooms for washing utensils and handling milk Facilities for steam	1		1		
Total	40		Total	60	

#### STANDARDS FOR SKIM MILK.

Requirements.

1. It shall contain not less than 8.75 per cent milk solids.

2. Control of sale of skim milk: Whether skim milk is sold in wagons or in stores all containers holding skim milk should be painted some bright, distinctive color and prominently and legibly marked "skim milk." When skim milk is placed in the buyer's container, a label or tag bearing the words "skim milk" should be attached.

#### PRODUCTION OF PASTEURIZED MILK

Pasteurized milk is milk that is heated to a temperature of not less than  $140^{\circ}$  F. for not less than 20 minutes, or not over  $155^{\circ}$  F. for not less than 5 minutes, and for each degree of temperature over  $140^{\circ}$  F. the length of time may be 1 minute less than 20. Said milk shall be cooled immediately to  $50^{\circ}$  F. or below and kept at or below that temperature.

#### Cow Stables.

Requirements.

The same as for the production of raw milk.

#### Recommendations.

The same as for the production of raw milk.

#### Milk Room.

#### Requirements.

The same as for the production of raw milk.

#### Recommendations.

The same as for the production of raw milk.

#### Cows.

# Requirements.

The same as for the production of raw milk, with the exception of the sections relating to the tuberculin test.

#### Recommendations.

That no cows be added to a herd excepting those found to be free from tuberculosis by the tuberculin test.

# Employees.

#### Requirements.

The same as for the production of raw milk.

Recommendations.

The same as for the production of raw milk.

# Utensils.

Requirements.

The same as for the production of raw milk.

Recommendations.

The same as for the production of raw milk.

# Milk for Pasteurization.

Requirements.

The same as for the production of raw milk, with the ex-1. ception of sections 1, 2, and 6b.

2. It shall be cooled to 60° F. or below within two hours after it is drawn from the cow, and it shall be held at or below that temperature until it is pasteurized. After pasteurization, it shall be held at a temperature not exceeding 50° F. until delivered to the consumer.

3. Pasteurized milk shall be distinctly labeled as such, together with the temperature at which it is pasteurized and the shortest length of exposure to that temperature and the date of pasteurization.

# Recommendations.

1. No milk shall be repasteurized. 2. The requirements governing the production and handling of milk for pas-teurization should be raised wherever practicable.

# Pasteurizing Plants.

Requirements.

The same as under "Receiving stations and bottling plants" for raw milk.

# Recommendations.

The same as under "Receiving stations and bottling plants" for raw milk.

#### Stores.

Requirements.

The same as for raw milk.

# Recommendations.

The same as for raw milk.

# General Regulations.

#### Requirements.

1. It is recommended that dairies producing milk which is to be pasteurized shall be scored on the United States Bureau of Animal Industry score card, and that health departments, or the controlling departments whatever they may be, strive to bring these scores up as rapidly as possible.

2. Milk from cows that have been rejected by the tuberculin test, but which show no physical signs of tuberculosis, as well as those which have not been tested, may be sold provided that it is produced and handled in accordance with all the other requirements herein set forth for pasteurized milk.

3. Ice used for cooling purposes shall be clean.

#### Recommendations.

The same as for raw milk.

#### PRODUCTION OF PASTEURIZED CREAM.

#### Requirements.

1. It shall be obtained only from milk that could legally be sold as milk under the requirements hereinbefore set forth.

2. Pasteurized cream, or cream separated from pasteurized milk, shall be labeled in the manner herein provided for the labeling of pasteurized milk.

# STANDARDS FOR PASTEURIZED MILK.

#### Requirements.

1. It shall not contain more than 1,000,000 bacteria per cubic centimeter before pasteurization, nor over 50,000 when delivered to the consumer.

2. The standards for the percentage of milk fat and of total solids shall be the same as for raw milk.

# Recommendations.

 $1. \$  The limits for the bacterial count before pasteurization and after pasteurization should both be lowered if possible.

#### STANDARDS FOR PASTEURIZED CREAM.

# Requirements.

1. No cream shall be sold that is obtained from pasteurized milk that could not be legally sold under the provisions herein set forth, nor shall any cream that is pasteurized after separation contain an excessive number of bacteria. 2. There shall be a bacterial standard for pasteurized cream corresponding to the grade of milk from which it is made and to its butterfat content.

3. The percentage of milk fat shall be the same as for raw cream.

# SANITARY INSPECTION OF MILK PLANTS AND DAIRY FARMS.

Blank forms of the latest United States Bureau of Animal Industry score cards which have been previously referred to, are shown on pages 251-252. Experience has shown that there is no system of sanitary inspection so efficient as that obtained by the use of these cards. Every condition pertaining to the milk is considered and rated mathematically according to its importance. The completed score gives an accurate survey of the facts in a comparative manner which may serve as a permanent record, far more reliable in character than is a mere inspection unaided by the score card. This system not only provides a uniform and systematic summary, but it also has a tendency to stimulate the producer to increased efforts in overcoming the defects which reduce his total score.

#### PRODUCTION OF AND STANDARDS FOR CERTIFIED MILK.

The methods and standards for the production and distribution of certified milk, adopted by the American Association of Medical Milk Commissions, May 1, 1912, contain all the necessary provisions for the preparation of this special milk which undoubtedly leads all classes of milk as a food for infants.

# Hygiene of the Dairy.

UNDER THE SUPERVISION AND CONTROL OF THE VETERINARIAN.

1. Pastures or paddocks.—Pastures or paddocks to which the cows have access shall be free from marshes or stagnant pools, crossed by no stream which might become dangerously contaminated, at sufficient distances from offensive conditions to suffer no bad effects from them, and shall be free from plants which affect the milk deleteriously.

2. Surroundings of buildings.—The surroundings of all buildings shall be kept clean and free from accumulations of dirt, rubbish, decayed vegetable or animal matter or animal waste, and the stable yard shall be well drained.

3. Location of buildings.—Buildings in which certified milk is produced and handled shall be so located as to insure proper shelter and good drainage, and at sufficient distance from other buildings, dusty roads, cultivated and dusty fields, and all other possible sources of contamination; provided, in the case of unavoidable proximity to dusty roads or fields, the exposed side shall be screened with cheesecloth.

4. Construction of stables.—The stables shall be constructed so as to facilitate the prompt and easy removal of waste products. The floors and platforms shall be made of cement or other nonabsorbent material and the gutters of cement only. The floors shall be properly graded and drained, and the manure gutters shall be from 6 to 8 inches deep and so placed in relation to the platform that all manure will drop into them.

5. The inside surface of the walls and all interior construction shall be smooth, with tight joints, and shall be capable of shedding water. The ceiling shall be of smooth material and dust tight. All horizontal and slanting surfaces which might harbor dust shall be avoided.

6. Drinking and feed troughs.—Drinking troughs or basins shall be drained and cleaned each day, and feed troughs and mixing floors shall be kept in a clean and sanitary condition.

7. Stanchions.—Stanchions, when used, shall be constructed of iron pipes or hardwood, and throat latches shall be provided to prevent the cows from lying down between the time of cleaning and the time of milking.

8. Ventilation.—The cow stables shall be provided with adequate ventilation either by means of some approved artificial device, or by the substitution of cheesecloth for glass in the windows, each cow to be provided with a minimum of 600 cubic feet of air space.

9. Windows.—A sufficient number of windows shall be installed and so distributed as to provide satisfactory light and a maximum of sunshine, 2 feet square of window area to each 600 cubic feet of air space to represent the minimum. The coverings of such windows shall be kept free from dust and dirt.

10. Exclusion of flies, etc.—All necessary measures should be taken to prevent the entrance of flies and other insects and rats and other vermin into all the buildings.

11. Exclusion of animals from the herd.—No horses, hogs, dogs, or other animals or fowls shall be allowed to come in contact with the certified herd, either in the stables or elsewhere.

12. Bedding.—No dusty or moldy hay or straw, bedding from horse stalls, or other unclean materials shall be used for bedding the cows. Only bedding which is clean, dry, and absorbent may be used, preferably shavings or straw.

13. Cleaning stable and disposal of manure.—Soiled bedding and manure shall be removed at least twice daily, and the floors shall be swept and kept free from refuse. Such cleaning shall be done at least one hour before the milking time. Manure, when removed, shall be drawn to the field or temporarily stored in containers so screened as to exclude flies. Manure shall not be even temporarily stored within 300 feet of the barn or dairy building.

14. Cleaning of cows.—Each cow in the herd shall be groomed daily, and no manure, mud, or filth shall be allowed to remain

upon her during milking; for cleaning, a vacuum apparatus is recommended.

15. *Clipping.*—Long hairs shall be clipped from the udder and flanks of the cow and from the tail above the brush. The hair on the tail shall be cut so that the brush may be well above the ground.

16. Cleaning of udders.—The udders and teats of the cow shall be cleaned before milking; they shall be washed with a cloth and water, and dry wiped with another clean sterilized cloth—a separate cloth for drying each cow.

17. Feeding.—All foodstuffs shall be kept in an apartment separate from and not directly communicating with the cow barn. They shall be brought into the barn only immediately before the feeding hour, which shall follow the milking.

18. Only those foods shall be used which consist of fresh, palatable, or nutritious materials, such as will not injure the health of the cows or unfavorably affect the taste or character of the milk. Any dirty or moldy food or food in a state of decomposition or putrefaction shall not be given.

19. A well-balanced ration shall be used, and all changes of food shall be made slowly. The first few feedings of grass, alfalfa, ensilage, green corn, or other green feeds shall be given in small rations and increased gradually to full ration.

20. *Exercise*.—All dairy cows shall be turned out for exercise at least 2 hours in each 24 in suitable weather. Exercise yards shall be kept free from manure and other filth.

21. Washing of hands.—Conveniently located facilities shall be provided for the milkers to wash in before and during milking.

22. The hands of the milkers shall be thoroughly washed with soap, water, and brush and carefully dried on a clean towel immediately before milking. The hands of the milkers shall be rinsed with clean water and carefully dried before milking each cow. The practice of moistening the hands with milk is forbidden.

23. *Milking clothes.*—Clean overalls, jumper, and cap shall be worn during milking. They shall be washed or sterilized each day and used for no other purpose, and when not in use they shall be kept in a clean place, protected from dust and dirt.

24. Things to be avoided by milkers.—While engaged about the dairy or in handling the milk employees shall not use tobacco nor intoxicating liquors. They shall keep their fingers away from their nose and mouth, and no milker shall permit his hands, fingers, lips, or tongue to come in contact with milk intended for sale.

25. During milking the milkers shall be careful not to touch anything but the clean top of the milking stool, the milk pail, and the cow's teats.

26. Milkers are forbidden to spit upon the walls or floors

of stables, or upon the walls or floors of milk houses, or into the water used for cooling the milk or washing the utensils.

27. Fore milk.—The first streams from each teat shall be rejected, as this fore milk contains large numbers of bacteria. Such milk shall be collected into a separate vessel and not milked onto the floors or into the gutters. The milking shall be done rapidly and quietly, and the cows shall be treated kindly.

28. *Milk and calving period.*—Milk from all cows shall be excluded for a period of 45 days before and 7 days after parturition.

29. Bloody and stringy milk.—If milk from any cow is bloody and stringy or of unnatural appearance, the milk from that cow shall be rejected and the cow isolated from the herd until the cause of such abnormal appearance has been determined and removed, special attention being given in the meantime to the feeding or to possible injuries. If dirt gets into the pail, the milk shall be discarded and the pail washed before it is used.

30. *Make-up of herd.*—No cows except those receiving the same supervision and care as the certified herd shall be kept in the same barn or brought in contact with them.

31. Employees other than milkers.—The requirements for milkers, relative to garments and cleaning of hands, shall apply to all other persons handling the milk, and children unattended by adults shall not be allowed in the dairy nor in the stable during milking.

32. Straining and strainers.—Promptly after the milk is drawn it shall be removed from the stable to a clean room and then emptied from the milk pail to the can, being strained through strainers made of a double layer of finely meshed cheesecloth or absorbent cotton thoroughly sterilized. Several strainers shall be provided for each milking in order that they may be frequently changed.

33. Dairy building.—A dairy building shall be provided which shall be located at a distance from the stable and dwelling prescribed by the local commission, and there shall be no hogpen, privy, or manure pile at a higher level or within 300 feet of it.

34. The dairy building shall be kept clean and shall not be used for the purposes other than the handling and storing of milk and milk utensils. It shall be provided with light and ventilation, and the floors shall be graded and water-tight.

35. The dairy building shall be well lighted and screened and drained through well-trapped pipes. No animals shall be allowed therein. No part of the dairy building shall be used for dwelling or lodging purposes, and the bottling room shall be used for no other purpose than to provide a place for clean milk utensils and for handling the milk. During bottling this room shall be entered only by persons employed therein. The bottling room shall be kept scrupulously clean and free from odors.

36. Temperature of milk.—Proper cooling to reduce the tem-

perature to  $45^{\circ}$  F. shall be used, and aerators shall be so situated that they can be protected from flies, dust, and odors. The milk shall be cooled immediately after being milked, and maintained at a temperature between  $35^{\circ}$  and  $45^{\circ}$  F. until delivered to the consumer.

37. Sealing of bottles.—Milk, after being cooled and bottled, shall be immediately sealed in a manner satisfactory to the commission, but such seal shall include a sterile hood which completely covers the lip of the bottle.

38. Cleaning and sterilizing of bottles.—The dairy building shall be provided with approved apparatus for the cleansing and sterilizing of all bottles and utensils used in milk production. All bottles and utensils shall be thoroughly cleaned by hot water and sal soda, or equally pure agent, rinsed until the cleaning water is thoroughly removed, then exposed to live steam or boiling water at least 20 minutes, and then kept inverted until used, in a place free from dust and other contaminating materials.

39. Utensils.—All utensils shall be so constructed as to be easily cleaned. The milk pail should preferably have an elliptical opening 5 by 7 inches in diameter. The cover of this pail should be so convex as to make the entire interior of the pail visible and accessible for cleaning. The pail shall be made of heavy seamless tin, and with seams which are flushed and made smooth by solder. Wooden pails, galvanized-iron pails, or pails made of rough, porous materials, are forbidden. All utensils used in milking shall be kept in good repair.

40. Water supply.—The entire water supply shall be absolutely free from contamination, and shall be sufficient for all dairy purposes. It shall be protected against flood or surface drainage, and shall be conveniently situated in relation to the milk house.

41. Privies, etc., in relation to water supply.—Privies, pigpens, manure piles, and all other possible sources of contamination shall be so situated on the farm as to render impossible the contamination of the water supply, and shall be so protected by use of screens and other measures as to prevent their becoming breeding grounds for flies.

42. Toilet rooms.—Toilet facilities for the milkers shall be provided and located outside of the stable or milk house. These toilets shall be properly screened, shall be kept clean, and shall be accessible to wash basins, water, nail brush, soap and towels, and the milkers shall be required to wash and dry their hands immediately after leaving the toilet room.

# Transportation.

43. In transit the milk packages shall be kept free from dust and dirt. The wagon, trays, and crates shall be kept scrupulously clean. No bottles shall be collected from houses in which communicable diseases prevail, unless a separate wagon is used and under conditions prescribed by the department of health and the medical milk commission.

44. All certified milk shall reach the consumer within 30 hours after milking.

# Veterinary Supervision of the Herd.

45. *Tuberculin test.*—The herd shall be free from tuberculosis, as shown by the proper application of the tuberculin test. The test shall be applied in accordance with the rules and regulations of the United States Government, and all reactors shall be removed immediately from the farm.

46. No new animals shall be admitted to the herd without first having passed a satisfactory tuberculin test, made in accordance with the rules and regulations mentioned; the tuberculin to be obtained and applied only by the official veterinarian of the commission.

47. Immediately following the application of the tuberculin test to a herd for the purpose of eliminating tuberculous cattle, the cow stable and exercising yards shall be disinfected by the veterinary inspector in accordance with the rules and regulations of the United States Government.

48. A second tuberculin test shall follow each primary test after an interval of six months, and shall be applied in accordance with the rules and regulations mentioned. Thereafter, tuberculin tests shall be reapplied annually, but it is recommended that the retests be applied semi-annually.

49. Identification of cows.—Each dairy cow in each of the certified herds shall be labeled or tagged with a number or mark which will permanently identify her.

50. *Herd-book record.*—Each cow in the herd shall be registered in a herd book, which register shall be accurately kept so that her entrance and departure from the herd and her tuberculin testing can be identified.

51. A copy of this herd-book record shall be kept in the hands of the veterinarian of the medical milk commission under which the dairy farm is operating, and the veterinarian shall be made responsible for the accuracy of this record.

52. Dates of tuberculin tests.—The dates of the annual tuberculin tests shall be definitely arranged by the medical milk commission, and all of the results of such tests shall be recorded by the veterinarian and regularly reported to the secretary of the medical milk commission issuing the certificate.

53. The results of all tuberculin tests shall be kept on file by each medical milk commission, and a copy of all such tests shall be made available to the American Association of Medical Milk Commissions for statistical purposes.

54. The proper designated officers of the American Association of Medical Milk Commissions should receive copies of reports of all of the annual, semiannual, and other official tuberculin tests which are made and keep copies of the same on file and compile them annually for the use of the association.

55. Disposition of cows sick with diseases other than tuberculosis.—Cows having rheumatism, leucorrhea, inflammation of the uterus, severe diarrhea, or disease of the udder, or cows that from any other cause may be a menace to the herd shall be removed from the herd and placed in a building separate from that which may be used for the isolation of cows with tuberculosis, unless such building has been properly disinfected since it was last used for this purpose. The milk from such cows shall not be used nor shall the cows be restored to the herd until permission has been given by the veterinary inspector after a careful physical examination.

56. Notification of veterinary inspector.—In the event of the occurrence of any of the diseases just described between the visits of the veterinary inspector, or if at any time a number of cows become sick at one time in such a way as to suggest the outbreak of a contagious disease or poisoning, it shall be the duty of the dairyman to withdraw such sickened cattle from the herd, to destroy their milk, and to notify the veterinary inspector by telegraph or telephone immediately.

57. *Emaciated cows.*—Cows that are emaciated from chronic diseases or from any cause that in the opinion of the veterinary inspector may endanger the quality of the milk, shall be removed from the herd.

#### Bacteriological Standards.

58. Bacterial counts.—Certified milk shall contain less than 10,000 bacteria per cubic centimeter when delivered. In case a count exceeding 10,000 bacteria per cubic centimeter is found, daily counts shall be made, and if normal counts are not restored within 10 days the certificate shall be suspended.

59. Bacterial counts shall be made at least once a week.

60. Collection of samples.—The samples to be examined shall be obtained from milk as offered for sale and shall be taken by a representative of the milk commission. The samples shall be received in the original packages, in properly iced containers, and they shall be so kept until examined, so as to limit as far as possible changes in their bacterial content.

61. For the purpose of ascertaining the temperature, a separate original package shall be used, and the temperature taken at the time of collecting the sample, using for the purpose a standardized thermometer graduated in the centigrade scale.

62. Interval between milking and plating.—The examinations shall be made as soon after collection of the samples as possible, and in no case shall the interval between milking and plating the samples be longer than 40 hours.

63. Plating.—The packages shall be opened with aseptic precautions after the milk has been thoroughly mixed by vigorously reversing and shaking the container 25 times.

Two plates at least shall be made for each sample of 64. milk, and there shall also be made a control of each lot of medium and apparatus used at each testing. The plates shall be grown at 37° C. for 48 hours.

In making the plates there shall be used agar-agar media 65. containing 1.5 per cent agar and giving a reaction of 1.0 to phenolphthalein.

The following is the method recommended by a committee of the American Public Health Association for the making of the media, modified, however, as to the agar content and reaction to conform to the requirements specified in section 65:

1. Boil 15 grams of thread agar in 500 c. c. of water for half an hour and make up weight to 500 grams or digest for 10 minutes in the autoclave at 110° C. Let this cool to about 60° C.

2. Infuse 500 grams finely chopped lean beef for 24 hours with its own weight of distilled water in the refrigerator.

3. Make up any loss by evaporation.

4. Strain infusion through cotton flannel, using pressure.

Weigh filtered infusion. 5.

6. Add Witte's peptone, 2 per cent.

7. Warm on water bath, stirring until peptone is dissolved and not allowing temperature to rise above 60° C.

8. To the 500 grams of meat infusion (with peptone) add 500 grams of the 2 per cent agar, keeping the temperature below 60° C.

9. Heat over boiling water (or steam) bath 30 minutes.

10. Restore weight lost by evaporation.

11. Titrate after boiling one minute to expel carbonic acid.

12.Adjust reaction to final point desired  $\pm 1$  by adding normal sodium hydrate.

Boil two minutes over free flame, constantly stirring. 13.

Restore weight lost by evaporation. 14

Filter through absorbent cotton or coarse filter paper, passing the filtrate 15. through the filter repeatedly until clear.

16. Titrate and record the final reaction.

17. Tube (10 c. c. to a tube) and sterilize in autoclave one hour at 15 pounds pressure or in the streaming steam for 20 minutes on three successive days.

66. Samples of milk for plating shall be diluted in the proportion of 1 part of milk to 99 parts of sterile water; shake 25 times and plate 1 c. c. of the dilution.

The committee on bacterial milk analyses of the American Public Health Association in Part IV of its report presented details with respect to plating apparatus and technique in part as follows:

Plating apparatus.—For plating it is best to have a water bath in which to melt the media and a water-jacketed water bath for keeping it at the required temperature; a wire rack which should fit both the water baths for holding the media tubes; a thermometer for recording the temperature of the water in the water-jacketed bath, sterile 1 c. c. pipettes, sterile Petri dishes, and sterile dilution water in measured quantities.

Dilutions.—Ordinary potable water, sterilized, may be used for dilutions. Oc-casionally spore forms are found in such water which resist ordinary autoclave sterilization; in such cases distilled water may be used or the autoclave pressure increased. With dilution water in 8-ounce bottles calibrated for 99 cubic centimeters all the necessary dilutions may be made. Short, wide-mouthed ''blakes'' or wide-mouthed French square bottles are more

easily handled and more economical of space than other forms of bottles or flasks.

Eight-ounce bottles are the best, as the required amount of dilution water only about half fills them, leaving room for shaking. Long-fiber nonabsorbent cotton should be used for plugs. It is well to use care in selecting cotton for this purpose to avoid short-fiber or dusty cotton, which give a cloud of lint-like particles on shaking. Bottles \* \* \* should be filled a little over the 99 c. c. \* \* \* to allow for loss during sterilization.

*Pipettes.*—Straight sides 1 c. c. pipettes are more easily handled than those with bulbs; they may be made from ordinary three-sixteenths inch glass tubing and should be about 10 inches in length.

Be about to incluse in longent Plating technique.—The agar after melting should be kept in the water-jacketed water bath between 40° C, and 45° C, for at least 15 minutes before using to make sure that the agar itself has reached the temperature of the surrounding water. If used too warm the heat may destroy some of the bacteria or retard their growth.

Shake the milk sample 25 times, then with a sterile pipette transfer 1 c. c. to the first dilution water and rinse the pipette by drawing dilution water to the mark and expelling; this gives a dilution 1 to 100.

\* \* \* Then with a sterile pipette transfer 1 c. c. to the Petri dish, using care to raise the cover only as far as necessary to insert the end of the pipette.

Take the tube of agar from the water bath, wipe the water from outside the tube with a piece of cloth, remove the plug, pass the mouth of the tube through a flame, and pour the agar into the plate, using the same care as before to avoid exposure of the plate contents to the air.

Carefully and thoroughly mix the agar and diluted milk in the Petri dish by a rotary motion, avoiding the formation of air bubbles or slopping the agar, and after allowing the agar to harden for at least 15 minutes at room temperature, place the dish bottom down in the incubator.

Plating should always be done in a place free from dust or currents of air.

In order that colonies may have sufficient food for proper development 10 c. c. of agar shall be used for each plate.

67. Determination of taste and odor of milk.—After the plates have been prepared and placed in the incubator, the taste and odor of the milk shall be determined after warming the milk to  $100^{\circ}$  F.

68. *Counts.*—The total number of colonies on each plate should be counted, and the results expressed in multiples of the dilution factor. Colonies too small to be seen with the naked eye or with slight magnification shall not be considered in the count.

69. Records of bacteriologic tests.—The results of all bacterial tests shall be kept on file by the secretary of each commission, copies of which should be made available annually for the use of the American Association of Medical Milk Commissions.

# Chemical Standards and Methods.

The methods that must be followed in carrying out the chemical investigations essential to the protection of certified milk are so complicated that in order to keep the fees of the chemist at a reasonable figure, there must be eliminated from the examination those procedures which, whilst they might be helpful and interesting, are in no sense necessary.

For this reason the determination of the water, the total solids and the milk sugar is not required as a part of the routine examination.

70. The chemical analyses shall be made by a competent chemist designated by the medical milk commission.

71. *Method of obtaining samples.*—The samples to be examined by the chemist shall have been examined previously by the

bacteriologist designated by the medical milk commission as to temperature, odor, taste, and bacterial content.

72. Fat standards.—The fat standard for certified milk shall be 4 per cent, with a permissible range of variation of from 3.5 to 4.5 per cent.

73. The fat standard for certified cream shall be not less than 18 per cent.

74. If it is desired to sell higher fat-percentage milks or creams as certified milks or creams, the range of variation for such milks shall be 0.5 per cent on either side of the advertised percentage and the range of variations for such creams shall be 2 per cent on either side of the advertised percentage.

75. The fat content of certified milks and creams shall be determined at least once each month.

76. The methods recommended for this purpose are the Babcock (a), the Leffmann-Beam (b), and the Gerber (c).

(a) Babcock test.—The Babcock test is based on the fact that strong sulphuric acid will dissolve the nonfatty solid constituents of milk, and thus enable the fat to separate on standing. It can be conducted by any of the Babcock outfits which are purchasable in the market.

"The test is made by placing in the special test bottle 18 grams (17.6 c. c.) of milk. To this is added, from a pipette, burette, or measuring bottle, 17.5 c. c. commercial subhuric acid of a specific gravity of 1.82 to 1.83. The contents of the bottle are carefully and thoroughly mixed by a rotary motion. The mixture becomes brown and heat is generated. The test bottle is now placed in a properly balanced centrifuge and whirled for 5 minutes at a speed of from 800 to 1,200 revolutions per minute. Hot water is then added to fill the bottle to the lower part of the neck, after which it is again whirled for two minutes. Now, enough hot water is added to float the column of fat into the graduated portion of the neck of the bottle, and the whirling is repeated for a minute. The amount of fat is read while the neck of the bottle is still hot. The reading is from the upper limits of the meniscus. A pair of calipers is of assistance in measuring the column of fat.'' (Jensen's Milk Hygiene, Leonard Pearson's translation.)

(b) Leff mann-Beam test.—The distinctive feature is the use of fusel oil, the effect of which is to produce a greater difference in surface tension between the fat and the liquid in which it is suspended, and thus promote its readier separation. This effect has been found to be heightened by the presence of a small amount of hydrochloric acid.

The test bottles have a capacity of about 30 c. c. and are provided with a graduated neck, each division of which represents 9.1 per cent by weight of butter fat.

Fifteen centimeters of the milk are measured into the bottle, 3 c. c. of a mixture of equal parts of amyl alcohol and strong hydrochloric acid added and mixed. Then 9 c. c. of concentrated sulphuric acid is added in portions of about 1 c. c.; after each addition the liquids are mixed by giving the bottle a gyratory motion. If the fluid has not lost all of its milky color by this treatment, a little more concentrated acid must be added. The neck of the bottle is now immediately filled at about the zero point with one part sulphuric acid and two parts water, well mixed just before using. Both the liquid in the bottle and the diluted acid must be hot. The bottle is then placed at once in the centrifugal machine; after rotation from one to two minutes, the fat will collect in the neck of the bottle and the percentage may be read off.

(c) Gerber's test.—This test is applied as follows: The test bottles are put into the stand with the mouths uppermost; then, with the pipette designed for the purpose, or with an automatic measurer, 10 c. c. of sulphuric acid are filled into the test bottle, care being taken not to allow any to come in contact with the neck. The few drops remaining in the tip of the pipette should not be blown out. Then 11 c. c. of milk are measured with the proper pipette and allowed to flow slowly onto the acid, so that the two liquids mix as little as possible. Finally, the amyl alcohol is added. (It is important to use the reagents in the proper order, which is—sulphuric acid, milk, amyl alcohol. If the sulphuric acid is followed by amyl alcohol and the milk last, then the result is sometimes incorrect.) A rubber stopper, which must not be damaged, is then fitted into the mouth of the test bottle, and the contents are well shaken, the thumb being kept on the stopper to prevent it coming out. As a considerable amount of heat is generated by the action of the sulphuric acid on the milk, the test bottle should be wrapped in a cloth.

The shaking of the sample must be done thoroughly and quickly, and the test bottle inverted several times, so that the liquid in the neck becomes thoroughly mixed. By pressing in the rubber stopper the height of the liquid can be brought to about the zero point on the scale.

Zero point on the scale. If only a few samples have to be analyzed and the room is warm, the test bottles can be put into the centrifuge without any preliminary heating, otherwise the test bottles must be warmed for a few minutes (not longer) in the water bath at a temperature of  $60^{\circ}$  to  $65^{\circ}$  C. When the temperature rises higher than this, say above  $70^{\circ}$  C., the rubber stopper is liable to be blown out of the test bottle. After the test bottles have been heated they are arranged symmetrically in the centrifuge and whirled for 3 to 4 minutes at a speed of about 1,000 revolutions per minute. When the centrifuge has a heating arrangement attached to it the preliminary minute. When the centrifuge has a heating arrangement attached to it, the preliminary warming is not, of course, necessary. When the test bottles are taken out of the centrifuge, they are again placed in the water bath at a temperature of 60° to 65° C., and left there for several minutes before being read; where the centrifuge is heated, the tubes can be read off as taken from the centrifuge.

By carefully screwing in the rubber stopper, or even by pressing it, the lower limit of the fat column is brought onto one of the main divisions of the scale, and then, by holding the test bottle against the light, the height of the column of fat can be accurately ascertained. The lowest point of the meniscus is taken as the level when reading the upper surface of the fat in a sample of whole milk, and the middle of the meniscus for separated milk. If the column of fat is not clear and sharply defined, the sample must be again

whirled in the centrifuge.

Each division on the scale is equivalent to 0.1 per cent, so it is very easy to read to 0.05 per cent, or, with a lens, to 0.025 per cent. If the number which is read off is multiplied by 0.1, then the percentage quantity of fat in the milk is obtained; e. g., if the number on the scale was 36.5, then the percentage of fat is 3.65. (Milk and Dairy Products, Barthel; translated by Goodvin, p. 71.)

77. Before condemning samples of milk which have fallen outside the limits allowed, the chemist shall have determined, by control ether extractions, that his apparatus and his technique are reliable.

78. Protein standard.—The protein standard for certified milk shall be 3.50 per cent, with a permissible range of variation of from 3 to 4 per cent.

The protein standard for certified cream shall corre-79. spond to the protein standard for certified milk.

80. The protein content shall be determined only when any special consideration seems to the medical milk commission to make it desirable.

81. It shall be determined by the Kjeldahl method, using the Gunning or some other reliable modification, and employing the factor 6.25 in reckoning the protein from the nitrogen.

Kjeldahl method.-Five cubic centimeters of milk are measured carefully into a flat-bottom 800 c. c. Jena flask, 20 c. c. of concentrated sulphuric acid (C. P.; sp. gr., 1.84) are added, and 0.7 gram of mercuric oxid (or its equivalent in metallic mercury); the mixture is then heated over direct flame until it is straw-colored or perfectly white; a few crystals of potassium permanganate are now added till the color of the liquid remains green. All the nitrogen in the milk has then been converted into the form of animonium sulphate. After cooling, 200 c. c. of aminonia-free distilled water are added, 20 c. c. of a solution of potassium sulphide (containing 40 grams sulphide Here and the solution of a solution of polassian subjunct (containing to grains subjunct per liter), and a fraction of a gram of powdered zine. A quantity of semi-normal HCl solution more than sufficient to neutralize the ammonia obtained in the oxidation of the milk is now carefully measured out from a delicate burette (divided into 1/20 c. c.) into an Erlenmeyer flask and the flask connected with a distillation apparatus. At the other end the Jena flask containing the watery solution of the ammonium

sulphate is connected, after adding 50 c. c. of a concentrated soda solution (1 pound "pure potash" dissolved in 500 c. c. of distilled water and allowed to settle); the contents of the Jena flask are now heated to boiling, and the distillation is continued for 40 minutes to an hour, until all ammonia has been distilled over.

The excess of acid in the Erlenmeyer receiving flask is then accurately titrated back by means of a tenth-normal standard ammonia solution, using a cochineal solution as an indicator. From the amount of acid used the per cent of nitrogen is obtained; and from it the per cent of casein and albumen in the milk by multiplying by 6.25. The amount of nitrogen contained in the chemicals used is determined by blank experiments and deducted from the nitrogen obtained as described. (Farrington and Woll, Testing Milk and Its Products, p. 221.)

82. Coloring matter and preservatives.—All certified milks and creams shall be free from adulteration, and coloring matter shall not be added thereto.

83. Tests for the detection of added coloring matter shall be applied whenever the color of the milk or cream is such as to arouse suspicion.

Test for coloring matter.—The presence of foreign coloring matter in milk is easily shown by shaking 10 c. c. of the milk with an equal quantity of ether; on standing, a clear ether solution will rise to the surface; if artificial coloring matter has been added to the milk, the solution will be yellow colored, the intensity of the color indicating the quantity added; natural fresh milk will give a colorless ether solution. (Testing Milk and Its Products, Farrington and Woll, p. 244.)

84. Tests for the detection of formaldehyde, borax, and boracic acid shall be applied at least once each month. Occasionally application of tests for the detection of salicylic acid, benzoic acid, and the benzoates is also recommended.

Test for the detection of formaldehyde.—Five cubic centimeters of milk is measured into a white porcelain dish, and a similar quantity of water added; 10 c. c. of HCl, containing a trace of Fes Cle, is added, and the mixture is heated very slowly. If formaldehyde is present, a violet color will be formed. (Testing Milk and Its Products, Farrington and Woll, p. 249.) Test for boracic acid (borax, borates, preservaline, etc.).—One hundred cubic centimeters of milk are made alkaline with a soda or potash solution, and then evaporated to dryness and incinerated. The ash is dissolved in water, to which a little hydrochloric.

Test for boracic acid (borax, borates, preservaline, etc.).—One hundred cubic centimeters of milk are made alkaline with a soda or potash solution, and then evaporated to dryness and incinerated. The ash is dissolved in water, to which a little hydrochloric acid has been added, and the solution filtered. A strip of turmeric paper moistened with the filtrate will be colored reddish brown when dried at 100°C. on a watch glass, if boracic acid is present.

If a little alcohol is poured over the ash to which concentrated sulphuric acid has been added, and fire is set to the alcohol, after a little while this will burn with a yellowish-green tint, especially noticeable if the ash is stirred with a glass rod and when the flame is about to go out. (Testing Milk and Its Products, Farrington and Woll, p. 247.) Test for salicylic acid (salicylates, etc.).—Twenty cubic centimeters of milk are

Test for salicylic acid (salicylates, etc.).—Twenty cubic centimeters of milk are acidulated with sulphuric acid and shaken with ether; the ether solution is evaporated, and the residue treated with alcohol and a little iron-chlorid solution; a deep violet color will be obtained in the presence of salicylic acid. (Testing Milk and Its Products, Farrington and Woll, p. 248.)

Test for benzoic acid.—Two hundred and fifty to five hundred cubic centimeters of milk are made alkaline with a few drops of lime or baryta water, and then evaporated to about a quarter of the bulk. Powdered gypsum is stirred into the remaining liquid until a paste is formed, which is then dried on the water bath. The gypsum only serves to hasten the drying, and powdered pumice stone or sand can be used equally well. When the mass is dry, it is finely powdered and moistened with dilute sulphurie acid and shaken out three or four times with about twice the volume of 50 per cent alcohol, in which benzoic acid is easily soluble in the cold, the fat only being dissolved to a very slight extent or not at all. The acid alcoholic liquid from the various extractions, which contains milk sugar and inorganic saits in addition to the benzoic acid, is neutralized with baryta water and evaporated to a small bulk. Dilute sulphuric acid is again added, and the liquid shaken out with small quantities of ether. On evaporation of the ether, the benzoic acid is left behind in almost pure state, the only impurities being small quantities of fat or ash.

The benzoic acid which is obtained is dissolved in a small quantity of warm water, a drop of sodium acetate and neutral ferric chloride added, and the red precipitate of benzoate of iron indicates the presence of the acid. (Milk and Dairy Products, Barthel; translated by Goodwin, p. 121.)

85. Detection of heated milk.—Certified milk or cream shall not be subjected to heat unless specially directed by the commission to meet emergencies.

86. Tests to determine whether such milks and creams have been subjected to heat shall be applied at least once each month.

Detection of heated milk.—Storch's method.—Five cubic centimeters of milk are poured into a test tube; a drop of weak solution of hydrogen dioxide (about 0.2 per cent) which contains about 0.1 per cent sulphuric acid, is added, and two drops of a 2 per cent solution of paraphenylendiamin (solution should be renewed quite often), then the fluid is shaken. If the milk or the cream becomes, at once, indigo blue, or the whey violet or reddish brown, then this has not been heated or, at all events, it has not been heated higher than 78° C. (172.5° F.); if the milk becomes a light bluish gray immediately or in the course of half a minute, then it has been heated to 79° to 80° C. (174.2° to 176° F.). If the color remains white, the milk has been heated at least to 80° C. (176° F.). In the examination of sour milk or sour buttermilk, lime water must be added, as the color reaction is not shown in acid solution.

Arnold's gualac method.—A little milk is poured into a test tube and a little tincture of gualac is added, drop by drop. If the milk has not been heated to  $80^{\circ}$  C. (176° F.) a blue zone is formed between the two fluids; heated milk gives no reaction, but remains white. The gualac tincture should not be used perfectly fresh, but should have stood a few days and its potency have been determined. Thereafter it can be used indefinitely. These tests for heated milk are only active in the case of milks which have been heated to 176° F. or 80° C. (Jensen's Milk Hygiene, Pearson's translation, p. 192.)

Microscopic test for heated (pasteurized) milk—Frost and Ravenel.—About 15 c. c. of milk are centrifuged for 5 minutes, or long enough to throw down the leucocytes. The cream layer is then completely removed with absorbent cotton and the milk drawn off with a pipette, or a fine-pointed tube attached to a Chapman air pump. Only about 2 mm. of milk are left above the sediment which is in the bottom of the sediment tube.

The stain, which is an aqueous solution of safranin 0, soluble in water, is then added very slowly from an opsonizing pipette. The important thing is to mix stain and milk so slowly that clotting does not take place. The stain is added until a deep opaque rose color is obtained. After standing 3 minutes, by means of the opsonizing pipette, which has been washed out in hot water, the stained sediment is then transferred to slides. A small drop is placed at the end of each of several slides and spread by means of a glass spreader, as in Wright's method for opsonic index determinations.

In an unheated milk the polymorphonuclear leucocytes have their protoplasm slightly tinged or are unstained.

In heated milk the polymorphonuclear leucocytes have their nuclei stained. In milk heated to 63° C. or above, practically all of the leucocytes have their nuclei definitely stained. When milk is heated at a lower temperature the nuclei are not all stained above 60° C. The majority, however, are stained.

87. Specific gravity.—The specific gravity of certified milk shall range from 1.029 to 1.034.

88. The specific gravity shall be determined at least each month.

The Quevenne lactodensimeter is recommended for the determination of the specific gravity. It is made like an ordinary aerometer and divided into degrees which correspond to a specific gravity from 1.014 to 1.040, or only 1.022 to 1.038, since by the latter division a greater space is gained between the different degrees without unduly lengthening the instrument. From such a lactodensimeter one can easily read off four decimal places.

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The milk the specific gravity of which is to be determined is well shaken and poured into a high glass cylinder of suitable diameter; the aerometer is dropped in slowly, in order to prevent its bobbing up and down. (The bulb should be free from adhering air bubbles.) The figures on the stem are the second and third decimals of the numbers of the specific gravity, so that 34 is to be read 1.034. For this examination, the temperature of the milk must be  $15^{\circ}$  C. ( $60^{\circ}$  F.); if it is not, the specific gravity of the milk at  $15^{\circ}$  C. must be calculated from the specific gravity found and from the temperature, for in milk inspection and analysis this is the standard.

## Methods and Regulations for the Medical Examination of Employees—Their Health and Personal Hygiene.

89. A medical officer, known as the attending dairy physician, shall be selected by the commission, who should reside near the dairy producing certified milk. He shall be a physician in good standing and authorized by law to practice medicine; he shall be responsible to the commission and subject to its direction. In case more than one dairy is under the control of the commission and they are in different localities, a separate physician should be designated for employment for the supervision of each dairy.

90. Before any person shall come on the premises to live and remain as an employee, such person, before being engaged in milking or the handling of milk, shall be subjected to a complete physical examination by the attending physician. No person shall be employed who has not been vaccinated recently or who upon examination is found to have a sore throat, or to be suffering from any form of tuberculosis, venereal disease, conjunctivities, diarrhea, dysentery, or who has recently had typhoid fever or is proved to be a typhoid carrier, or who has any inflammatory disease of the respiratory tract, or any suppurative process or infectious skin eruption, or any disease of an infectious or contagious nature, or who has recently been associated with children sick with contagious disease.

91. In addition to ordinary habits of personal cleanliness all milkers shall have well-trimmed hair, wear close-fitting caps, and have clean shaven faces.

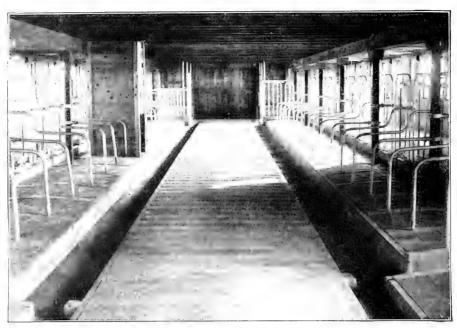
92. When the milkers live upon the premises their dormitories shall be constructed and operated according to plans approved by the commission. A separate bed shall be provided for each milker and each bed shall be kept supplied with clean bedclothes. Proper bathing facilities shall be provided for all employees on the dairy premises, preferably a shower bath, and frequent bathing shall be enjoined.

93. In case the employees live on the dairy premises a suitable building shall be provided to be used for the isolation and quarantine of persons under suspicion of having a contagious disease.

The following plan of construction is recommended:

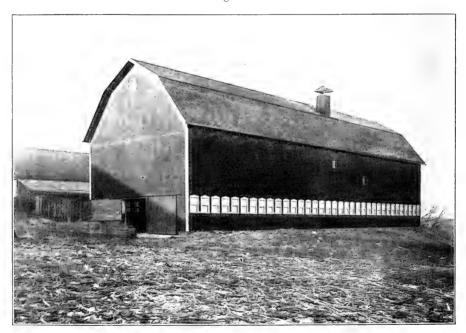
The quarantine building and hospital should be one story high and contain at least two rooms, each with a capacity of about 6,000 cubic feet and containing not more than three beds each, the rooms to be separated by a closed partition. The doors

Fig. 27.



A practical, convenient, sanitary stable. (Photo by Dr. Cassius Way.)

Fig. 28.



A well lighted, well ventilated dairy barn. (Photo by Dr. Cassius Way.)

opening into the rooms should be on opposite sides of the building and provided with locks. The windows should be barred and the sash should be at least 5 feet from the ground and constructed for proper ventilation. The walls should be of a material which will allow proper disinfection. The floor should be of painted or washable wood, preferably of concrete, and so constructed that the floor may be flushed and properly disinfected. Proper heating, lighting, and ventilating facilities should be provided.

In the event of any illness of a suspicious nature the 94. attending physician shall immediately guarantine the suspect. notify the health authorities and the secretary of the commission, and examine each member of the dairy force, and in every inflammatory affection of the nose or throat occurring among the employees of the dairy, in addition to carrying out the abovementioned program, the attending physician shall take a culture and have it examined at once by a competent bacteriologist approved by the commission. Pending such examination, the affected employee or employees shall be quarantined.

95. It shall be the duty of the secretary, on receiving notice of any suspicious or contagious disease at the dairy, at once to notify the committee having in charge the medical supervision of employees of the dairy farm upon which such disease has developed. On receipt of the notice this committee shall assume charge of the matter, and shall have power to act for the commission as its judgment dictates. As soon as possible thereafter. the committee shall notify the commission, through its secretary, that a special meeting may be called for ultimate consideration and action.

96. When a case of contagious disease is found among the employees of a dairy producing certified milk under the control of a medical milk commission, such employee shall be at once quarantined and as soon as possible removed from the plant, and the premises fumigated.

When a case of contagion is found on a certified dairy it is advised that a printed notice of the facts shall be sent to every householder using the milk, giving in detail the precautions taken by the dairyman under the direction of the commission, and it is further advised that all milk produced at such dairy shall be heated at 145° F. for 40 minutes, or 155° F. for 30 minutes, or 167° F. for 20 minutes, and immediately cooled to 50° F. These facts should also be part of the notice, and such heating of the milk should be continued during the accepted period of incubation for such contagious disease.

The following method of fumigation is recommended: After all windows and doors are closed and the cracks sealed by strips of paper applied with flour paste, and the various articles in the room so hung or placed as to be exposed on all sides, preparations should be made to generate formaldehyde gas by the use of 20 ounces of formaldehyde and 10 ounces of permanganate of potash for every 1,000 cubic feet of space to be disinfected.

For mixing the formaldehyde and potassium permanganate a large galvanized-iron pail or cylinder holding at least 20 quarts and having a flared top should be used for mixing therein 20 ounces of formaldehyde and 10 ounces of permanganate. A cylinder at least 5 feet high is suggested. The containers should be placed about in the rooms and the necessary quantity of permanganate weighed and placed in them. The formadehyde solution for each pail should then be measured into a widemouthed cup and placed by the pail in which it is to be used.

Although the reaction takes place quickly, by making preparations as advised all of the pails can be "set off" promptly by one person, since there is nothing to do but pour the formaldehyde solution over the permanganate. The rooms should be

kept closed for four hours. As there is a slight danger of fire, the reaction should be watched through a window or the pails placed on a noninflammable surface.

97. Following a weekly medical inspection of the employees, a monthly report shall be submitted to the secretary of the medical milk commission, on the same recurring date by the examining visiting physician.

The following schedule, filled out in writing and signed by himself, is recommended as a suitable form for the attending physician's report:

This is to certify that, on the dates below indicated, official visits were made to the ----- dairy, owned and conducted by ----- of ---- (indicating town and State), where careful inspections of the dairy employees were made. (a) Number and dates of visits since last report.

(b)Number of men employed on the plant.

(e)Has a recent epidemic of contagion occurred near the dairy, and what was its nature and extent?

(d)Have any cases of contagious or infectious disease occurred among the men since the last report?

(e)Disposition of such cases.

(f)report? What individual sickness has occurred among the men since the last

(g)Disposition of such cases. -

(h)Number of employees now quarantined for sickness.

(i)Describe the personal hygiene of the men employed for milking when prepared for and during the process of milking.

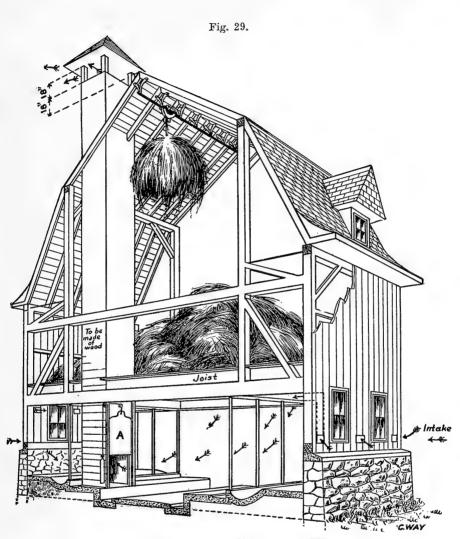
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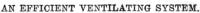
What facilities are provided for sickness in employees? \_\_\_\_\_. General hygienic condition of the dormitories or houses of the em-(k)ployees.

- (l)Suggestions for improvement.
- What is the hygienic condition of the employees and their surroundings? (m)
  - (n)How many employees were examined at each of the foregoing visits? -Remarks. (0)

Date, \_\_\_\_.

Attending Physician.





Perspective view of one center vent of barn, showing relation of ventilator to the hay fork, the timbers of the barn, the butter, and the relation of the fresh air intakes to the foul air out-take or ventilator. The outlet chute should be built on the ratio of 5 or 6 cows to the square foot. There should be enough intakes about one-half square foot in area evenly distributed around the outside of the stable to nearly equal the area of the outlet shaft. There should be four square feet of window area per cow. Slide A can be adjusted to regulate the size of the opening in accordance with the temperature of the barn. This system is practical, inexpensive and will work absolutely perfect.

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