(b) *B. influenzae*.—As the *B. influenzae* is usually regarded as without lethal effect upon the lower animals, except when introduced directly into the brain tissue, no virulence estimations were attempted. Inoculations were, however, made with strains of *B. influenzae* isolated from two of the "mild" cases. Two small rabbits of 500 grammes each were subjected to intrapulmonary inoculations—about three loopfuls of a 24-hours old blood-agar culture emulsified in 0.1 c.c. sterile saline solution being injected into the lung tissue by means of a hypodermic syringe provided with fine needle, which was plunged through the chest wall directly into the substance of the lung. One experiment was a complete failure, as the needle passed through the right lower lobe and injured a large vessel, causing a right-sided hæmothorax, and the animal died some 12 hours later. The second inoculation was more successful, the inoculum being deposited in the centre of the right upper lobe. At the autopsy, performed on the fifth day, the right pleural cavity contained rather more than 2 c.c. of opalescent fluid, the centrifugalised deposit from which showed large numbers of polynuclear cells, many of which were packed with influenza bacilli, eosinophile cells, and some mononuclears and endothelial cells. Cultures gave a rich growth of *B. influenzae*. The upper lobe of the right lung was enveloped in a layer of lymph which extended between the lobes. Cultures from the lung tissue gave a pure growth of *B. influenzae*. Cultures from the heart blood yielded a luxurious growth of *B. influenzae* which contained many thread forms. Cultures from the spleen gave a good growth of *B. influenzae*, the individual bacilli in which were fairly uniform in length. It was intended to test further the pathogenetic activities of *B. influenzae*, but during the absence of one of us from the laboratory owing to illness the various strains died out, and this portion of the investigation had perforce to be abandoned.

General Bacteriological Conclusions.

It seems not unlikely that these patients suffer from a primary invasion of their lung tissues by the *B. influenzae*, and that pneumococci, present at the same time, are at first of low virulence. The earlier symptoms seem to be attributable to a *B. influenzae* toxæmia. Exaltation of the virulence of the pneumococci by symbiotic growth with *B. influenzae* would appear to follow, a fatal termination being due to pneumococcal septicæmia.

Mortality.

Although the above eight cases constitute but a small portion of the total number that have been in the hospital altogether, they were consecutive cases, not selected in any way, and they show a mortality of 25 per cent., sufficiently indicating the seriousness of the disease. When a patient has become decidedly cyanosed no treatment that we have been able to devise appears to do any good. Loss of men from the malady is likely to be prevented not so much by means of treatment when the disease is well established as by getting the men into hospital early when they are in a condition of what in civil practice would be regarded perhaps as a quite trivial bronchial catarrh. Seeing that a large percentage of our cases came from one unit—6 out of 8 in the above series, although the Connaught Hospital admits patients without selection from almost every unit in the command—and seeing that the bacterial infection appears to be primarily an influenzal one, another line of treatment which suggests itself is isolation of the cases and disinfection in the hope of preventing the spread by contact of this serious form of influenzal infection, and thus consequent loss of life. We could emphasise again that although we have dealt with only eight cases in detail we have had a much larger number altogether, and we have heard that the malady has been by no means confined to our command.

Our best thanks are due to Lieutenant-Colonel William Turner, C.M.G., officer commanding the Connaught Hospital, for permission to publish these cases.

Note.—Since the above article was completed a more thorough and elaborate publication on the subject has appeared in THE LANCET of July 14th, 1917, p. 41, in which Hammond, Rolland, and Shore, in the investigation of cases occurring among troops in France, have recorded conclusions almost identical with ours. It is probable that the disease in question is more widespread than has hitherto been recognised. We have, however, published our own results, if only to confirm the findings of Hammond, Rolland, and Shore (*vide* THE LANCET, July 21st, p. 98).

TRENCH FEVER, OR P.U.O., ASSOCIATED WITH THE PRESENCE OF A HÆMOGREGARINE.

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THE presence of a small hæmogregarine resembling in many respects the *Hæmogregarina gracilis* found by Wenyon¹ in a lizard host, *Mabuia quinquetæniata*, has been noted in the venous blood, the blood from liver, spleen, and lung punctures in 12 cases of P.U.O. or trench fever. As a prolonged investigation of the capillary blood by means of thin films and the Ross thick-film method failed to give uniform positive results, an examination of the venous blood was undertaken.

An enormous number of films were examined, and at frequent intervals forms were discovered which by their shape and staining reactions suggested the possibility that they might be hæmogregarines. As these bodies were obviously present in small numbers, even when the blood was examined upon the day or two preceding the febrile stages of the disease, the following technique was adopted in order to obtain the necessary number for purposes of diagnosis and observation.

Technique.—The specimens of blood from the cases of trench fever, which included 8 of McNee, Renshaw, and Brunt's² Class A and 4 of their Class B, or periodically relapsing type, were invariably examined, together with the blood of controls in whose case trench fever and even the contact with lice could be excluded. Thirteen such control cases have been so examined. The distilled water used was invariably filtered through porcelain, and all the glass apparatus and slides were specially well cleaned, sterilised, and the latter kept in absolute alcohol and flamed before use in order to exclude extraneous organisms such as yeasts, &c. All blood films, however prepared, were immediately covered with glass shades in order to avoid the possibility of any organisms gaining access thereto from the air.

Two c.c. of the venous blood was obtained by means of a 2 or 5 c.c. syringe from the median basilic vein and at once syringed into a sterile filtered solution of 1.8 per cent. sodium citrate. To this was added 2, 3, or 4 c.c. of a 0.5 per cent. solution of saponin previously filtered and sterilised in order to cause complete hæmolysis of the red corpuscles and more or less complete cytolysis of the leucocytes according to the amount added. The clear red fluid was then placed in the boats of a centrifuge and centrifugalised for 1½ to 2 hours, when all the supernatant clear fluid was poured off and the deposit spread on clean flamed glass slides by means of a sterile pipette and allowed to dry in the air.

An unstained preparation was in addition always mounted in the following way. A thin circle of sterile paroline was traced out on a slide and a drop of the deposit placed in the centre of a coverslip which was then gently dropped upon the paroline. By this means a specimen was obtained in which the movements of the organism could be observed for considerable periods of time, as the thin layer of paroline surrounding the drop of the deposit prevents evaporation which rapidly takes place when the ordinary fresh preparation is being examined on the warm stage. During the periods just preceding pyrexia when the protozoa are most numerous, the following modification is used. Three or four c.c. of the suspension of the 2 c.c. of blood in sodium citrate solution are added to 12 to 15 c.c. of the sterile, filtered distilled water. The water completely hæmolyses the red corpuscles, but has the advantage that apart from causing swelling up of the leucocytes, it leaves them more or less intact and so allows observation to be made as to the presence or not of parasites within the leucocyte cytoplasm.

The movements of the stained parasites, &c., can be followed on the warm stage by the azur I-saline-paroline method and of the unstained parasite thrown into relief against a red background by the trypan-red-saline-paroline method described in "The Blood Platelets in Health and Disease."³ In the case of trypan-red in certain dilutions there appears to be a definite slowing and cessation of the characteristic movements of the parasite, as is also the case when trypan-blue and trypan-flavine are used. Clinically these dyes are being tried, and already the intravenous exhibition of the latter has given good results.

In addition to the above technique, diagnosis has been confirmed by prolonged study of ordinary thick and thin films of the venous blood.

Diagrams Showing a Few of the Types of the Protozoon of Trench Fever seen in Venous Blood and the Blood and Cells from Spleen and Liver Punctures.

In order to study the cycle of development of the organism the blood and cells from liver, spleen, and lung punctures have been examined. At Lieutenant R. M. Wilson's suggestion the cerebro-spinal fluid from six of the cases was centrifugalised and the deposit examined. The intrathecal pressure of the fluid varied from 10-20 mm. of mercury; the number of cells present varied from one to five or six in 4 c.c., and were invariably lymphocytes. No signs of any hæmogregarines were noted, but the relief of the "shin pain," the hyperalgesia of the skin of middle third of front of legs, the pains in right shoulder, and spleen pains, &c., was so marked, while there was no tendency whatsoever for the pain to return when from 10-12 c.c. of the fluid were withdrawn, that it is now adopted as a routine method of treatment in such cases of severe pain when other simpler measures have failed to afford relief.

One precaution has to be observed in the after-treatment of lumbar puncture when the amount of fluid and its pressure are normal—viz., to keep the patient in bed, the foot-end of which has been raised 8-10 inches, for at least 36-48 hours in order to avoid the typical lumbar puncture headache which usually follows if the patient is allowed to sit up too soon after the operation. This period of rest can be reduced to 6-10 hours if the 10-12 c.c. of cerebro-spinal fluid removed is at once replaced by a similar volume of sterile normal saline.

As the inguinal lymphatic glands were noted to be enlarged and somewhat shotty in certain cases the blood from gland puncture in several of the patients was examined, and in one or two the presence of the hæmogregarine was detected with difficulty and after prolonged examination of thin and thick films. (Major W. Byam informs me that this shotty condition of the inguinal glands is common in the soldier, and that such enlargement cannot be associated with trench fever. In some of the cases, however, axillary and cervical glands were somewhat hard and shotty, and in these specific disease could apparently be excluded.)

The difficulty of finding the protozoon in the lymphatic glands suggests that such are not their habitat as is the liver, and to a less extent the spleen and lung and probably the bone marrow. The examination of the blood withdrawn from the periosteal thickenings of the tibia in certain of the cases is being followed up, but with indefinite results up to the present. The blood films were stained by one or other of the following methods: Giemsa's, Leishman's, or Wright's, and in certain cases Heidenheim's iron-hæmatoxylin method.

A rapid staining method has also been used with excellent results—viz., 0.228 gramme of eosin azur ground up and dissolved in 50 c.c. of pure methyl alcohol. This is allowed to stand for 10-12 days, and the supernatant fluid centrifugalised and used to fix and stain the films. After three minutes' action of the stain it is rapidly washed away by quickly drawing the slide through distilled water once or twice, and then quickly and vigorously shaking dry in order to avoid prolonged action of the water which removes the blue from the structures stained therewith.

Short Description of Protozoon.

The two more common types of the hæmogregarine encountered in the venous blood a day or two before the onset of pyrexia are (1) macromerozoites, 7-13 μ long (these are generally free in the plasma), and (2) micromerozoites, 3-4 μ long, more commonly encysted, and in the red corpuscles, but may be free. In addition to the micromerozoites, &c., which are slender, not more than 1 μ wide at the most, there are other free or encysted "sausage-shaped" forms 4-6 μ long by 3-4 μ wide. Within their transparent cyst the blue-stained cytoplasm stands out clearly, and the deep-red-stained nucleus consists of small pyriform masses of chromatin

DESCRIPTION OF ILLUSTRATIVE PLATE.

I. to V.—A few of the stages of the development of the parthenogenetic gametocyte in the venous blood, liver, and spleen, showing division of the nucleus with its dense chromatin into ultimately 20 merozoites. The blue-stained, coarsely reticular, cytoplasm and the nucleus *de reliquat* are very distinct in all the stages figured. The merozoites at sporulation are drawn double the actual size (Fig. V.) (Stained by Wright's method.)

VI.—Fully developed merozoite. (Stained by Wright's method.)

VII.—Female gametocyte. (Stained by Wright's method.)

VIII.—Encapsulated micromerozoites within the red corpuscles. (Stained by eosin-azur II method; see text.)

N.B.—The upper scale of microns applies to Figs. I.-VI., the lower scale to Figs. VII. and VIII. only.

so arranged as to give it a rosette appearance. Other types of "sausage" bodies are encountered in the blood of these cases similar in size to the preceding, but whose round or oval nucleus consists of a thin red-stained circumference with a single deep-red chromosome at its centre. One or other or all of these types of merozoite may be present according to the stage of the disease in relation to the pyrexia, being most numerous just preceding the febrile stage. They gradually increase in number up to the height of fever, when they disappear, and the day after the pyrexia none of these forms can be found in the blood, the only form of parasite then present being the parthenogenetic gametocyte. This gametocyte can be found in different stages of development according to the time elapsed subsequent to the pyrexial stage, and ultimately just before the rise of temperature gives rise to 20 merozoites.

A rough diagram of the cycle of development of this parthenogenetic origin of the merozoites is appended, as during the latent stages of trench fever a diagnosis can be made with absolute certainty by finding any of these characteristic bodies in the venous blood, spleen, or liver. Personally I have found spleen puncture the quickest way to establish the diagnosis of trench fever.

In brief, this gametocyte of the hæmogregarine of trench fever is a round, oval, or bluntly triangular body in shape, which in the first stages of development is 4-6 μ long by 3-5 μ wide. The cytoplasm is stained an intense blue, and is coarsely reticular in specimens stained by Giemsa's method. There is a large, diffuse, purple nucleus *de reliquat*, and at the periphery of the cell there is a dense, deep-red-stained nucleus rich in chromatin. This is usually situated at an opposite pole to the nucleus *de reliquat*, and in the first stages of development it is round, later oval, and then rod-shaped. As development continues this nucleus divides into two, then four, eight, until when 20 nuclei are formed sporulation commences with the setting free of 20 small merozoites. These merozoites at the moment of sporulation are generally 0.75-1.2 μ long, and gradually become larger until the mid-stages of pyrexia, when they disappear from the venous blood. In distinction from the gametocyte of latent malaria (*H. vivax* infection) this gametocyte of trench fever possesses no pigment or hæmozoin. The size of the gametocyte at the moment of sporulation is 13-15 μ . In addition to this form of development of the merozoites, the micromerozoites develop by a process of schizogony, the schizont when first noted in the liver or blood being an oval purple-stained body 5.5 μ long by 4.5 μ broad, the periphery of which is crenated and just within which crenations is a row of small nuclei which completely encircle the schizont.

There is also evidence that the merozoites develop asexually with the production of a second type of schizont in the liver. In the liver and occasionally in the venous blood what are apparently the gametocytes, or sexual forms, are to be noted. The female gametocyte consists of a circular dense ring-shaped nucleus with a central chromosome. On either side of the nucleus are two semicircular uniform masses of cytoplasm, one of greater transverse diameter than the other. The measurements of a typical example are: length 5 μ , width of wider cytoplasmic mass 3.4 μ , of the smaller 2.2 μ , and the diameter of the nucleus 1.7 μ . The male gametocyte is a round or egg-shaped body 3-3.5 μ in diameter with a centrally situated nucleus usually with three chromatin masses. Both these two bodies are very distinct, easily recognised in fresh preparations, and when stained by Giemsa's method the cytoplasm stains an azure blue and the nucleus a very distinct deep red.

The fact that this protozoon of trench fever is a hæmogregarine suggests the extreme probability that the *Pediculus corporis*, or what is even more likely that a louse allied to the *Hæmatopinus stephensi*, described by Newstead and Christophers as parasitic on the Indian field rat, is the carrier of the protozoon. The presence of such large numbers of rats in the trenches which come into close association with our troops seems to favour this conclusion. An effort is being made to trace out the cycle of sporogony in the stomach cells of the different types of lice commonly present in the under-clothing of our soldiers.

I would like to take this opportunity of thanking Major Byam, R.A.M.C., to whom I am indebted for bringing together in one hospital the clinical material upon which the present work is being done. In addition, I would like to

thank Miss Brad and Corporal Haylock, my assistants, for their help in carrying out this research. I am also much indebted to Lieutenant Wilson, R.A.M.C., who has clinical charge of these cases, for much help and valuable suggestions.

Since writing the above paper I have had opportunities of confirming the presence of the hæmogregarine in a number of other cases of trench fever, and also, in addition, in cases of D.A.H. complicated with some of the clinical signs of P.U.O. and one or two cases of definite benign tertian malaria.

In the cases of double infection, with the parasites of malaria and trench fever, a significant feature was the fact that quinine was powerless to subdue completely the pyrexial attacks which we now know were partly due to the protozoon of malaria and partly to the hæmogregarine of trench fever.

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A CASE OF INFLUENZAL MENINGITIS.

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PATIENT, a female child, aged 14 months, admitted on March 7th, 1917, into East London Hospital for Children, Shadwell, under care of Dr. T. Fisher. The child had then been ill ten days with "bronchitis" and had been treated outside. The illness started with a sore-throat; the child had vomited and gone blue in lips on first day of illness. It was coughing during five days previous to admission. The right shoulder, left wrist, and left ankle-joints were noticed to have swollen on the day before admission. No tuberculous family history.

The child looked obviously ill and semi-conscious and not quite intelligent. It would not grasp a finger put into its hand. It was very restless, jerking the limbs about now and then, and frequently uttering a sharp short cry without apparent external cause. Head was strongly retracted and neck rigid; altogether a moderate degree of opisthotonos. Arms were adducted over chest, elbows semi-extended, wrists strongly dorsiflexed, fingers bent into palms, and the whole limbs rotated so that dorsal aspects of wrists faced each other. Toes bent into soles; ankles hyper-extended. Knee-jerks very exaggerated, tending to clonus, plantar reflexes extensor. Kernig's sign was very evident in both limbs. Supinator jerk present on right side; not looked for on left. Abdominal wall lax; abdominal reflex present. Lungs quite normal: no sign of bronchitis. Heart's action rapid; nothing else abnormal about heart or pulse; rate 140; respirations 36; temperature 101° F. Spleen not palpable: liver one inch below costal margin in right nipple line. The abdominal skin was slightly inelastic. Pupils reacted normally to light. Fontanelle not bulging. The throat was slightly redder than normal; tonsils slightly enlarged. The external genitals and perianal region generally were much excoriated. Doughy swellings were present in right shoulder, left wrist and left ankle-joints; no fluid detected. Movement was practically perfect in range, but evidently painful and resisted.

On account of these swellings, and the evident meningitic condition the child was admitted into hospital with the diagnosis of tuberculous meningitis. Lumbar puncture was done soon after admission.

The cerebrospinal fluid was very slightly turbid; under slightly increased pressure. Cells 150 per c.mm.; 54 p.c. polymorphonuclears, 46 p.c. lymphocytes. In counting these 100 cells 18 "endothelial" cells also seen. Globulin slightly increased; no reducing substance present. The film made from c.s. fluid for differential count of leucocytes showed typical influenza bacilli in large numbers, both short isolated bacilli and long chain forms. These long chain forms were found to consist of the short bacilli joined end to end by a cell membrane. This resolution needed an objective of N.A. 1.3, and was better shown with an objective of N.A. 1.4. The bacilli were almost all extracellular, very few indeed being seen within the polymorphonuclears. Cultures showed the organism to be a typical bacillus of influenza. Subsequently on another lumbar puncture a culture was taken on blood agar and sent to Dr. P. N. Panton, who very kindly examined it and pronounced the culture sent to be "a typical bacillus of the influenza type."