THE BACTERIOLOGY OF HUMAN MILK.

By LEONARD S. DUDGEON, C.M.G., C.B.E., F.R.C.P. (Lond.),
Professor of Pathology, University of London,
and REGINALD C. JEWESBURY, M.A., M.D. (Oxon.), F.R.C.P. (Lond.),
Physician in Charge of the Children's Department, St Thomas's Hospital.

This enquiry into the bacteriology of human milk was prompted by our recent investigation of a case of entero-colitis in a breast-fed infant. In this case an abundant growth of a streptococcus was obtained from the faeces of the infant which was identical as far as we could determine with a streptococcus isolated from the mother's milk.

On turning to the literature, remarkably little work seems to have been done on this subject; in fact most of the modern text-books of paediatry either ignore it completely or dismiss it in a few lines. There is no reference to the subject in the English text-books to which we have referred. In America, Holt and Howland (1922, p. 146) write: "Under normal conditions woman's milk may contain a few bacteria; they are chiefly cocci derived from the internal milk ducts and are of no importance. In suppurative inflammation of the mammary gland, numerous bacteria may be found in the milk; also in some cases of puerperal sepsis. Tubercle bacilli have been demonstrated by Roger and Garnier in the milk of women with advanced tuberculosis, but ordinarily they are not present unless the gland is the seat of the disease."

Hess (1923, p. 282) believes that infection of the infant may be caused by lack of asepsis in the care of the maternal breasts.

Von Reuss (1914) states that it is impossible to prevent bacteria entering the oral cavity and intestine of the infant, even with the most extreme care on the part of the attendants, because they may come from the milk ducts or the genital canal of the mother. He also says that infections due to streptococci derived from the maternal circulation before birth (which in itself may be the cause of premature birth), or to secondary infections through the mother's milk, lochia, or other products entering the gastro-intestinal tract by way of the mouth, are among the most virulent. Von Reuss considers that if there is a possibility that the mother's milk is the source of infection, it should be obtained from another source, or sterilised.

Köstlin (1897) investigated the secretion of the breast in 100 pregnant women, and the breast milk in 137 lying-in cases. In the 100 pregnant women he found *Staphylococcus albus* present in all cases, *S. aureus* in 23, and "bacilli" in 25. In 14 cases the secretion was sterile. In the 137 cases from women during the puerperium *S. albus* was present in 132, *S. aureus* in 79 and bacilli in 22.

https://doi.org/10.1017/S0022172400008470 Published online by Cambridge University Press

Cohn and Neumann (1891) collected breast milk after disinfection of the nipple with sublimate, alcohol, and ether, into sterile test-tubes; from 5 drops to 1 c.c. of milk were taken and the first and last portions were examined. They found that the milk from healthy breasts at which strong infants had just suckled contained very few or even no bacteria, but that in cases where the children were weakly or diseased the bacterial content varied from 16 to 150 colonies. If suckling was discontinued the numbers of bacteria in the milk increased, but as the secretion of milk diminished the number of bacteria also decreased. The bacterial content was greater in milk first drawn off than in the last portion. They found that the number of organisms depended on two circumstances:

(1) The time interval since the last act of suckling.

(2) The quantity of milk drawn off immediately before the test sample.

The shorter the time the milk was allowed to remain in the breast, the less the number of organisms present in it.

In the milk of 48 women they found *Staphylococcus albus* in pure culture in 36, *S. aureus* in 1, *S. albus* and streptococci in the remainder. In their opinion milk from healthy mammary glands contains cocci, especially *S. albus*, which are present in varying numbers and penetrate from outside into the milk.

Work on this subject has also been done by Honigmann (1893) who found *S. albus*, and to less extent *S. aureus*, in the milk of healthy women.

Ringel (1893) has investigated the breast-milk from 25 cases. He discarded the first milk withdrawn, and then cleaned the nipple with alcohol and perchloride of mercury. He considers that human milk nearly always contains *S. albus* and less commonly *S. aureus*, and that the organisms penetrate from without into the mammary gland without causing any ill effect.

Before going further we wish to state most emphatically that the results of our investigations do not throw any doubt upon the unquestionable value of breast feeding, which is the only proper method of feeding the infant, but at the same time it is well to realise that infection of the infant from the mother's milk is a greater possibility than has been believed, and that valuable information may be obtained from the bacteriological and cytological examination of the mother's milk and infant's faeces.

TECHNIQUE.

Samples of breast milk were collected from 100 different women, the majority taken during the puerperium, but others throughout all stages of lactation. The milk was expressed by hand, taking care not to allow the hand to come into contact with the nipple or surrounding tissues, and was received direct into sterilised bottles. About 2 c.c. of milk were usually taken.

In some cases the nipple was not previously prepared; in others it was cleaned with sterile dry sponges, ether, hydrogen peroxide 10 volumes, and then kept covered for two days with sterile dressings, 2 per cent. iodine in spirit, carbolic lotion 1 in 60, and perchloride of mercury 1 in 1000 and 1 in

2000. In some cases milk was taken in two separate portions from the same breast and the first and second samples examined separately. The majority of the samples of milk were taken from healthy women with normal breasts, but some also from women with pyrexia from various causes, such as puerperal sepsis, and from women with varying degrees of inflammation of the breast culminating in breast abscess. The stools and urine of infants and the urine of mothers were examined when considered necessary.

Milk (1).

Each sample of milk was sent to the laboratories in sterile glass-stoppered bottles. On arrival it was well mixed, and 0·025 c.c. withdrawn in a sterile pipette and spread all over the surface of an agar plate and the same amount on litmus lactose agar. Each plate was incubated aerobically at 37° C. for twenty-four hours, the colonies counted, differentiated and subcultured as considered necessary; the plates were then discarded. 0·5 c.c. of milk was added to a tube of 1 per cent. lactose, and glucose, and 1·0 c.c. incubated in a sterile tube at 37° C. for twenty-four hours, when films were made, and if the results obtained appeared to differ from the surface colonies on the plates referred to, then further plating was done and the results compared with the original sample of milk. This method was of undoubted value as instances occurred when streptococci and *B. coli* were cultivated in one or more of the liquid media, although absent on the direct plates. Whenever streptococci were cultivated from a sample of milk, one or more colonies were selected and passed through the usual media, tested for haemolysis, morphological appearances, and in some instances for heat resistance. Staphylococci were tested for evidence of haemolysis, and in many instances titrated with *S. aureus* and *S. albus* anti-sera. All strains of *Bacillus coli* were examined in detail culturally for evidence of haemolysis, and serologically with the colon anti-sera.

The remainder of the milk was centrifugalised and film preparations were made from the deposit and from the cream. It is essential to make films from both sources as although the cells may be equally abundant in each portion, yet, wide variations occur.

The wet films were fixed in Schaudinn's solution and stained with Mayer's haemalum. No counter stain was employed in most instances; occasionally mercurochrome, saffranin, or Bordeaux red was used. Perfect film preparations were obtained by this method of fixation, so that cell differentiation was readily obtained, and the usual difficulties experienced by previous workers with cow and human milk, when dried films were employed, were avoided. The cells are readily differentiated and the true polymorphs stand out distinctly.

Total cell counts can be made by the Strong-Seligman method. It may be possible to make the "drops" from the well-mixed undiluted milk, but if the cells are numerous dilution in formalised citrated saline, as employed for total leucocyte counts, is satisfactory. If the drops are made from undiluted milk, it is necessary to fix in the wet in Schaudinn's fixative.

BABY'S FAECES (2).

If from the bacteriological observations of the mother's milk it was considered necessary to examine the infant's faeces, a sample of the latter was obtained. This was dried by Dudgeon's method (*vide* Wordley, 1921) on unglazed tiles, and the dried powder spread on plates of blood agar, litmus lactose agar, and plain agar, and added to tubes of milk, starch, and Robertson's medium.

Full investigations of the bacteria cultivated from the faeces were made as employed for the bacteria isolated from the milk.

TO SHOW THE DIFFERENCE IN THE ACTION OF BACTERIA ON HUMAN AND COW'S MILK.

Four strains of *B. coli* isolated from the urine of cases of pyelo-cystitis, three strains of *B. coli* obtained from infected human milk, and three strains of streptococci isolated from a similar source were cultivated in tubes of 5 c.c. of human and cow's milk, which had been steamed at 100° C. for one hour. In every instance both samples of milk were acidified in forty-eight hours, but at the end of one week's growth at 37° C. each sample of cow's milk was firmly clotted and each tube of human milk was acidified, but no clotting had occurred. The human milk used in these experiments was collected in the usual way from four mothers, and was pooled as each supply was normal. Other samples of human milk were obtained and tested with other strains of *B. coli* and *S. aureus*, but clotting did not occur. Certain strains of *B. proteus* peptonised human milk, but apart from this effect produced by *proteus* bacilli, the only change recorded with all the bacterial strains employed by us has been acid formation, and with cultures of *B. paratyphosus* B. subsequent alkaline formation. This difference between human and cow's milk is distinctive, and it may be a question of practical importance in the feeding of infants when cow's milk is substituted for human milk. In a similar way it is a well-recognised fact that human milk when treated with rennet does not form the firm solid clot such as occurs with cow's milk.

BACTERIOLOGICAL OBSERVATIONS ON THE MOTHER AND CHILD.

The results of the investigations of the mother and child, referred to in detail below, indicate that the aerobic intestinal flora of the breast-fed infant may be very abnormal, both as regards numbers and strains of the bacteria which are isolated.

In cases 1 and 6 very strong evidence was obtained that the abundant growth of haemolytic colon bacilli in the faeces of the baby was due to infection from the mother's urine.

In case 1, the child was severely ill from a gastro-intestinal infection for many weeks, as already described by us (*vide* Jewesbury and Dudgeon, 1923), and, in our opinion, it cannot be too forcibly emphasised that the new-born infant may be directly infected owing to cystitis in the mother.

Our bacteriological observations described in this report have compelled

us to realise that although some samples of mother's milk actually consist of a mixture of pus and milk heavily infected with *Staphylococcus aureus*, *Bacillus coli* and streptococci, yet no ill effect on the infant may be apparent during the period of observation. We offer the suggestion, however, that the passage of masses of bacteria such as referred to along the intestinal tract of babies for days at a time, or even weeks, may result in the lodgment of these organisms in the tissues, and form a focus of infection at a subsequent period. This view is accepted in the case of cow's milk in relation to the subject of tuberculosis.

We have seen cases of severe boils in breast-fed infants for which no satisfactory explanation was forthcoming, although breast feeding had been replaced by cow's milk because it was believed, for want of a better explanation, that the human milk was too "rich."

The experimental work of Moody and Irons (1923), however, does not lend support to our suggestion; when they introduced by the stomach tube, suspensions of *B. pyocyaneus*, *B. prodigiosus* and *Streptococcus haemolyticus*, they were unable to cultivate these organisms from the blood, chyle or viscera.

To over-estimate the importance of the presence of bacteria in human milk is a very serious error, as in many instances the bacterial flora is without significance however numerous the bacteria may be, and in the vast majority of instances the bacterial flora of human milk is so infinitely better than the best cow's milk, that to offer comparisons would be absurd. It is necessary, however, to call attention to the bacterial defects of human milk, more especially as the literature on this subject is so scanty, and such defects as may occur can be remedied. It is well, therefore, to realise, as we have shown in this communication, that human milk may be heavily infected with pyogenic bacteria, which no cleansing of the nipple and areola will prevent.

Dudgeon (1924), in his Presidential Address to the Section of Tropical Diseases and Parasitology (R.S.M.), in 1924, explained the importance of an accurate knowledge of a patient's diet, if deductions are to be made from an examination of the faeces as to intestinal infection in the human subject.

The investigations of the bacterial flora of infants' faeces have shown that it closely corresponds to the bacterial content of the infant's food, and whenever abnormal findings are obtained from an examination of the faecal flora in children, the diet factor must be fully investigated bacteriologically. It is especially in infants that such investigations can be undertaken most efficiently (cases 1–14).

The constant occurrence of *S. albus* in human milk is readily explained and in the majority of instances is without significance, but it is of interest to record that Todd (1922) showed that this organism was present in 55 per cent. out of 101 examinations of the faeces of infants.

Case 1. MOTHER. *Milk:* Polymorphs +. Abundance of *Streptococci* and some *Staphylococci*. *Streptococcus* is non-haemolytic and forms short chains.

Urine and Lochia: Pure *B. coli* which is haemolytic and agglutinates with haemolytic colon anti-serum (Dow).

https://doi.org/10.1017/S0022172400008470 Published online by Cambridge University Press

BABY. *Faeces:* Very abundant growth of apparently same *Streptococcus* as in the milk and same haemolytic strain of *B. coli* as in mother's urine.

Vomit: Same result as in case of faeces.

Clinical History: Baby very ill for several weeks from acute gastro-intestinal infection.

Case 2. MOTHER. *Milk:* Polymorphs 46 per cent. *S. albus* + + +.

Urine: Pure bacilluria due to *B. coli* (N.H.).

BABY. Never breast fed.

Vomit: Streptococci +, *S. albus* + + +.

Faeces: B. coli (N.H.). *B. coli* in faeces of baby and urine of mother not apparently same strain.

Case 3. MOTHER. *Milk:* Clumps of polymorphs filled with diplococci. *S. aureus* + + + +. (Breast prepared with 1 in 2000 perchloride of Hg.)

BABY. *Faeces: S. aureus* + + +.

Case 4. MOTHER. *R. breast* prepared with ether. Polymorphs 26 per cent. *S. albus* + + +. Liquid media gave *B. coli* in addition to *S. albus.*

L. breast. Abundant growth of *B. coli* and *S. albus.*

B. coli colonies from right and left breast are not haemolytic and do not agglutinate with the colon anti-sera. All milk *S. albus* colonies agglutinate with *S. albus* anti-serum prepared from case No. 19.

Urine: sterile.

BABY. *Faeces: S. albus* + + +. It agglutinates with *S. albus* anti-serum No. 19.

Case 5. MOTHER. *Milk:* Nipple cleaned with ether. No polymorphs. Very feeble growth of *S. albus.*

BABY. *Faeces: S. albus* +, *B. coli* + + +, *Streptococci* + + +.

Case 6. MOTHER. *Milk:* Breast cleaned with ether. No polymorphs. *S. aureus* + + +, *S. albus* +. *S. albus* culture agglutinates with *S. albus* anti-serum No. 19.

Urine: Pus and bacilli. Pure culture *B. coli* (H.) which agglutinates with colon anti-serum, haemolytic type (Dow).

BABY. *Faeces: B. coli* (H.) + + +, *S. albus* + +. This haemolytic strain of *B. coli* also agglutinates with same colon anti-serum (Dow).

Case 7. MOTHER. *Milk: R. breast* prepared with ether. Polymorphs + + +, *S. albus* + + +, *B. coli* +.

L. breast, unprepared; no polymorphs. *S. albus* + + +, *B. coli* (N.H.) +.

Urine: No *B. coli* obtained.

Milk re-examined fourteen days later. *No B. coli* found, but pure *S. albus.*

BABY. *Faeces: B. coli* (N.H.) + + +, *S. albus* +. Colon strain inagglutinable with the anti-sera.

Case 8. MOTHER. *R. breast* prepared. Polymorphs + + +, *S. albus* + + +, *S. aureus* +, *B. coli* (N.H.) + +, *Streptococci* +.

L. breast, unprepared. *S. albus* + + +, *B. coli* (N.H.) + +, *Streptococci* +.

Urine: No *B. coli* isolated. Pus and masses of cocci which gave pure growth of *S. albus.*

BABY. *Faeces: S. albus* + + +, *B. coli* (N.H.) +.

Milk and faecal strains of *B. coli* do not correspond.

Case 9. MOTHER. *Milk:* Polymorphs + + +, *Staphylococci* + + +, pure *S. aureus* + + +.

BABY. *Faeces: S. aureus* + + +, *S. albus* few, *B. coli* + + +.

Case 10. MOTHER. *Milk:* Pus present and masses of *Staphylococci. S. aureus* + + +, Long chain *Streptococcus* +.

BABY. *Faeces: S. aureus* + + +, *B. coli* (N.H.) +.

This strain of *B. coli* agglutinates with a colon anti-serum.

Case 11. MOTHER. First supply of milk (half an ounce), *S. aureus* + + + +, *S. albus* +. Second supply of milk, *S. aureus* +, *S. albus* + + + +.

BABY. *Faeces: S. aureus* + + +, *S. albus* + + +, *Streptococci* + +, *B. coli* +.

Case 12. MOTHER. *L. breast* cleaned with ether. No polymorphs. *S. albus* + + +, *Streptococci* +.

R. breast, same result.

BABY. *Faeces: S. albus* + + +, *S. aureus* + +, *Streptococci* + +.

Case 13. MOTHER. *R. breast,* first supply of milk: very few polymorphs. *S. aureus* + + +. Second supply of milk, after cleaning with iodine: *S. aureus* + + +, *S. albus* +, *Streptococci* few.

L. breast, pure *S. aureus.*

BABY. *Faeces: S. aureus* + + +, *S. albus* + +, *Streptococci* very few.

Case 14. MOTHER. *R. breast,* Mastitis. Polymorphs + +, *S. aureus* + + +, and *Streptococci* (N.H.).

Milk obtained just before abscess opened: *S. albus* + +, *S. aureus* +, *Streptococci* + (N.H.).

L. breast, "normal." Polymorphs + +, *S. aureus* + + +, *Streptococci* + + + (N.H.). Left breast suppurated fourteen days later.

Urine: Sterile.

Cervical Swab: Streptococci (H.) + + +, *S. albus* + + +, *S. aureus* +.

Blood culture: Sterile.

BABY. *Faeces: S. aureus* and *S. albus.*

H. = Haemolysis. N.H. = No haemolysis. + + +, + +, + signify degrees of bacterial activity.

STREPTOCOCCI.

Streptococci were isolated from 49 per cent. of the samples of human milk obtained from our cases. The positive findings, however, among the specimens of normal milk were approximately the same as we obtained from the cases with evidence of inflammation in the breast, although the number of streptococci isolated from some of the samples of milk which showed a high percentage of polymorphs was far greater than occurred with the normal milk. No positive evidence was obtained of a direct relationship between the streptococci isolated from the milks and those obtained from the blood and tissues in puerperal fever.

Streptococci were isolated from 0·025 c.c. of milk plated direct on agar and litmus lactose agar, or in some cases the primary growth occurred only in the tubes of dextrose or lactose to which 0·5 c.c. of milk had been added, or in the 1 c.c. of incubated milk. These strains of streptococci were tested for evidence of haemolysis on human red cells in liquid media by the method employed by Dudgeon and his co-workers (1921), for their morphological characteristics, and the action on lactose, dextrose, salicin, mannitol, inulin and cow's milk. Every strain was gram-positive, and in all but two instances failed to haemolyse human red cells.

There was only a "trace" of haemolysis in the tubes of 0·5 and 0·85 per cent. of sodium chloride in the two positive cultures. The samples of milk from which the haemolytic streptococci were cultivated gave a very low bacterial content, no polymorphs, and nothing else abnormal in the milk, while the mothers' breasts and the infants were healthy. Streptococci with different morphological and cultural reactions were isolated in several instances from the same samples of milk. A method of classifying these streptococci was not obtained from a

study of the morphological and such cultural reactions as referred to, and from haemolytic evidence. For this reason the results are grouped as shown in Table I, as a detailed description of each individual streptococcus is of no practical utility.

Table I.

No. of strains	Haemolytic test		Morphology				Cultural reaction				
	H.	N.H.	Long chain	Short chain	Diplo-cocci		Lac-tose	Man-nite	Salicin	Inulin	Milk
						+ with long chains:	28	4	12	4	
						+ with short chains and diplococci:	9	5	6	0	
						Total +	37	9	18	4	Acid 11 Acid and clot 31
43	2	41	31	10	2	Total −	6	34	25	39	1

The great majority of these streptococci formed a granular deposit in those carbohydrate media which were employed for these tests, and the same average of results was obtained with the long and short chain varieties.

Bacillus Coli.

In ten cases, strains of *B. coli* were cultivated from samples of human milk, and in four out of the ten polymorphs were abundant in film preparations of the centrifugalised milks. In each instance, with one exception, the colon strains were non-haemolytic, but from the milk of this case both haemolytic and non-haemolytic strains were obtained. Positive findings occurred more frequently from samples of 1·0 c.c. of milk incubated in sterile tubes, or in tubes of dextrose and lactose to which 0·5 c.c. of milk had been added, than from milks plated direct. The haemolytic strain was agglutinated with the haemolytic colon anti-serum (Dow), prepared by Dudgeon, Wordley and Bawtree (1922), while only one of the non-haemolytic strains agglutinated with a non-haemolytic colon anti-serum. None of these milks were abnormal in appearance, and did not produce any ill effect on the infants so far as we could ascertain. The cultural reactions of these ten strains were without significance, although 83 per cent. fermented saccharose.

The Effect on the Bacterial Content of the Milk from Cleaning the Nipple and Areola with dry Sterile Wool, or with various Chemical Preparations, or by "Flushing" the Ducts with Milk.

Observations were made on the bacterial content of the milk obtained direct from the breast without preparation and on subsequent samples after cleaning the nipples and areola, with iodine, ether, perchloride of mercury 1:1000 or 1:2000, or by rubbing with sterile wool, or by "flushing" the ducts with the first milk which was discarded. The breasts were prepared at the time of the examination, or for one or two days previously. A comparison was made with milk obtained from the same side before and after preparation, or from samples taken from the unprepared and prepared breasts, although the

former procedure is the more satisfactory as it is less open to fallacies. Milk collected in the usual manner from prepared and unprepared breasts was plated on agar and litmus lactose agar. It is obvious that cleansing of the skin, as far as it concerns the number of staphylococci, especially *S. albus*, will produce a diminished bacterial growth, but in cases of infection this reduction does not occur, or only to a limited degree. The actual number of colonies of *S. albus* present in milk is generally of no importance, as they are derived from the areola and skin around the openings of the ducts, and partly from the infant. It is now known that this organism is frequently present in the faecal flora of infants (Todd), as referred to in this communication. In cases which show a high polymorph count in the milk, the predominant organisms are *S. aureus*, and to a less extent *S. albus*, streptococci and *B. coli*.

Table II.

The bacterial content of milk obtained from "prepared" and "unprepared" breasts.

(In all these experiments 0·025 c.c. of milk was plated on the surface of the medium.)

A. Both examinations made on same side.

	Unprepared					*Prepared*			
	No. of colonies					No. of colonies			
No.	Agar	L L.[1] Agar	Predominant organism	Polymorphs	Method of preparation	Agar	L.L.[1] Agar	Predominant organism	Polymorphs
1.	1600	480	*S. albus*	–	"Flushing" ducts	1280	2784	*S. albus*	–
2.	3000 +[2]	46	,,	–	,,	3000 +	—	,,	–
3.	1860	236	,,	–	,,	2500	—	,,	–
4.	3000 +	1024	*S. aureus*	–	Iodine	1184	672	*S. aureus*	–
5.	3000 +	184	*S. albus*	–	,,	—	—	,,	–
6.	1168	176	,,	–	,,	22	7	*S. albus*	
7.	—	74	*S. aureus*	–	Ether	—	93	*S. aureus*	
8.	3000 +	—	*S. albus*	–	,,	17	—	*S. albus*	–
9.	3000 +	—	,, *B. coli*	+	,,	1700	—	,, *B. coli*	+

B. Examinations made from opposite sides.

1.	7	5	*S. albus*	–	Ether	3000 +	292	*S. aureus* *S. albus*	+ + +
						(Breast abscess subsequently)			
2.	2800	—	,,	–	Ether for 2 days	5	—	*S. albus*	–
3.	3000 +	212	,,	–	Iodine	3000 +	112	,,	+

[1] =litmus lactose.

[2] + =over 3000 colonies present, beyond which number it was impossible to estimate.

Cytology.

Many observations have been made on the varieties of cells met with in human milk, and discrepancies have occurred as in the case of cow's milk, which is largely due, in our opinion, to the methods employed in the preparation of the films. We have found, as already stated, that the most satisfactory method for the study of the cells met with in human milk is to centri-

fugalise a fresh sample and make film preparations from the deposit and the cream, as it is essential to examine both portions. In most cases the cells appear to be evenly distributed in the deposit and in the cream, but very definite exceptions occurred. Film preparations made from the centrifugalised deposit or from the surface cream were fixed *in the wet* in Schaudinn's solution, and subsequently stained with Mayer's haemalum and counter-stained if necessary. Film preparations which are allowed to dry before they are fixed are useless. Czerny (1890) stated that in his opinion when the breast function is upset leucocytes pass into the milk. He showed in the case of a cat that the lymph glands nearest to the mammary gland were full of the same type of leucocyte as found in the milk.

Berka (1911) from his examination of human milk concluded that the cells are lymphocytes of the large uninuclear variety, but from disintegration become polymorphic. They are capable of absorbing fat and carrying it to the nearest lymph gland.

Film preparations were made from the centrifugalised deposit and from the "cream" obtained from the 100 samples of milk in this series, and total cell counts were made in some instances. One sample of colostrum was examined and film preparations were made and fixed in the wet. Polymorphs were found in large numbers, free and in clumps, while large mononuclear cells filled with small fat droplets, or with one large fat droplet which compressed the nucleus, small mononuclear cells, and free fat droplets were also present.

In 15 cases out of the 100, polymorphs were found in the milk in relatively large numbers, either as individual cells or in small or large clumps. In a few instances differential counts were made, but the difficulties experienced outweighed any possible advantages which might be derived from so laborious a study.

In four instances the percentages of polymorphs in the film preparation of the milks were as follows: 29, 65, 46 and 26 per cent. The presence of polymorphs in large numbers in milk is of practical importance, as, after the fourth day of lactation, it is an indication of inflammation in the breast, although there may not be at the time any clinical evidence in favour of this.

In five cases in this series polymorphs were found in large numbers in the milk, although the breasts were believed to be normal. During the course of the next few days clinical evidence of inflammation was present and finally suppuration occurred.

It would appear, however, from one single examination of colostrum, and from several examinations of "milk" during the first three or four days of lactation, that the presence of numerous polymorphs at this stage is not necessarily an indication of acute inflammation or suppuration. By the 7th or 8th day and later, normal human milk shows extremely few cells, and such cells as are present are degenerated epithelial cells with a few or abundance of fat droplets. Small mononuclear cells may be found, but polymorphs are so scarce as to be a negligible quantity.

In two cases polymorphs were present in large numbers in film preparations of milk, lying singly and in large clumps together with masses of cocci. Pure cultures of *S. aureus* were obtained from one case, and *S. aureus* and streptococci from the other, and in both cases suppurative mastitis developed.

The bacteriological findings in the sixteen cases which showed numerous polymorphs in the film preparations of milks are of sufficient importance to refer to at some length, as the results confirm our view that the presence of large numbers of polymorphs in milk, quite apart from the clinical condition of the patients, is of considerable significance; in fact, the presence of numerous polymorphs in human milk after the 4th day of lactation is an indication to keep careful observation on the mother's breasts for an acute inflammatory complication. The very large number of colonies on agar plates in 13 cases out of 16 exceeding the maximum limit of 3000 colonies from 0·025 c.c. of milk, which could be accounted for accurately, is far in excess of the average number of colonies obtained from normal cases or cases without a polymorph increase. The high percentage of *S. aureus* cases is also a distinctive feature. The strains of *S. aureus* obtained from milk were haemolytic on blood agar. Many strains grown on agar slopes were very difficult to emulsify in saline for agglutination tests, even when repeated subcultures on agar were made.

Table III.

No.	No. of colonies on agar from 0·025 c.c. of milk	Bacteria present in order of frequency	Clinical course	
			At time	Subsequent
1.	84	*S. albus*, streptococci	nil	—
2.	3000	*S. aureus*, streptococci	,,	—
3.	3000	*S. albus*, *S. aureus*, and streptococci	,,	—
4.	3000	*S. albus*, *S. aureus*	Mastitis	Abscess
5.	3000[1]	*S. aureus*, streptococci[2]	—	—
6.	1700	*S. albus*, *B. coli*	nil	—
7.	3000	*S. aureus*, *S. albus*	,,	—
8.	3000	*S. albus*	—	—
9.	3000	*S. albus*	—	—
10.	3000	*S albus*, *S. aureus*, *B. coli*	Mastitis	—
11.	3000	*S. aureus*, *S. albus*, streptococci	nil	—
12.	3000	*S. aureus*, streptococci[2]	Mastitis	Abscess
13.	3000	*S. aureus*[2]	,,	,,
14.	3000	*S. aureus*	,,	,,
15.	3000	*S. aureus*, *S. albus*	nil	—
16.	492	*S. albus*	,,	—

[1] Nipple prepared for 2 days with 1 in 2000 perchloride of mercury.

[2] Cocci present in film preparations of the milk.

Conclusions.

(1) The cleansing of the nipple and areola, before samples of milk are withdrawn, with chemicals such as iodine and ether may effect a considerable reduction in the bacterial content of normal milk.

(2) If a high bacterial content is associated with numerous polymorphs in the milk, cleansing of the nipple with chemicals or flushing of the ducts will not greatly reduce the number of bacteria.

(3) The most important organism in samples of milks from cases of mastitis, suppurative mastitis, and those in which polymorphs are numerous, is the *Staphylococcus aureus*.

(4) Streptococci were isolated from 49 per cent. of the samples of human milks. Only 2 per cent. of these streptococci were haemolytic. There is no evidence that the presence of streptococci in human milk is an indication of a pathological process in the breast, although the number of streptococci present in milks with a high polymorph count was greater than in the normal milk. The haemolytic streptococci were not associated with any apparent abnormal condition in mothers or infants.

(5) *B. coli* strains were isolated from ten cases. In nine instances the organisms were non-haemolytic and in one haemolytic. The haemolytic strain reacted with a haemolytic colon anti-serum. In four out of the ten cases polymorphs were abundant in the milk. There was no evidence of ill health in the infants fed on these milks.

(6) Normal human milk may contain *S. albus*, *S. aureus*, streptococci or *B. coli*. The commonest organism is *S. albus* which may be associated with one or more of the bacteria referred to.

(7) The cytological examination of fresh human milk must be made a routine procedure in all cases if a bacteriological investigation is necessary. We recommend centrifugalisation of the milk and the fixation of the *wet films* prepared from the deposit and the cream in Schaudinn's solution. After the 4th day of lactation, polymorphs are relatively scarce in normal milk, but if found to be numerous, lying separately and in clumps, the mother's breasts should be carefully watched for clinical evidence of inflammation and the milk should be examined bacteriologically. The presence of numerous polymorphs in a sample of fresh milk obtained after the 4th day of lactation, is an indication of infection which may not be associated with any clinical manifestations although may subsequently give rise to mastitis.

(8) Actual pus may be present in milk, before there is clinical evidence of mastitis.

(9) The cultures of *S. aureus*, *B. coli* and streptococci employed by us failed to clot human milk.

(10) The bacteriological flora of an infant's faeces may serve as a definite indication of the bacterial content of the mother's milk.

REFERENCES.

BERKA (1911). Untersuchungen über Menschliches Kolostrum. *Virchow's Archiv*, CCV. 59.

COHN, M. and NEUMANN, H. (1891). *Ibid.* CXXVI. 391.

CZERNY (1890). Ueber das Kolostrum. *Prager med. Wochenschr.* 401–416.

DUDGEON, L. S. (1924). Certain problems which concern Intestinal Infection and the Intestinal Flora. Presidential Address, Sect. Trop. Dis. and Parasitology. *Roy. Soc. Med.* London. XVII. 1–6.

DUDGEON, L. S., WORDLEY, E. and BAWTREE, F. (2. x. 1921 and 2. xii. 1922). On *Bacillus coli* Infections of the Urinary Tract, especially in relation to Haemolytic Organisms, *Journ. Hygiene*, XX. 137, and XXI. 168.

HESS, J. H. (1923). *Premature and Congenitally Diseased Infants.*

HOLT, E. L. and HOWLAND, J. (1922). *The Diseases of Infancy and Childhood.*

HONIGMANN, F. (1893). *Zeitschr. f. Hygiene u. Infectionskr.*, Leipzig, XIV. 207.

JEWESBURY, R. C. and DUDGEON, L. S. (1923). Acute *Bacillus coli* Infection of the Gastro-Intestinal Tract in a new-born baby, contracted from its Mother. *Lancet*, II. 118.

KOSTLIN, R. (1897). *Archiv. f. Gynakol.*, Berlin, LIII. 201.

MOODY, W. B. and IRONS, E. E. (3. iii. 1922). Invasion of body by bacteria from Intestinal Tract. *Journ. Infect. Dis.* XXXII. 226.

VON REUSS (1914). *Die Krankheiten des Neugeborenen*, Berlin.

RINGEL, T. (1893). *München med. Wochenschr.*

TODD, E. W. (1922). The Haemolytic Organisms of Normal Infants' Faeces. *Journ. Hygiene*, XXI. 37.

WORDLEY, E. (15. vii. 1921). A new Method for the Isolation of Organisms from faeces and sputum with some Observations on Haemolytic Streptococci in Faeces obtained by this Method. *Journ. Hygiene*, XX. 60.