Clinical and other Notes.

SIXTEEN CASES OF INFECTION WITH *BACILLUS AERTRYCKE*. CLINICAL AND LABORATORY OBSERVATIONS. A NOTE ON THE RELATIONSHIP OF *B. AERTRYCKE* AND *B. PARATYPHOSUS* B.

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Cases of this infection have been comparatively rare during the late war.

The outbreak differed little in its nature, save perhaps in the moderation of the clinical signs and symptoms in all cases, from what is generally observed when a group of people have taken material in which *Bacillus aertrycke* has been living and its toxines have developed therein or have become infected by taking in the bacillus without added toxic material.

The sixteen cases to be described were all V.A.D. nurses coming out from England and transhipping in the eastern Mediterranean for Malta. They had not been ashore for about fourteen days prior to onset of their illness.

Cognizant of the rôle played by food and carriers in this disease, searching inquiry to determine any common factor was undertaken by Colonel J. R. Robertson, I. M.S., Special Sanitary Officer, and by Colonel G. B. Price, A.M.S., who commanded the hospital in which the cases were treated. Up to the time of the ship leaving Malta no conclusive epidemiological finding could be made, and before the arrival of the hospital ship at the next port of call it was sunk.

The number of cases formed but a third of the total of nurses travelling under the same conditions on the ship, and being nurses, they were able to assist greatly in the investigation. No special relationship to sleeping places, seats at one table, attendance by one servant or use of one bathroom, could be traced. With regard to food it was really remarkable how impossible it was to determine a dish or drink taken by all during a few days prior to their illness.

A chart showing the clinical conditions on admission and progress under treatment has been prepared in collaboration with Dr. Gertrude Dobrashian under whose immediate care the patients were; and laboratory findings of direct interest to clinical workers are included. It may be emphasized that the disease was in all but one case of mild form, of concurrent incidence and short duration and was characteristic in presenting the form of a gastro-enteritis rather than of a septicæmic infection. Evidence showed proliferation of the bacillus in the intestinal canal and its early disappearance after the cessation of symptoms. Blood sowings made on the second or third day after onset remained sterile. All patients had received T.A.B. vaccine a month or two before.

Animal Experiments.—Cultures made from the *B. aertrycke* isolated from two of the patients gave similar results. Full grown guinea-pigs were used.

Peritoneal injection of $\frac{1}{20}$ of a twenty-four hours' growth on an agar slope from a test tube of seventeen centimetres by seventeen millimetres. Death

Summary of Clinical Observations.

Date of onset of symptoms	Initial symptoms	Physical examination, etc., on admission	Character of temperature	Date when temperature first normal	Further progress	Character of stools with microscopic examinations. The consistency of the stool was most frequently attributable to the line of treatment	Result of stool culture for *B. aertrycke*	Blood cultures
6.10.16 a.m.	Shivering, headache.	Oct. 7, tongue very furred, spleen just felt. Temperature 100·2°, pulse 88	Oct. 8, a.m., normal p.m., 99·2°	Oct. 9 ..	Oct. 13, headache, constipated. Thereafter uninterrupted recovery	9.10.16 Liquid. Rare mucus flakes, white blood cells, rarer epithelial cells 20.10.16 and later, normal ..	— 9.10.16 — 20.10.16	Ten c.c. were taken from cases in hospital with raised temperatures. No growth resulted.
6.10.16 a.m.	Colicky pains, shooting pain down left leg, headache. Shivering later in day	Oct. 7, rigor on admission. Temperature 103°. Headache over vertex, abdomen slightly distended, tongue very furred	Fell by lysis	Oct. 10 ..	Tongue remained very furred until Oct. 15. No appetite until that date. Thereafter uninterrupted recovery	8.10.16 Liquid. Rare mucus flakes, white blood cells, rarer epithelial cells 18.10.16 and later, normal ..	+ 8.10.16 — 18.10.16	
6.10.16 p.m.	Shivering, headache. Aching all over	Temperature 103°, pulse 112. Tongue very furred. Nothing else abnormal	Sharp fall ..	Oct. 9, evening	Tongue remained coated until Oct. 12, did not seem well until this date. Debilitated	10.10.16 Liquid. Mucus flakes, white blood cells, rarer epithelial cells 20.10.16 and later, normal ..	— 10.10.16 — 20.10.16	
6.10.16 a.m.	Headache, backache, slight diarrhœa, nausea	Temperature 100·8°, pulse 90. Tongue slightly furred, abdomen tender all over	Oct. 8, a.m., normal p.m., 99·2°	Oct. 9 ..	Oct. 12, appeared quite fit again. Not constipated. Oct. 18, facial neuralgia. Thereafter uninterrupted recovery	10.10.16 Soft. Mucus flakes, white blood cells, rarer epithelial cells 18.10.16 and later, normal ..	— 10.10.16 — 18.10.16	
6.10.16	Headache, backache, slight diarrhœa and pain	Temperature 100·6°, pulse 92. Eyes suffused, tongue furred	Gradual fall	Oct. 10, a.m.	Oct. 12, clinically quite well again	9.10.16 Nil. Abnormal ..	— 9.10.16	
6.10.16 p.m.	Shivering, headache, aching all over, epigastric pain	Temperature 102°, pulse 96. Perspiring, slight epigastric tenderness, tongue furred	Fell by lysis	Oct. 10, a.m.	Very constipated, bowels acted only after strong purgative or saline given	9.10.16 Liquid. No mucus, white blood cells, rarer epithelial cells 18.10.16 and later, normal ..	+ 9.10.16 + 18.10.16	

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5.10.16	Oct. 4, out of sorts. Oct. 5 (p.m.), abdominal pain. Oct. 6, headache, vomiting, bowels loose	Temperature 100·2°, pulse 100. Slight epigastric tenderness. Gurgling, iliac fossa. Tongue slightly furred	Sharp fall ..	Oct. 8 ..	Oct. 20, bilious attack, headache, vomiting. Thereafter uninterrupted recovery	9.10.16 Liquid. Much glairy mucus, white blood cells, rarer epithelial cells. Many Gram-neg. curved bacilli which did not grow on aerobic cultures 18.10.16 Soft. With little mucus, white blood cells, few epithelial cells. Later normal	+ 9.10.16 — 18.10.16	Ten c.c. were taken from cases in hospital with raised temperatures. No growth resulted.
6.10.16 a.m.	"Tired," with headache towards evening, bowels loose, vomited once. Shivered at night	Temperature 100°, pulse 108. Face flushed, slight epigastric tenderness, tongue very furred	Remained at 100° till Oct. 9, a.m.	Oct. 10 ..	Continued to have colicky pains and to pass mucus till Oct. 15. Thereafter uninterrupted recovery	9.10.16 Soft. Fragments of mucus, rarer white blood cells and epithelial cells 18.10.16 Normal	— 9.10.16 — 18.10.16	
6.10.16 a.m.	a.m.: Nausea, drowsiness. p.m.: Vomiting, diarrhœa, abdominal pain	Temperature 100·4°, pulse 108. Tongue very furred. Vomited bile on admission. Epigastric tenderness	Fell by lysis	Oct. 13, a.m.	Nausea continued till Oct. 12. Some colicky pain with mucus in stools till Oct.	10.10.16 Soft. Patches of dull opalescent mucus, many white blood cells and epithelial cells, few red cells 18.10.16 Soft. Plaques of firm mucus as seen in mucous colitis 23.10.16 Soft fæces and further mucous plaques 27.10.16 Soft. Few pieces of glairy, but no firm placques of mucus	+ 10.10.16 + 18·10.16 — 23.10.16 — 27.10.16	
6.10.16 p.m.	Nausea and shivering Oct. 7, diarrhœa, motion every half hour, headache, nausea	Temperature 101·2°, pulse 92. Perspiring very frequently, offensive stools	Fell by lysis	Oct. 10, a.m.	Rapid convalescence after Oct. 12	8.10.16 Liquid. Some flakes of glairy mucus, few epithelial cells, white and red blood cells 18.10.16 and later, soft. Normal	+ 8.10.16 — 18.10.16	

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SUMMARY OF CLINICAL OBSERVATIONS.—*Continued*

Date of onset of symptoms	Initial symptoms	Physical examination, etc., on admission	Character of temperature	Date when temperature first normal	Further progress	Character of stools with microscopic examinations. The consistency of the stool was most frequently attributable to the line of treatment	Result of stool culture for *B. sertrycke*	Blood cultures
7.10.16 a.m.	Headache, shivering, nausea, shooting pains in wrists and in ankles	Temperature 102°; pulse 104. Gurgling in right iliac fossa. Tongue very furred	Sharp fall ..	Oct. 9, a.m.	Shaky and easily tired up to Oct. 17. Thereafter uninterrupted recovery	9.10.16 Soft. Some flakes of mucus, white blood cells, rarer epithelial cells 18.10.16 and later, normal ..	— 9.10.16 — 18.10.16	Ten c.c. were taken from cases in hospital with raised temperatures. No growth resulted.
6.10.16 p.m.	Headache, shivering, pains in neck, abdominal pain, bowels loose but only two motions	Temperature 102·5°, pulse 96. Perspiring, slight epigastric tenderness, tongue very furred	Gradual fall	Oct. 11, a.m.	Rapid convalescence ..	9.10.16 Fluid. Normal .. 18.10.16 Soft. Flakes of glairy mucus, white blood cells, epithelial cells. Later normal	+ 9.10.16 — 18.10.16	
6.10.16 a.m.	Diarrhœa, malaise, headache, aching all over by afternoon, vomited by Oct. 7	Temperature 102°, pulse 104. Tongue very furred	Gradual fall	Oct. 10, a.m.	Clinically appeared normal except that mucus was seen in stools up to 17th	8.10.16 Soft. Flakes of mucus, white blood cells, epithelial cells 18.10.16 Soft. Flakes of mucus, white blood cells, epithelial cells 23.10.16 Formed. Rare threads of mucus showing on the surface	+ 8.10.16 — 18.10.16 — 23.10.16	

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6.10.16 p.m.	Diarrhœa (four motions in six hours), vomiting, headache and general aching	Temperature, 100·4°, pulse 92. Tongue very furred	Oct. 8, a.m., 99°; p.m., 99·8°	Oct. 9, a.m.	Rapid convalescence..	9.10.16 Soft. Flakes of mucus and of blood, many epithelial and white blood cells. Numerous non-motile thin small Gram-negative bacilli also present. No aerobic growth thereof 18.10.16 and later, soft. Normal	+ 9.10.16 — 18.10.16	Ten c.c. were taken from cases in hospital with raised temperatures. No growth resulted.
6.10.16 p.m.	Abdominal pain, vomited once, bowels loose, general aching, shooting pains in limbs	Temperature, 102·4°, pulse 98. Skin hot and dry, slight epigastric tenderness, tongue furred	Oct. 9, 99·2°, with rise to 99° each evening	Oct. 12, p.m.	Oct. 9, spleen just palpable; Oct. 18, tongue clean. Patient shaky Thereafter rapid convalescence	9.10.16 Soft. Flakes of mucus, epithelial and white blood cells 18.10.16 Liquid. Very rare flakes of clear-looking mucus, white blood cells and epithelial cells. Later normal	+ 9.10.16 — 18.10.16	
11.10.16 p.m.	Languid, feverish, slight colicky pain, following day diarrhœa and vomiting. Oct. 15, blood and mucus	Oct. 16, admitted Imtarfa. Temperature 99°, pulse 52, slightly irregular. Tongue furred, slight epigastric tenderness, stools loose	..	Normal Oct. 17, a.m.	Stools never frequent, but loose, with pain. Oct. 20, normal stool. Rapid convalescence	17.10.16 Soft. Streaks of mucus and blood, epithelial and white blood cells 28.10.16 Soft. Normal	— 17.10.16 — 28.10.16	

followed in fifteen to twenty hours. The *B. aertrycke* was found generalized and was isolated in pure culture from the gall bladder, heart's blood and peritoneal fluid. The peritoneal cavity was full of an opaque watery fluid with strands of lymph especially over the liver. The exudate contained numerous cells. There was also an excess of pleural fluid. No other obvious changes.

Subcutaneous Injection.—Same dose. Death twenty-eight hours later. The animal appeared to be in great pain for half an hour prior to death, and showed convulsive movements. No diarrhœa. The *B. aertrycke* was again found to be generalized. No marked inflammatory condition existed at the site of the infection. Both peritoneal and pleural cavities showed excess of fluid which was turbid and showed strands of lymph and microscopically many cells and bacilli. Broth cultures were made, and after four weeks' growth were well centrifuged. One cubic centimetre of the supernatant broth unheated and heated to 80° C. for ten minutes did not lead to death or emaciation of the guinea-pigs, which were inoculated subcutaneously.

Feeding with almost a whole culture of a slope agar growth of twenty-four hours gave a negative result.

Agglutination and Absorption.—The importance of these reactions in the determination of the *B. aertrycke* and the question of its relationship to the *B. paratyphosus* B Group of micro-organisms justified some detailed investigation on these points during the favourable opportunity offered for research.

The following outstanding results are given as a summary of the research carried out. All patients had been inoculated with T.A.B. vaccine at a recent date, and agglutinins to the micro-organisms were present at the first examination of their blood sera two or three days after onset of the *B. aertrycke* infection. But agglutinin for the *B. aertrycke* isolated did not appear in any till after the sixth day, and was present in all cases on the ninth day. It persisted in fourteen out of the sixteen cases for one month, but in eight out of eleven examined after two months a very definite fall in amount was observed. The agglutinins for *B. typhosus*, *B. paratyphosus* B and *B. paratyphosus* A present in response to the inoculations were not obviously increased as the result of the *B. aertrycke* infection; and in three cases the titre for the *B. paratyphosus* B was less on some days on which the test was done and showed a fluctuation of marked degree. *B. enteriditis* Gaertner was not agglutinated by any of the patients' blood sera at any examination.

The sensibility to agglutination by specific antiserum shown by the colonies from positive stool cultures varied, and preliminary examination was made to select for experimental work that most true to type in its specific agglutinability in all patients' sera and in an animal anti-aertrycke serum prepared by inoculations of a known typical strain of *B. aertrycke*, and in being but slightly agglutinated in paratyphosus B anti-serum. This bacillus will be termed, *B. aertrycke* "Maltmann." It showed agglutination in the anti-aertrycke serum, 1/4,000 (the full titre of serum); anti-Gaertner serum 1/100 complete and 1/200 incomplete (titre 1/8,000); anti-paratyphosus B Schottmüller serum 1/800 incomplete (titre 1/8,000). Time, two hours at 37° C.

The absorption test results and method employed are tabulated.

In the absorption test on the sera of patients and inoculated people much care

must be taken to avoid errors of results and interpretations that may follow the supersaturation of human serum with bacterial emulsions. *The quantity of agglutinin present in a patient's serum for the micro-organisms to be tested should be determined, and a fixed quantity of agglutinin, as well as of the bacterial emulsion employed.* The technique has been described in a previous report to Command Headquarters.[1] It is based on the fact that when the supersaturation method was carried out on human sera emulsions of certain bacilli of allied and, in some instances, of non-allied groups have removed the specific agglutinin for another micro-organism *when this specific agglutinin was small in amount.* When the specific agglutinin content in the dilution of serum is great as it is in specific animal anti-serum of high titre the supersaturation method may be employed. As an illustration of the above statements an example from notes is given, following a table showing the absorptive activities of the strains employed tested with animal specific antisera.

Animal specific antiserum	24 hours at laboratory temperature	Agglutination after absorption		
		G	B	AM
Anti-Gaertner titre 1/8000	3 drops serum dilution 1 in 100 + 3 drops of *B. aertrycke.* "Maltman" emulsion	IC	Trace	—
	3 drops serum dilution 1 in 100 + 3 drops of *B. ent.* Gaertner emulsion	—	—	—
	3 drops serum dilution 1 in 100 + 3 drops of *B. paratyphosus* B emulsion	IC	—	—
Anti-paratyphosus B titre 1/8000	3 drops serum dilution 1 in 100 + 3 drops of *B. aertrycke.* "Maltman" emulsion	—	IC	—
	3 drops serum dilution 1 in 100 + 3 drops of *B. ent.* Gaertner emulsion	—	IC	Partial
	3 drops serum dilution 1 in 100 + 3 drops of *B. paratyphosus* B emulsion	—	—	—
Anti-aertrycke titre 1/4000	Dilution 1 in 50 + 3 drops of *B. aertrycke.* "Maltman" emulsion	—	—	—
	Dilution 1 in 50 + 3 drops of *B. ent.* Gaertner emulsion	—	Trace	IC
	Dilution 1 in 50 + 3 drops of *B. paratyphosus* B emulsion	—	—	IC

IC = Agglutination immediate and complete.

A patient examined early in the disease with a small quantity of specific agglutinin developed for *B. aertrycke*, and with large quantity of agglutinin specific for *B. paratyphosus* B due to inoculation.

Time	*B. paratyphosus* B				*B. ent.* Gaertner				*B. aertrycke* "Maltman"			
	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400
Two hours at 37° C.	+++	++	+	—	—	—	—	—	+	+	—	—

[1] Later published in the JOURNAL OF THE ROYAL ARMY MEDICAL CORPS, October, 1918.

Same serum after supersaturation with:	*B. paratyphosus* B				*B. aertrycke* "Maltman"			
	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400
B. paratyphosus B..	—	—	—	—	—	—	—	—
B. ent. Gaertner	+++	++	+	—	—	—	—	—
B. aertrycke "Maltman" ..	+++	++	+	—	—	—	—	—

Another patient with a larger quantity of specific agglutinin for *B. ent. aertrycke* :—

Time	*B. paratyphosus* B				*B. ent.* Gaertner				*B. aertrycke* "Maltman"			
	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400
Two hours at 37° C.	+++	++	+	—	—	—	—	—	++++	++++	++	+

Same serum after supersaturation with:	*B. paratyphosus* B				*B. ent.* Gaertner				*B. aertrycke* "Maltman"			
	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/60	1/100	1/200	1/400
B. paratyphosus B	—	—	—	—	—	—	—	—	++++	++++	++	+
B. ent. Gaertner	+++	++	+	—	—	—	—	—	++++	++++	++	+
B. aertrycke "Maltman"	+++	++	+	—	—	—	—	—	—	—	—	—

With the following technique which includes titration of agglutinin content of sera the results are very satisfactory, and lead to the elimination of certain factors of error which may occur in the supersaturation test on human sera.

Dilutions of the sera were prepared wherein the agglutinin actions thereof were respectively equal, and equal quantities of the dilution of serum necessary were employed. Standard emulsions of equal density (9,000 million bacilli to 1 cubic centimetre) were used. For standardizing the agglutinin content of sera my time governed agglutination method was employed. That dilution which showed agglutination of an emulsion on a slide in one minute was taken.

Supposing that for *B. paratyphosus* B it was 1 in 20, and for *B. aertrycke* it was 1 in 10, then the absorption test was carried out as follows :—

(A) 3 drops of serum, 1 in 10, plus 3 drops of emulsion, *B. paratyphosus* B.
" " " " " *B. aertrycke.*
(B) " " 1 in 5 " " *B. paratyphosus* B.
" " " " " *B. aertrycke.*

After contact for twenty-four hours at laboratory temperature, or eight hours at 37° C. and one or two shakings of the tubes during that time, or two hours at 55° C., each series A and B was tested for its agglutinability on the slide as before. *All* specific agglutinin may not be absorbed by this method; *co- or*

hetero-agglutinins are and the results show a complete absence or marked retardation of specific agglutins following the agglutination test similarly practised before and after the absorption test, and are easy of interpretation and accurate.

The dilution of serum to be employed for the absorption test can be selected from those used in an agglutination test carried out by the high titre testing methods while maintaining the principle of my quantitative method.

Group Classification.—The classification as *B. aertrycke* or *B. paratyphosus* B of a strain isolated during an epidemic wherein the majority of persons have presented signs of acute gastro-enteritis and some the signs and symptoms of a generalized septicæmia, may be difficult of determination, even with the aid of the absorption and agglutination tests.[1] The following experimental results are given in support thereof and for their practical interest.

The strain to be described is termed *B. wrexham*, and was given to me as the causal agent of the well known "food poisoning" epidemic that occurred there. The *B. wrexham*, when inoculated, produced agglutinins in sera which acted upon itself and not upon a *B. paratyphosus* B until the titre for the former became high. The converse findings were obtained from inoculation of a *B. paratyphosus* B stock strain. An emulsion of *B. wrexham* was tested for agglutinability at the same examinations as the stock strain of *B. paratyphosus* B with sera of approximately 100 cases of paratyphoid B infections. Five of these sera agglutinated the *B. wrexham* in a higher dilution than they agglutinated the *B. paratyphosus* B. The remainder agglutinated it in an equal or, much more frequently, in a lower dilution only.

The agglutination test on the sera of the first three cases of the above epidemic gave in brief these results (they had recently received T. A. B. vaccine) :—

Serum	*B. paratyphosus* B			*B. aertrycke* Maltman			*B. wrexham*		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
"U" ..	+	+	−	++++	+++	+	++++	+++	+
"W" ..	+	−	−	+++	+	−	++	+	−
"K" ..	+++	+	+	+	−	−	+	−	−

The absorption test on the first serum carried out by the supersaturation method showed that although the emulsion of *B. paratyphosus* B removed the agglutinin for *B. wrexham* the converse did not equally hold, and that *B. aertrycke* "Maltman" and *B. wrexham* had an absorbing fraction common to both.

Saturation of patient "U" serum with :	Agglutination after absorption		
	B. paratyphosus B	*B. maltman*	*B. wrexham*
B. paratyphosus B	−	+	−
B. aertrycke Maltman	+	−	−
B. wrexham	+	−	−
B. ent. Gaertner	+	+	+

[1] In the *Lancet*, August 24, 1914, I published experiments that showed that the inoculation of a strain of *B. paratyphosus* B produced agglutinin first detectable on itself and later, when increased, acting upon allied strains.

Comparative findings resulted from the test with the same emulsions and specific animal anti-sera :—

Saturation of anti-*paratyphosus* B serum with :	Agglutination after absorption		
	B. paratyphosus B	*B. maltman*	*B. wrexham*
B. paratyphosus B	—	+	+
B. aertrycke Maltman	+	—	—
B. wrexham	+	—	—
B. ent. Gaertner	+	+	+

Saturation of anti-*aertrycke* serum with :	Agglutination after absorption		
	B. paratyphosus B	*B. maltman*	*B. wrexham*
B. paratyphosus B	—	+	—
B. aertrycke Maltman	—	—	—
B. wrexham	—	—	—
B. ent. Gaertner	Trace	+	+

In an outbreak due to one of the two groups the etiological findings and clinical manifestations, also post-mortem findings in a case of death, are factors of value in the classification of the causal strain as *B. paratyphosus* B, or *B. ent. aertrycke*. Biological and serological examinations of the strain will in the very great majority show that it belongs to that group with which experience has taught us to associate characteristic etiological, clinical and pathological findings. In the above described outbreak the clinical symptoms are characteristic of the infection and biologically and serologically the strain is *B. ent. aertrycke*. But in rare instances the conjoint picture is not so completely true to type. Each group has a number of strains or "types" and some of these show the close relationship of the two groups as serological reactions testify, and as we see with *B. wrexham*.

A case of *B. ent. aertrycke* infection *may* clinically present the signs and symptoms of an insidious onset, continued fever and septicæmia, and a case of *B. paratyphosus* B *may* show an acute onset of severe vomiting and mucous diarrhœa with fever and a toxæmia that is dependent upon the relative toxicity of the strain, the amount of infected material ingested and the length of time the bacillus has been living therein, and I have made a post-mortem examination on a man from whom a *B. paratyphosus* B conforming to type culturally and serologically, was isolated from the intestines, gall bladder, heart's blood and spleen, and the only lesion in the intestines was an inflammatory condition of the mucous membrane with congestion of the lymph follicles in the large part.

The ferment activities of strains on the cultural media generally employed are similar for both groups and show but rare variants from characteristic findings. Amongst about 300 strains examined I have failed to detect or produce motility in one, and this, moreover, needed repeated subculturing in dulcite-peptone water to produce acid and gas. It gave all other characters of *B. paratyphosus* B, and was isolated from the blood of a clinically paratyphoid fever case.

Summarizing, the two groups are closely allied and within each there exist

strains that vary in a slight or marked degree from type in biological, physical or chemico-physical activities. Amongst such intragroup varieties are strains which approximate in these activities strains of the other group, and thereby present a difficulty in classification. A discussion on the mutability or permanence of strains is not within the scope of this article.

In conclusion, I wish to mention the valuable assistance given me in the laboratory work by Q.M. Serjt. R. J. Dermody, R.A.M.C.

THE STAINING OF BLOOD SMEARS FOR THE MALARIA PARASITE.

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The type of smear most commonly used in searching for the malaria parasite is what is known as the "thin smear." Such smears are made as thin as possible, and stained with one or other of the Romanowski stains, most commonly the Leishman. For the examination of the morphological details of the parasite this is without doubt the ideal method according to our present knowledge, but for the routine examination of smears it has several drawbacks, the most outstanding being that when the parasites are scanty a lengthy examination is necessary before any parasites are seen at all. In many cases examined by this method it is only at the end of a long search that a parasite is seen, and one is left with the impression that a number of smears returned as negative would be found to contain parasites if a more prolonged search were made.

Many use the thick smear method in searching for crescents but it seems to be the exception for this method to be used as a routine for all types of malaria, yet this is the method one came to adopt, after very considerable experience in the routine examination of smears, as being at once the easier and more accurate method.

Technique of the "Thick Smear" Method.

A large drop of blood (many times as large as the drop used for making a thin smear) is received on a clean slide from the pricked lobe of the ear, and is spread over an area of from half to three-quarters of an inch square. This is dried, moderate heat being used to hasten the process if so desired. The slide is then covered with the following solution, which simultaneously hæmolyses the red cells and fixes the remainder of the smear.

Formalin..	20 parts
Glacial acetic acid	2 ,,
Aq. dist.	78 ,,

This is left for at least ten minutes. The colourless smear is then gently washed in tap-water (being somewhat fragile it is easily washed off) and stained either with borax methylene blue or Loeffler's methylene blue for two minutes or longer. It is then gently washed in tap-water, and is dried by heat—not by blotting—when it is ready for examination.