ON THE DIGESTIVE ACTION OF LEUCOCYTES, PUS AND BODY EXUDATES.

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Noel Fiessinger (1923) states that the first definite idea of a proteolytic ferment in leucocytes is due to Leber, who noticed the digestion of fibrin and the liquefaction of gelatin by the aseptic pus from a hypopyon.

The first extensive work on the leucocytic enzymes seems to have been that of Achalmé (1899), who claimed to have demonstrated the presence of lipase, amylase, trypsin, casease, a ferment liquefying gelatin, oxidase and a ferment decomposing hydrogen peroxide in pus and in inflammatory effusions. He states that "milk, to which has been added several drops of pus, forms first a soft clot having no tendency to retract; then after some hours, the liquid clears, becoming transparent and the filtrate is no longer coagulated by acetic acid."

We have been quite unable to substantiate this statement using sterile milk and sterilised pus. It is possible that Achalmé obtained his results with milk and pus which had not been sterilised so that the clotting was due to the contained organisms, and though the subsequent digestion may have been due to the leucocytic protease, even then the digestion of milk was exceptionally rapid. Achalmé considered it possible that the digestion of fibrin, albumen and casein was due to the action of one and the same enzyme which was distinct from that liquefying gelatin, as this enzyme often was almost completely absent when trypsin was abundant.

Briot (1899) demonstrated that normal horse serum would inhibit the clotting of milk by rennet, and that the substance in serum having this action was thermolabile, non-dialysable and was precipitated by ammonium sulphate and alcohol.

Opie (1905) began an extensive series of investigations into the ferments of leucocytes in inflammatory exudates and the inhibition of their action by blood serum; he showed that the proteolytic enzymes of the leucocytes of inflammatory exudates acted both in acid and alkaline media, but better in the latter; he also showed that the serum of an inflammatory exudate had the power of inhibiting the action of these enzymes, and that this power was shared by normal blood serum; in the later stages of inflammation the inhibitory power of the serum exudate was diminished; this anti-enzyme was inactivated

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at 75° C. and also in an acid medium. Opie produced inflammatory exudates in the pleural cavities of dogs by injecting aleuronat. He differentiated two types of cells producing proteolytic enzymes, the polymorph and the "macrophage" of Metchnikoff; the proteolysis was measured by estimating the non-coagulable nitrogen in blood serum heated to 70° C., both before and after incubation with suspensions of the cells. Opie found also that the proteolytic power of cells was preserved in glycerine, and that a glycerine extract of purulent tuberculous sputum digested fibrin and digested coagulated albumen; a dried powder of the cells also had full digestive action. His conclusions were that two proteolytic enzymes occurred in white cells, the one from the polymorphs which acted in a neutral or alkaline medium, and which he designated leucoprotease, and the other from the large non-granular cells which acted in an acid medium and to which he gave the name lymphoprotease. Both these enzymes were destroyed by exposure for half an hour at 70–75° C.; the same time at 55–70° C. diminished the activity of lymphoprotease, but did not materially affect leucoprotease.

Opie, in a later publication (1906), put forward the hypothesis that, as inflammation proceeds, the inhibitory action of the serum exudates is progressively neutralised by the enzymes of the disintegrated leucocytes, until finally the whole pus itself, without separation of the serum, is capable of active autolysis and has the power to digest foreign protein. He showed, by producing subcutaneous abscesses in dogs, that when the serum was separated from the white cells in the pus it was no longer inhibitory to the proteolytic action of the white cells. He found also that pus itself was capable of further autolysis at 37° C.

Opie (1907) further confirmed the above conclusions by work on the resolution of fibrinous exudates in artificially produced pleurisy. In an attempt to discover the portion of the blood serum having an inhibitory action on leucocytic enzymes, Opie and Barker (1907) found that the albumen fraction of the serum inhibited the enzyme of the polynuclear leucocytes; the globulin fraction, on the contrary, contained an enzyme which digested protein in neutral or alkaline medium and resembled leucoprotease, but was normally held in check by the anti-enzyme in the albumen fraction which is in considerable excess in normal serum.

Müller and Jochmann (1906) introduced a simplified method of demonstrating the presence of proteolytic ferments by the use of inspissated serum plates. By this means they found that leucocytes, both of normal blood and in myeloid leukaemia, digested the serum, which lymphocytes did not, and further, that the pus from tuberculous abscesses seldom showed any digestive action. They tried using varying temperatures above that of the body, and found that the pancreas digested serum very rapidly at 50° C. Müller (1907) found that the presence of digestive ferments in exudates depended upon there being a large proportion of polymorphs in the exudate; he also concluded that the natural inhibitory power of the serum exudate was proportional to the

amount of albumen in the fluid, and was gradually neutralised as the cells increased and were destroyed, liberating their ferments. Bergel (1909) investigated lymphocytes with a view to determining the presence of a fat-splitting enzyme, and found one acting both at 37° C. and at 53–54° C., from which he concluded that its action was not dependent upon the living state of the cells, but was liberated after their death. He inserted small quantities of wax under the skins of various animals which, when removed in 24–48 hours, were often found to be infiltrated with "mononuclear white blood corpuscles" and crystals of fatty acid due to the digestion of the wax. Bradley (1910), using cells dried with alcohol and ether, from an empyema, confirmed much of Opie's work and determined the thermal death-point of leucoprotease at 67–71° C., though the quantity of protein present causes the exact point to vary. Jobling and Strouse (1912), besides demonstrating the two proteases as in Opie's work, found a third ferment acting like erepsin, which was inactivated at 70° C. and by drying and freezing the leucocytes; this ferment, they concluded, acted on the digestion products of the other two proteases.

Pochmann and Lockemann (1908) described a method for the preparation of a dried powder of leucocytes. Pus was dried with alcohol and ether, suspended in 50 per cent. glycerine, reprecipitated with alcohol and ether, and the precipitate allowed to dry *in vacuo*; this powder was readily soluble and digested protein. Fujimoto (1918) investigated the antitryptic power of normal serum and found that it was present in both globulin and albumen fraction, though more so in the latter, and that crystalline serum albumen is antitryptic. Husfeldt (1931) found trypsin and cathepsin-like ferments in polymorphs from exudates; he also investigated the isoelectric points of the granules in the polymorphs of various animal species and decided that they correspond roughly to the point at which the ferments of each species exert their action most strongly.

TECHNIQUE.

The specimens of pus, 87 in number, were obtained by us from all forms of septic inflammations in man including tissue abscess, pleurisy, peritonitis, arthritis and meningitis. The infections were of recent duration and also of long standing.

The samples of pus were examined fully cytologically and bacteriologically as soon as they were collected, and after heating they were tested for sterility and stored in the ice safe.

Specimens of tuberculous pus were examined in a similar manner and for the presence of tubercle bacilli, either by the direct method or after treatment with antiformin in a concentration of 20 per cent. Throughout this paper the term pus is used to denote the products of inflammation caused by the pyogenic bacteria as opposed to tuberculous pus.

In many instances a fraction of the pus was heated, and the remainder centrifugalised to separate the serum exudate. The thick cellular deposit was then heated and its activity was contrasted with the whole pus, while the serum

exudate was either filtered through a Seitz E.K. filter or heated at 60° C. without filtering. These exudates were then investigated for tryptic and anti-tryptic properties as will be described subsequently.

The causative microbes were commonly Streptococci and *Staphylococcus aureus*, although samples of pus produced by other bacteria were included. The microbes did not appear to influence the proteolytic activity.

The pus, in amounts varying from 2 to 20 c.c., was heated in test-tubes of $6 \times \frac{5}{8}$ or 6×1 in the water bath for 2 hours at 60° C. as a routine, although periods of 1 hour and 4 hours were employed occasionally. It was found that heating the pus as described above for 2 hours was sufficient to ensure sterility as a general rule, and did not reduce its proteolytic action.

Samples of pus, or pus and bacteria, added to the various media were incubated at 37° C., except gelatin, or when otherwise stated.

Preparation of glycerine extracts of pus.

Two methods which were employed by us for the preparation of glycerine extracts of pus will be described in detail.

(A) 1 c.c. of pus was added to every 10 c.c. of pure acetone and left in contact for 30 min. The acetone was filtered off and the deposit dried on unglazed porcelain tiles at 20° C. The dry residue was scraped off the tile, crushed, and equal parts of glycerine and saline added. The whole was allowed to stand for 4 days with frequent stirring.

The glycerine mixture was then centrifugalised at high speed and 0·1 c.c. of the supernatant fluid used for each experiment. It was necessary at the outset to store the glycerine extracts for about 7 days in the ice safe to ensure sterility; if, however, air contamination occurred during the preparation the extract was discarded.

(B) Samples of pus were dried with acetone as applied to the previous method. The dried powder obtained was extracted several times with ether for a period of an hour or so at a time, then dried in the air, and the powder suspended in 50 per cent. glycerine in saline for 5 days at 20° C. The glycerine mixture was centrifugalised, and 0·1 c.c. of the sterile supernatant fluid was used for the experiments.

Method of examination.

Each sample of pus was planted on various media, incubated at 37° C. and examined as a routine at the end of 1, 2, 3, 5 and 7 days respectively.

The pus was planted on the media with a large loop, 0·25 in. in diameter, unless otherwise stated. For obvious reasons a loop of fluid pus may give a different result from that of a loop of solid pus, and the samples of solid pus vary in their tryptic action.

ACTION ON THE MEDIA.

Cow's milk. Milk inoculated with pus commenced to clear in from 1 to 3 days and by 7 days the clearing would be very obvious, but if the milk was shaken the turbidity reappeared until the particles were redistributed, though this result was not constant. The reaction always remained alkaline.

When pus in quantities amounting to 0·1 or 0·2 c.c. was added to tubes of sterile milk no clotting occurred, but some digestion of the milk was first noticed in 24 hours and was well marked in 3 days.

Inspissated blood serum. In 24 hours at 37° C. definite pitting or liquefaction of blood serum occurred which rapidly increased until it was totally liquefied. The rate of liquefaction varied, but it occurred usually at a very early date.

Hydrocele saline. This medium was a slightly opalescent liquid prepared by diluting hydrocele fluid with saline 1 : 25.

Pus produced a granularity followed by precipitation of the granules and clearing of the medium in 1–7 days.

Gelatin. Planted with the pus and incubated at 22° C. liquefaction occurred in from 1 to 3 days.

Dorset egg medium. A slope of the medium was grooved by the pus in from 1 to 5 days and gradually softened.

1 per cent. dextrose broth and agar were unaffected.

EXPERIMENTAL OBSERVATIONS WITH PUS.

The pus from 87 septic cases in man was examined in detail by the technical methods already referred to and by additional methods which were employed in individual cases. These investigations have shown that every specimen of pus, with one exception, whether from an acute or chronic pyogenic infection, acts in a more or less similar manner on the media, more especially if the cellular deposit is employed after centrifugalising the pus. With thick pus the results are similar to those obtained with the cellular deposit.

When pus is incubated at 37° C. with the media referred to—cow's milk, inspissated blood serum, Dorset egg slopes, gelatin, and hydrocele saline—the first digestive action is observed in 24 hours. Blood serum, which is generally the first medium to show evidence of digestion, may be pitted, softened and ultimately liquefied. Egg is grooved and then softened. Hydrocele saline is transformed from an opalescent solution to a granular suspension and ultimately precipitation of the granules occurs—milk which is gradually digested shows a deposit in about 5 days and a thick surface layer above a clear alkaline solution. Gelatin is pitted and ultimately liquefied at 22° C. All these media are digested in 24–48 hours with the most active samples of pus, and show complete digestion in about 5 days—with less active pus the results are retarded.

The effect on the media of a good average specimen of pus is shown in Table I.

Table I. *Pus. Osteomyelitis of femur.*

Masses of polymorphs in pus and Gram-negative bacilli. Pure cultures of *B. mucosus capsulatus*. Pus sterilised at 60° C. for 2 hours. One large loop of pus added to media.

Media	Days 1	2	5
Milk	—	—	Digested
Inspissated blood serum	Liquefaction	—	—
Hydrocele saline ...	—	Granular	Precipitated
Jelly	Pitted	Liquefied	—
Egg	Grooved	Soft	Soft and partial liquefaction

A glycerine extract of pus from a large subcutaneous abscess due to *Staph. citreus* was prepared. The pus itself was active, and the glycerine extract had a most rapid and intensive digestive action as shown in Table II.

Table II. *Glycerine extract of pus.*

Media	Days 1	5
Inspissated blood serum	Liquefied	—
Milk	—	Digested
Egg	Pitted	Very soft
Hydrocele saline ...	Granular	Complete precipitation
Jelly	Liquefied	Complete liquefaction

We found that when pus and certain strains of *Staph. albus* were added to sterile cow's milk, the milk was clotted although the strains of *albus* alone did not clot milk. Having fully confirmed this observation we observed the effect of several strains of typhoid and paratyphoid bacilli, *B. gaertner*, and of Shiga's bacillus on milk containing pus. Sterile cow's milk inoculated with these microbes and a large loop of the various specimens of pus was clotted in from 1 to 5 days. It is necessary to employ several strains of each microbe, as clotting of milk may not occur with each individual strain or the action may be delayed with one strain more than another. The most active samples of pus will clot all or nearly all the milks inoculated with each strain of typhoid, the paratyphoids, Gaertner's bacillus and Shiga's bacillus within 2 or 3 days, while the least active may clot some of the milks inoculated with typhoid and paratyphoid B strains, while the other milks are unaffected.

It was found that when milk was acidified by the addition of acetic acid to approximately the same reaction as would be produced by a 48 hours' growth of *B. typhosus*, and pus then added, clotting did not occur at 37° C., which shows that the clotting of milk is not due to the degree of acidity produced by the organism.

B. paratyphosus A was grown in milk for 48 hours to allow fermentation of the constituents which produce acidity in the milk; the tubes were then heated for 1 hour at 60° C. to kill the organism, and the reaction of the milk was brought back to neutral by the addition of an alkali. The medium was then

inoculated with *B. typhosus* or *B. paratyphosus* B and pus; clotting occurred in exactly the same way as in untreated milk, showing that it is not dependent on the presence of substances in the milk fermented by the microbes.

Tubes of 1 per cent. lactose and saccharose were inoculated with several strains of the microbes and a large loop of pus; the reaction produced on incubation at 37° C. was identical with that produced by the microbes alone.

THE INOCULATION OF STERILE COW'S MILK WITH PUS AND WITH TYPHOID-PARATYPHOID AND DYSENTERY BACILLI.

The organisms used in our experiments were six strains of *B. typhosus*, two *B. paratyphosus* A, three *B. paratyphosus* B and C, two *B. gaertner* and five *B. shiga*. The cultures were grown on agar at 37° C. for 24 hours.

Throughout this paper these organisms will be referred to as "the microbes"; any other organisms used will be referred to in detail.

The changes produced in milk are expressed as follows:

A, acidity; *Alk.*, Alkaline; *C*, small clot; C_1, partial clot; C_2, solid clot.

In Table III the results obtained with a very active sample of pus from a case of acute arthritis due to *Staph. aureus* are recorded. Each tube of milk was inoculated with one large loop of sterile pus and the microbes and incubated at 37° C. The results were read after 1–3 days at 37° C.

Table III.

Microbe	Days 1	2	3
B. typhosus 1	*A*	C_1	C_2
,, ,, 2	*A*	C_1	C_2
,, ,, 3	*A*	C_1	C_2
,, ,, 4	*A*	C_1	C_2
,, ,, 5	*A*	*A*	C_2
,, ,, 6	*A*	*A*	C_2
B. paratyphosus A 1	*A*	*A*	C_1
,, ,, ,, 2	*A*	*A*	C_1
,, ,, B 1	*A*	C_2	C_2
,, ,, ,, 2	*A*	C_2	C_2
,, ,, C 1	*A*	C_2	C_2
B. gaertner 1	*A*	C_1	C_2
,, ,, 2	*A*	C_2	C_2
B. shiga 1	*A*	C_2	C_2
,, ,, 2	*A*	C_2	C_2
,, ,, 3	*A*	C_1	C_2
,, ,, 4	*A*	C_1	C_2
,, ,, 5	*A*	C_1	C_2

Glycerine extracts were prepared of many of the specimens of pus and the method of extraction previously referred to in the technique as *A* was usually adopted. Glycerine extracts were more potent than the original pus, often to a very considerable degree, and by means of glycerine extracts a definite estimate of the proteolytic activity of pus can be determined.

Several specimens of sputum from cases of abscess of lung were examined and found to be strongly active. All samples of sputum were given 2 hours at

60° C., but it was necessary at times to prolong the heating to 4 hours at 60° C., and occasionally to secure sterilisation of the sputum a temperature was required which destroyed the proteolytic action. When the sputum had been heated it was allowed to remain in the ice safe for some hours and then centrifugalised so as to remove the fluid portion from the solid sputum. It was always the purulent deposit which was used in our experiments, and which had such strong proteolytic action.

Inactivation of pus by heat.

Although prolonged heating of pus at 60° C., even up to 6 hours, did not diminish its proteolytic activity appreciably, a temperature of 80° C. for 20 min., or 100° C. for 10 min., completely inactivated it. This inactivation on heating was the same for every sample of pus, and applied to all manifestations of its fermentative properties as tested for by us.

Action of pus in the ice chest and at 22° C.

Several specimens of pus and of glycerine extracts of pus were added to the media and incubated for 5 days at 22° C. and in the ice chest (about 10° C.).

At 22° C. blood serum was digested as quickly as at 37° C., with egg and hydrocele saline the digestion was slow and feeble, but when transferred to 37° C. the digestion proceeded normally. In the ice chest serum was digested slowly and feebly, egg and hydrocele medium remained unaffected. In order to test the effect on milk with the microbes at low temperatures, strains of the microbes were grown in milk at 37° C. for 24 hours; specimens of pus and glycerine extracts of pus were then added, some were stored at 22° C. and some in the ice chest, where they remained for 5 days. At 22° C. a small proportion of the tubes showed clotting after 3–4 days; of those which had not clotted at the end of 5 days the tubes containing *B. typhosus* or *B. paratyphosus* A were acid and showed some digestion, but when incubated at 37° C. clotting occurred in 24–48 hours; those tubes containing *B. paratyphosus* B or *B. gaertner* were very alkaline and the milk was digested almost to clearness at the end of 5 days, so that on transferring to 37° C. no clotting occurred. It was noticeable that the clots which formed in milk at 22° C. were quite soft, while those which formed subsequently at 37° C. were hard and firm.

In the ice chest, clotting or digestion of the milks was prevented, but when incubated subsequently at 37° C., clotting occurred in the normal manner.

Milk tubes which had contained *B. paratyphosus* B or *B. gaertner* at 22° C. were extremely alkaline and digested, but when inoculated with strains of *B. coli* the medium was acidified, and a soft clot had formed in 24 hours, showing that, in spite of the digestion to almost complete clearness, enough casein was still present to furnish a clot on changing the reaction of the milk from alkaline to acid.

EXPERIMENTS ON THE INOCULATION OF COW'S MILK WITH PUS AND KILLED MICROBES.

Milk tubes were inoculated with various strains of the microbes and incubated at 37° C. for 5 days. The majority of the tubes were heated in a water bath for 1 hour at 60° C. and subcultured to prove their sterility; a few were kept unheated as controls. The milks were then inoculated with a large loop of pus and incubated at 37° C.; in those tubes which contained *B. typhosus* and *B. paratyphosus* A, clotting occurred in 24–48 hours in every case, irrespective of whether the organism was alive or dead. With tubes containing either *B. paratyphosus* B or *B. gaertner*, in which the reaction was alkaline, clotting did not occur in the tubes containing the living or dead microbes and pus, but extremely rapid digestion took place so that the milk was practically clear and very alkaline in 24 hours.

EXPERIMENTS ON THE CLOTTING OF HUMAN MILK.

Tubes containing 2 c.c. of sterile human milk, with phenol red added as an indicator, were inoculated with several specimens of pus and with strains of *B. typhosus*, *B. paratyphosus* B, *B. coli* and *Staph. aureus* and incubated at 37° C. Clotting did not occur except in some of those tubes which contained both *B. coli* and pus; control tubes of *B. coli* alone were acidified, but without clotting of the milk.

The above experiments were repeated, using human milk to which calcium chloride had been added, in order to raise the normal calcium content of 0·034 per cent. to that of cow's milk, *i.e.* 0·124 per cent., but no difference in the results was obtained.

Dudgeon and Jewesbury (1924) found that various strains of *B. coli* and *Staph. aureus* did not clot human milk, and the above experiments show that, even with the addition of pus and various microbes, clotting only took place in the case of *B. coli*.

ON THE PROTEOLYTIC ACTIVITY OF THE SERUM EXUDATE FROM PUS.

A considerable number of observations were made with the serum exudate obtained after centrifugalising pus. A portion of each sample was centrifugalised as fully as possible and then sterilised at 60° C. for 2 hours and another portion was filtered through a Seitz E.K. filter. Some of the filtered exudate was heated for varying times at 60° C., so as to compare it with the unheated. 0·1 c.c. of an active exudate acted on the media and with the microbes, but with feeble exudates 0·75 c.c. might be required for this purpose.

An experiment described in Table IV shows the comparative merit of the heated cellular deposit of pus with its heated or filtered serum exudate.

Table IV. *Empyema due to a haemolytic streptococcus.*

Thick green pus which gave about one-quarter of its bulk in supernatant fluid with a *p*H of 7·5. The solid portion consisted of polymorphs, degenerated polymorphs and large mononuclears. The pus was divided into three portions:

(1) Cellular deposit heated for 2 hours at 60° C.
(2) Supernatant fluid heated for 1 hour at 60° C.
(3) Supernatant fluid filtered through a Seitz E.K. filter.

(1) Cellular deposit heated for 2 hours at 60° C. 1 loop added to media

Media	Days 2	5
Inspissated blood serum	Liquefied	—
Egg	Soft	Pitted
Hydrocele saline ...	Granular deposit	Precipitated
Jelly	Pitted	Liquefied
Milk	—	Digested

(2) Supernatant fluid. 1 hour 60° C. 0·1 c.c. added to media.

Media	Days 2	5
Inspissated blood serum	Liquefied	—
Egg	Pitted	—
Hydrocele saline ...	Granular deposit	—
Jelly	Pitted	Liquefied
Milk	—	Digested

(3) Filtered supernatant fluid. 0·1 c.c. added to media.

Media	Days 2	7
Inspissated blood serum	Pitted	Liquefied
Egg	—	Pitted
Hydrocele saline ...	—	Granular deposit
Jelly	Pitted	Liquefied
Milk	—	—

(4) Filtered supernatant fluid added to milk which was then inoculated with the microbes and incubated at 37° C.

Amount of fluid, c.c.	Microbe	Result Day 1	Day 2
0·1	*B. typhosus*	*A*	C_1
0·25	,, ,,	*A*	C_2
0·5	,, ,,	*A*	C_2
0·1	*B. paratyphosus* B	*A*	C_2
0·25	,, ,, ,,	*A*	C_2
0·5	,, ,, ,,	C_2	C_2

When one loop of the heated sterile pus was added to tubes of milk together with several strains of the microbes the milks were acidified and clotted in 1, 2 or at the latest 3 days at 37° C.

A glycerine extract of the pus and of the heated supernatant fluid were prepared and 0·1 c.c. of each was added to the media, and to milk which had been inoculated with the microbes. Both of these extracts gave strong positive reactions throughout.

When the pus and supernatant fluids were heated at 80° C. for 20 min. they were rendered inactive.

The pus heated at 60° C. for 2 hours was stored in the ice safe for several

weeks. The supernatant fluid which had separated off was centrifugalised, and 0·1 c.c. was added to the media, and to milk together with the various microbes. The media were digested and the milk was clotted as with the fresh supernatant fluid.

The serum exudates from a specimen of pus obtained from a large subcutaneous abscess due to a haemolytic streptococcus and *Staph. aureus*, and from a large bone abscess due to the same bacteria were fully investigated. In each instance the results were similar. The samples of supernatant fluid filtered through a Seitz E.K. filter digested the media and produced a solid clot in milk *without* the microbes, in amounts varying from 0·05 to 0·25 c.c. within 3 days at 37° C., and similar results were obtained after the filtered fluids had been heated at 60° C. for 30, 60 and 120 min., but after 20 min. at 80° C. they were inactive. When these filtered serum exudates were mixed with normal human serum in the proportion of 1 in 6 and incubated for 2 hours at 37° C. and the mixtures added to milk, clotting was prevented, but in the proportion of 1 in 4 the clotting of milk was merely delayed. These two filtered exudates are the only two examples we have met with of exudates causing solid clotting of milk, otherwise gradual digestion only has taken place. The deposits from these specimens were very actively proteolytic.

Numerous experiments with the serum exudates have shown that the filtered and heated exudate and the pus itself may correspond in activity on the media and in the inoculated milks, but in some cases a filtered exudate obtained from a very active pus may be inactive or only feebly active.

The cerebro-spinal fluid was examined from four cases of acute meningitis, two of which were due to the meningococcus, one to the *Streptococcus mucosus lyticus* and one to a haemolytic streptococcus. In every case the heated supernatant fluid was inactive, but the pus added to the media and to milk tubes inoculated with the microbes was feebly proteolytic. In each case, however, the amount of available pus was very limited.

LEUKAEMIA: WHITE CELLS.

White cells from the blood of several cases of leukaemia were investigated in a like manner to pus, and glycerine extracts were prepared.

The white cells were examined as follows: 5–10 c.c. of oxalated leukaemic blood was centrifugalised so as to separate off the white cells from the plasma and red cells. The leucocytes were then heated for 2 hours at 60° C. and in a few cases the plasma was passed through a Seitz E.K. filter and tested for inhibitory action.

Types of leukaemia.

(1) *Chronic myeloid leukaemia* (three cases).

In these cases the blood picture was one of typical chronic myeloid leukaemia, with a total of 100,000 white cells, or more, per c.mm., of which the majority were cells of the granular series.

In two cases the heated cells when added to the media acted exactly like pus, digesting the media and clotting milk which had been inoculated with the microbes.

In the third case, the white cells from the first sample of blood taken, though digesting the media, caused clotting of milk with a few of the microbes only, but when a glycerine extract was prepared from these cells it acted exactly like an extract of pus; a second sample of white cells, taken from the same case after several doses of X-rays, acted on the media and with the microbes precisely like pus.

(2) *Myeloblastic termination of myeloid leukaemia* (two cases).

These cases, which had given the blood picture of chronic myeloid leukaemia for years, were being treated with X-rays. The total number of white cells suddenly increased, and changed from a high proportion of granular cells to between 80 and 90 per cent. of myeloblasts. Blood was taken from these patients in this terminal myeloblastic stage.

The heated white cells in each case digested the media feebly, but milks inoculated with the cells and microbes were completely negative in one case and with the other clotting occurred in a small proportion.

Glycerine extracts of the cells were prepared and were found to digest the media feebly, but clotted the milks with the microbes.

(3) *Chronic lymphatic leukaemia* (three cases).

In these cases the differential white count showed over 90 per cent. of lymphocytes. The heated white cells and the glycerine extracts were inactive on the media, except for slight digestion of blood serum, and milk was not clotted with the microbes and the extracts.

(4) *Acute leukaemia* (three cases).

These cases ran a very rapid course, with a high leucocyte count, of which over 90 per cent. consisted of primitive cells, mononuclear in type and with non-granular cytoplasm; the nature of these cells has for long been in dispute, and in one of the cases here referred to it was thought that the cells were lymphocytes, in the other two myeloblasts.

The white cells were heated and glycerine extracts prepared, but the cells and the glycerine extracts in each case were inactive, *and thus correspond to extracts of lymphoid or non-granular cells.*

Schumm (1904) is stated to have demonstrated that the incoagulable nitrogen of leukaemic blood resulted from enzymotic activity; this autolysis occurring in leukaemic blood was confirmed by Pfeiffer (1906).

Müller and Jochmann (1906), by the use of Loeffler's serum plates, showed that proteolysis takes place with myeloid leukaemic blood, but not with lymphoid leukaemia, while Stern and Eppenstein (1906–7), using gelatin as their medium, arrived at similar conclusions.

Morris and Boggs (1911) stated that there were no observations on the ferments of the lymphocytes of normal blood, but that a protease is present in the lymphocytes of chronic lymphoid leukaemia and in the leucocytes of

acute and chronic myeloid leukaemia, and of pus. They considered that there was no biological difference between myeloid cells and the lymphocytes of chronic lymphoid leukaemia in neutral media.

Jochmann and Ziegler (1906) investigated the spleen, lymph glands and bone marrow in leukaemia and pseudo-leukaemia. These tissues from myeloid leukaemia showed strong fermentative action, and specimens preserved in formalin for as long as seven months are stated to have retained this action completely. They refer to a publication of Longcope and Donhauser (1908) on the proteolytic ferments of the large lymphocytes in a case of acute leukaemia, in which they describe the cells as "typical large lymphocytes"; proteolytic enzymes acting in much the same way as those of pus and of myelogenous leukaemia were demonstrated and the authors conclude that "these results seem to show that the large cells of the so-called acute lymphatic leukaemia are not true lymphocytes, but are nearly related to the granular myelocytes and should probably be considered as forerunners of these cells."

The method of estimating the activity of the proteolytic enzyme in this case was to measure the quantity of non-protein nitrogen in a given volume of blood before and after incubation at 37° C. for a given time.

Longcope and Cooke (1911) described a similar case to the above and obtained the same results.

Husfeldt (1931) found various proteinases in the white cells from a case of myeloid leukaemia, and also in glycerine extracts of these cells; qualitatively these enzymes corresponded to those found in the normal granular white cells of the blood.

Cooke (1931) investigated five cases of chronic myeloid leukaemia and thirteen cases of acute leukaemia; in the former there was a large increase in the protease, and in the latter higher quantities, on the average, than in normal blood.

The same author (1932) investigated further cases, including one of chronic lymphatic leukaemia in which the protease was decreased, and came to the conclusion that "the rather high protease activity" of the non-granular cells of acute leukaemia was evidence that they are more closely related to granular myelocytes than true lymphocytes.

DISCUSSION.

It will be seen from these results that the white cells from the blood of chronic myeloid leukaemia act in every way like pus, while those from chronic lymphatic leukaemia show no fermentative action whatever with the exception of slight digestion of blood serum, which we have learnt to disregard; and moreover, glycerine extracts of the cells were completely inactive.

Between these two extremes we have the myeloblastic termination of chronic myeloid leukaemia, in which the majority of cells were regarded as myeloblasts, and which gave a weak reaction both on the media and in milk with the microbes, and the two cases of acute leukaemia, in which the primitive non-granular cells failed to show any reaction at all, and their glycerine extracts

were also inactive, thus corresponding to the cells of chronic lymphatic leukaemia.

These results in acute leukaemia are not in agreement with those of Longcope, Donhauser and Cooke, referred to above, who found evidence of relationship between the primitive cells of acute leukaemia, and white cells of the granular series.

Tissue extracts.

In one case of chronic lymphatic leukaemia, the bone marrow obtained at autopsy was heated at 60° C. for 2 hours, and a glycerine extract was also prepared, but each preparation gave completely negative results with our methods of investigation.

A cervical gland was removed from a case of acute leukaemia a few hours after death and a glycerine extract was made from the minced gland. This extract had no proteolytic action on the media, but milk tubes inoculated with the extract and the microbes were clotted, in spite of the fact that the white cells from the patient were inactive.

In view of this result, it was decided to test out the activity of glycerine extracts of various other tissues, both normal and pathological. Normal human rib marrow obtained at autopsy was found to act, both on the media and in milk, like pus. Glycerine extracts of liver, kidney and spleen from normal guinea-pigs were prepared. These extracts had no digestive power on the media, but milks inoculated with the extracts and the microbes were clotted. An extract of the thyroid gland from a case of exophthalmic goitre, shown by microscopy to be free of polymorphs, had a similar action, and also an extract made from carcinoma of the breast.

Thus, it will be seen, that with the glycerine extracts of the tissues tested by us, apart from normal bone marrow, no digestive action on the media occurred, but in the majority of instances clotting of milk with the microbes was observed.

Opie (1905) showed the presence of proteolytic enzymes in normal bone marrow, Hedin and Rowland (1901) in the spleens, lymph glands and other organs of various animals, and Opie (1906) in inflamed lymph glands containing many large mononuclear cells.

Longcope and Donhauser (1908) found proteolytic enzymes in the lymph glands from a case of acute leukaemia with large lymphocytes in the blood, and Longcope and Cooke (1911) in the bone marrow of a similar case; Schumm (1904) found them in the spleen in acute leukaemia, and Jochmann and Ziegler (1906) in the bone marrow of acute leukaemia, and many organs in myeloid leukaemia.

Müller and Jochmann (1906), using Loeffler's serum plates, found that normal spleen digested the plates while normal lymph glands did not, and remarked upon this difference in tissues histologically somewhat similar.

Bergel (1909) demonstrated lipase in the spleen and lymph glands of a tuberculous guinea-pig.

Rondoni (1932) showed the presence of proteolytic enzymes in mouse cancer, but concluded that they were in no greater quantity than in some normal organs.

The inhibitory action of human blood serum and body exudates.

Samples of blood serum obtained from normal humans and those suffering from acute infections, and exudates from various sources, were examined for inhibitory action. The serum exudates were used before and after filtering, and before and after heating at 60° C.

The pleural effusion from a case of pulmonary tuberculosis, the cellular deposit of which consisted chiefly of lymphocytes, was found to be sterile. It was filtered through a Seitz E.K. filter and the filtrate was added in amounts varying from 0·05 to 0·25 c.c. to 5 c.c. tubes of milk inoculated with a strain of *B. typhosus* and one large loop of pus from three cases of sepsis. Nine experiments conducted on these lines showed that the pleural fluid in the amounts stated completely inhibited the clotting of milk, but with a very active glycerine extract of pus larger amounts of pleural fluid were required. When the filtered pleural fluid was heated at 60° C. for 30 min., the inhibitory action was lost.

In a case of tuberculosis of the knee joint the supernatant fluid even up to 0·5 c.c. had no inhibitory action although inactive in itself. The exudate from an acute pleural effusion due to *Staph. aureus*, in which the cells were chiefly lymphocytes, and large mononuclears, with a few polymorphs, was filtered and tested. It had no digestive action on media and 0·05–0·5 c.c. added to milk with the microbes failed to cause clotting. It was devoid of inhibitory action. A case of pleurisy with abscess of lung was examined. The cells were chiefly polymorphs, a few lymphocytes and large mononuclears, and the exudate was found to be sterile. The filtered fluid was devoid of digestive action, but in amounts varying from 0·25 to 1·0 c.c. it inhibited the action of several samples of pus. When heated at 60° C. from 30 to 60 min. the inhibitory action was lost.

Fresh hydrocele fluid filtered through a Seitz E.K. filter was found to be strongly inhibitory in amounts varying from 0·1 to 0·25 c.c.

A slightly turbid sterile pleural fluid was obtained from a case of abscess of lung. It showed a deposit consisting mainly of polymorphs but with some lymphocytes and large mononuclears. 0·05–0·5 c.c. of this filtered fluid completely inhibited the action of pus and the microbes in milk. When the filtered fluid was heated at 60° C. for $\frac{1}{2}$, 1 or 2 hours it required 1·0 c.c. to inhibit proteolytic activity.

Samples of normal human serum and of serum from cases of septicaemia were examined on the same lines as the exudates. Filtering the sera through Seitz E.K. filters did not alter their action. Quantities varying from 0·05 to 1·0 c.c. were examined. 0·05 c.c. might reduce the activity of pus, but 0·1–1·0 c.c. of normal serum was completely inhibitory, while sera from acute infections was less active.

A very strongly active pus was extracted with glycerine and a still more

active agent was obtained. This digested the media readily and clotted milk in the presence of the microbes rapidly. When however 0·1–0·25 c.c. of normal human serum was added to the milk tubes, together with the microbes and 0·1 c.c. of the glycerine extract of the pus, no clotting took place even after several days at 37° C.

Tuberculosis.

Twenty-two specimens of tuberculous pus obtained from subcutaneous and deep abscesses and from the pleural cavity in cases of tuberculous pyo-pneumothorax, and seven specimens of tuberculous sputum, were investigated on identical lines as employed for pyogenic pus.

Every specimen of tuberculous pus was examined cytologically, for tubercle bacilli and for the presence of pyogenic bacteria.

The important question whether polymorphs were present in small or large numbers mixed with the usual débris found in tuberculous pus was studied in detail. In five cases out of twenty-two, polymorphs were present in large numbers, although in every instance some polymorphs were found. On eight occasions tubercle bacilli were present, and it would appear that when they are numerous polymorphs are also numerous. With one exception, a case of tuberculous pyo-pneumothorax, cultures from the pus on various media were sterile for pyogenic bacteria. The one exception gave a growth of staphylococci.

The samples of tuberculous pus planted on the same media as employed for pyogenic pus, after heating at 60° C. for 2 hours, were found to be sterile. One large loopful, or 0·1 c.c., was added to tubes of sterile milk inoculated with the microbes. In eight cases the media were unaffected and milk was not clotted in the presence of the microbes and pus, in nine cases there was a very slight pitting of blood serum and in a few of the milk tubes slight clotting had occurred. In the five remaining cases the media and milk tests were positive and the reactions were as definite as when pyogenic pus was employed. These results show that, in seventeen cases out of twenty-two, tuberculous pus can be distinguished readily from pyogenic pus by these reactions. In these five positive cases polymorphs were numerous in the tuberculous pus, and one of these was complicated by a secondary infection.

In a typical example of tuberculous pus the reactions were as follows:

Tuberculous abscess of neck.

Pus. Few polymorphs and lymphocytes, chiefly débris.
Tubercle bacilli numerous. Cultures sterile.
Pus heated for 2 hours at 60° C.
Media were inoculated with a large loop of pus and incubated at 37° C. for 5 days.

Media	Days		
	1	3	5
Milk	—	—	—
Inspissated serum	—	Slight pitting	Digested
Egg	—	—	—
Jelly	—	—	—
Hydrocele saline	—	—	—

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Milk inoculated with the microbes and pus was not clotted at the end of 5 days.

Milk inoculated with the microbes and pus occasionally showed a few positive results, but even then the reactions were delayed. In some instances tuberculous pus was centrifugalised and the débris was tested correspondingly with the original pus, but the results were similar.

In four instances a glycerine extract was prepared in the same way as for pyogenic pus—using method (A) (see p. 102). In one case the pus and the glycerine extract were found to be inactive, in another the glycerine extract was infinitely more active than the original pus, in the remaining two the pus and glycerine extracts were similar, but much less active than pyogenic pus.

Tuberculous sputum. Sputum was obtained from nine cases of pulmonary tuberculosis, of which two were suffering from early tuberculosis and seven from advanced chronic disease. In every instance polymorphs were numerous in films of the sputum together with débris, mononuclear cells, tubercle bacilli and other microbes.

A specimen of sputum from each case was heated for 2 hours at 60° C., or 4 hours, if necessary, to secure sterility, centrifugalised at high speed and one large loop of the solid deposit was added to the media and to milk inoculated with the microbes. The results obtained in both groups of experiments were similar to those obtained with pyogenic sputum or pus, and similarly were rendered inactive at 80° C. for 20 min.

SUMMARY.

1. Eighty-seven specimens of septic pus obtained from patients suffering from recent and old infections were examined fully bacteriologically and cytologically and were sterilised at 60° C. for 2 or 4 hours. The heated samples of pus were used for our experiments.

2. Glycerine extracts were prepared of the pus by two methods referred to in detail in the text.

3. The digestive action of the samples of sterile pus and of their glycerine extracts were tested on inspissated blood serum, gelatin, milk, egg and hydrocele saline. Digestion occurred on all these media in varying degrees with the specimens of pus, but glycerine extracts were more active.

4. It was found that the addition of pus or glycerine extracts of pus with various strains of *Bacillus typhosus*, *B. paratyphosus* A, B and C, *B. gaertner* and *B. shiga* to milk, produced clotting of the medium at 37° C. in the majority of our experiments.

5. If pus was heated at 60° C. for as long as 6 hours its digestive manifestations were not affected, but 80° C. for 20 min. or 100° C. for 10 min. completely destroyed the digestive activity.

6. At 22° C. the digestive action of pus was weakly manifested, while in the ice chest no reaction whatever took place.

7. Clotting of the medium occurred when pus was added to killed growths of *B. typhosus* and *B. paratyphosus* A in milk, but not with *B. paratyphosus* B or *B. gaertner*.

8. Human milk did not clot with pus and these organisms.

9. A large number of observations were made with serum exudates from pus, either after filtering through a Seitz E.K. filter or after heating at 60° C.

It was found that the filtered or heated fluids might have a similar or a weaker action as compared with pus, or they might be inhibitory.

10. The white cells from several cases of chronic myeloid leukaemia, chronic lymphatic, the myeloblastic termination of chronic myeloid and acute leukaemia, were examined in the same manner as pus. The cells of chronic myeloid leukaemia acted in every way like pus, those of chronic lymphatic and their glycerine extracts were quite inactive; those of acute leukaemia corresponded to chronic lymphatic, while those of a myeloblastic termination gave a weak digestive reaction.

11. Specimens of blood serum from normal human beings and from patients with acute infections, and some specimens of body fluids before and after filtering, were found to inhibit the clotting of milk inoculated with pus and the microbes.

12. Twenty-two specimens of tuberculous pus obtained from abscesses and from pyo-pneumothorax, and seven samples of tuberculous sputum, were examined fully, but with special reference to their cytology.

In eight cases the pus showed no digestive action and no clotting of milk with the microbes, in nine cases a feeble reaction occurred, and in five cases the reactions were like those of pyogenic pus. Thus seventeen of the specimens could be distinguished readily from pyogenic pus; in the five positive specimens polymorphs were numerous, but one of these cases was complicated by a secondary infection. In every sample of tuberculous sputum polymorphs and tubercle bacilli were numerous and the reactions corresponded to those of pyogenic pus.

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