Sir David Bruce's notebook re cholera literature, compiled when he was Assistant Professor of Pathology at the Army Medical School at Netley

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MANUFACTURERS

TO HER MAJESTY'S GOVERNMENT.

David Bruce.

David Bruce.

Cholera. Literature.

Temperature.

Optimum 30° to 40° C. Below 17° Very slight. Below 16° None. −10° C retained vitality. Koch: vide Klein Bac. as Chap 13.

Klein No 1 p 150 — 16° to 40°

XXIV Kitasato. Can withstand temp of 50° & 60°.

Heim L.

22. Über das Verhalten der Krankheitserreger der Cholera &c in Milch, Butter, Molken und Käse: Arb: aus dem Kais Gesund. 1889.

23. Hesse. W. Unsere Nahrungsmittel als Nahrböden &c. Z fur H. V, 3. Cen. V p 545

24. Kitasato. Die Widerstandsfähigkeit der C B gegen das Eintrocknen und gegen die Hitze.

25. Paslein. Sulla vaccinazione del Cholera: Rivista Clinica, Archivio italiano di clinica medica 1890 Central: VII p 682

26. Tizzoni et Cantani. Recherches sur le Cholera asiatique: vide review in Central. V 572

27. D.D. Cunningham: Are Choleraic Comma-Bacilli even granting that they are the proximate cause of Cholera Symptoms, really efficient in determining the epidemic diffusion of Cholera.

28. G.F. Dowdeswell. Note on the Morphology of the Cholera Comma Bacillus Lancet 28 June 90. p 1419

29. D.D. Cunningham. On the association of several distinct species of Comma Bacilli with cases of Cholera in Calcutta. Indian Med. Gazette May 90.

30. Stevenson J. The salol treatment of Cholera. Indian Med. Gaz. May 1890.

31. Nicolson F.C. Treatment of Cholera with Salol. Indian Med. Gaz: Sept. 89.

32. Revue Generale de clinique et therapeutique 9 Juillet 1890.

33. Scientific memoirs by Med. Offrs. of the Army of India: On Milk as a medium for Choleraic Com. Bac. by Surg Maj. D D Cunningham M.B. F.R.S.

34. On some species of Choleraic C.B. occurring in Calcutta: Sanitf. Mem. of the Med. Off. of the Army of India. Centralblatt fur Bakt et Par Nr. 23, page 763. 1889.

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5. Pleomanias & Leucomanias Vaughan & Novy p 1888.

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11. Hueppe. Sur la virulence des parasites du Cholera: Centrab V. 583. 1889. C.R. de l'Ac des S. 1889. p 105

12. Lowenthal: Sur la virulence". Central: p 735 Band V. 1889. CR de l'Ac S 1889. p 192

13. Gamaleia. " p 73; 1890. Band VIII – Annal: de l'Instit: Pas: 1889. No 11. p 669

14 do. Sur la vaccin: Cholerique " p 196; 1890 Band VIII. 30. 11. 89.

15. Hueppe: Ueber die Verwendung v. Eiern zu Culturen: p 80, 1888. " IV

16. Macleod & Wilkes: An Inquiry into the causation of Asiatic Cholera: Lancet Mch 2nd 89.

17. Woodhead & Wood. Actions, antidotal & Summative, that the products of B.t. Lancet 223 Feb 90.

18. A: Lustig. Bac: Studien: über Chol. Asiatic" Zeitsch: für Hygiene Bd: 3rd p 174. 1888

19. Zur Aetiologie der Cholera Asiatica. Von Ferdinand Hueppe. Berlin. Klin. Woch No 9. 3 Mch 90.

20. Enzyme Action in Lower Organisms. G. E. Wood. Proceed. Roy. Soc. Ed: vol XVI

21. Berckholtz: Untersuchungen über den Einfluss des Eintrocknens auf die Lebensfähigkeit der Cholera-bacillen. Arbeiten aus dem Kais: Gesundheitsamte. Band V. S 1. Central: Bd VII p 258

Air. Aerobiosis Anaerobiosis.

Koch. Cease to grow when deprived of air. p. 12 No I. ¶336.

Kruppe IX. Woods, Holschewnikow's & Kruppe's show that CB can grow in the absence of air, if a proper choice of an albuminate is made (raw albumen, chopped meat &c). & that thereby they reach in a short time the highest grade of virulence —

XIX Kruppe. Fundamental fact - contrary to earlier statements - the CB. with proper choice of Nourishing materials can ~~live~~ live when air is excluded, and that they there form their poison more energetically and quicker, than by the ordinary method of cultivating them in the presence of air.

The cholera-process in man & S. P. in the intestine takes place under conditions of Anaerobiosis. The reducing characteristics of this bacterium, which have gradually come to be known, offer further support to this.

Wood has found out the fact that facultative anaerob. bac. in condition of Anaerobiosis towards External agencies are much more suscpt. than when in cond. of aerobiosis: This from influence of Membrane formation.

XX Wood. Now the mode of existence of organisms in the intestine must be from the first practically an ~~anaerobic~~ anaerobiotic one. — In these conditions produce poison in ⅓ quantity.

Carbonic Acid Gas.

Koch. Remain undeveloped in stream of CO_2. Do not die. p 13. N° I.

Growth among other Bacteria.

Koch. Damp linen or earth. at first stifle other species. In 2 or 3 days die off. No. I p. 14. # 338.
- # 338. Easily disappear in intestine. under certain circumstances.

Acids

Koch. As soon as the gelatine shows a trace of Acid reaction the growth of B. is very stunted. If the reaction be in a marked degree acid, the development completely ceases. Exception on surface of boiled potato. Potato acid from Malic Acid. No. I p15. ¶ 339.

Klein. Grow fairly well in faintly but distinctly acid broth, but amt. of turbidity + ppt. so far less than in alkaline or neutral broth.

XIX Stuppe. Wood has found out that C.B. in condition of anaerob: are much more susceptible to acids than when grown aerobically.

Drying.

Koch. Dried on coverglasses died 55. in 2 hours. 1 in 3 hrs. Never longer than 24 hrs. Mitt. I. p. 16. II 342

XXI Macleod. p. 471. p 416. Appearance as of Sp. form: drying 48 hrs did not kill. Did not live longer.

XIX Hueppe. CB. in dried condition under certain circumstances retains vitality for a long time.

XXI. Buckholtz: Coverglasses: 27 cases. Either dead in less than an hour, or at longest 2 days.
Glass plates(?). 26 " At most 7 days.
do " 16 " in exsiccator:
Erlenmeyer's flasks. 17 cases — similar result. longest 7 days.
Garden earth. 1 case 2 days.
Linen. 3 " 3.5 " 3 x 39 days in exsiccator.
Silk threads. 1 — 23 - 30 " 1 x 50 x 167 x 187 do.

Agar Agar. 1 days growth.
Gelatine Mod. 3 - (begin 1 - 3 days in). } Most resistant

Temperature. { 1 to 3 days at 37° more resistant than at 22° C.
at 5 days this was reversed.

Koch: 3 days at 35° C = 8 days
" — — 37° C = 3 "

XXII Kitasato.
Coverglasses M. Damp chamber 85 — 100 days.
Silk threads " 200 days and longer.
" Dried in air. longest 4 days.
" Exsiccator. 14 "

Spore Formation.

Koch: In my opinion there is no prospect of finding a permanent state. No 1. p. 18. // 343.

" // 383.

XVI. p 416.

XIX Hueppe. My opponents say
The lasting-form depends not on formation of a lasting form, but by formation of a Zoogloea — H. still sticks to his arthrospore formation.
H. gives 3 grades of resistance power. ① Anaerobiosis - ② Aerob: vegetat form.
③ Arthrospore-Zoogloea.

XXI Beckhold: Considers there is no Spore formation — only difference in conditions of drying give diff. in duration of vitality.

XXII Kitasato. Goes against Spore formation. —

Occurrence in Cholera cases

Koch. "Comma bac: are never found absent in cases of Chol: Nº 1 p 18. p 115.
Klein. In every case during first days the intestinal discharges contain the C.B. Nº 1. p 23.
Klein. Thinks there is no definite relation between the number of C.B. present in the stools & the severity of the disease
" If p.m. exam. delayed the number of CB & other bac: was likely to be greater than when p.m. examined after death. Nº 1 p 27
Wesenberg Frank. 31 cases dead within 24 hrs. 14. CB scarce: 1 none cd be found. 16. [numerous]?
24 " " 24 to 48 hrs 9 " " 1 " " -1? 13. do? Nº 1. p 30.
Klein. In no intestinal disorder in man have Kochs CB. been found. Nº 1 p 114
Koch. Nº II 344 - Egypt 10 cases microscop. - India 42 p ms - Present in all. 32 dejections also present in all.
In all about 100 cases present in all.
Nº II 173 - Roy - Completely absent in some cases - Did not look for it in premon. diarrhoea.
XVI - Macleod Mullen. In all 44 Cases: [30 fatal]. 6 p ms - [14 Recovered]

40 cases CB found: 1. CBs seen microsc. but not by plate as Carbol: Ac: has been added.
3. Non char cases & among first investigated.
"Later than collapse stage, when stools no longer typical but opaque. organisms seldom found & then with difficulty."
"P. M. Exam: alone is not sufficient to determine the absence of the organism for a given case but must be supplemented by failure to find it in the typical stools during life.
"Roy 16th Conclusions seem to have been based on P.M. exams alone."

XVIII Dieting: 170 cases: CB fd. in all. Gives details of period &c of disease:

5 cases exam? daily. In all CB exclusively during first two days. In 3 cases the Coll. were still found on 6th day - In 1 case in which the stools had become semi solid and poriculent the CBs were f? on 14th day.
In 2 cases which died of Complications (Croupous pneum) after some weeks no CBs were found.
These investig: show that the CB. is present in all slight or severe cases of Cholera not only during the height but also during the stage of Asphyxia whether case has been slow or rapid in development.

XXIX Cunningham. Agents are several sp. of CB assoc? with Cholera in Calcutta
XXXIII do. "one out of 11 successive spec: ~~derived from~~ of Choleraic materials derived from as many reputed cases of the disease yielded a Crop of Comma Bacilli on cultivation".
XXXIV do. In many undoubted cases of Cholera, CBs have not been found.

Stools.

Koch. Man had Chol. 6 weeks prev.y died of anoemia, no Bac. in stools.
" " " 8 days prev.y no Ch. Bac. in stools. V.I.p19.

Klein.

XVI. After collapse stage, in "lipid stage, when stools no longer char. M.O difficult to find & rarely found.
"Rice water not good term. R.W. is ~~opaque~~ non-transp. & has finely dispersed granular matter
Cholera stool. is almost transparent
In all non-typical stools several organisms present. in typical stool almost a pure culture of CB frequently –

XVIII The M.Os in the dejections were not numerous. By far most prom.t & in some cases the only one present was Koch's CB.

Presence in mucous membr.

Klein: Such appearances as are described & figured by Koch were not met with: No 1. p 33.
The CB. do not take the lead (in depth & numbers) in penetrating the M. M. as maintained by Koch. do p 35
Babes. Found CB in tissue after preserved in alcohol for years. No 1. p 35
Watson Cheyne — Fd CB in tissue. — List of writers given who question Koch's statement. No 1 p 35.
Klein. States that bacilli can penetrate into healthy M. M. & quotes B 33 03 ero. No 1. p 35
Koch. II 330 —
XVI Macleod: Agrees with Koch in regard to presence in intestine.
XXVI Tizzoni et Cantani. In rapid cases. found CB. in muc. mem & even in sub. mucous tissue.

Presence in blood & organs.

Klein. No CB. or other Bac. occur in blood or organs. Confirms Koch in this. No. 1 p38.

XVIII Lustig. Koch Ceci & others negative results.
Nicati & Rietsch fd. in Gall-duct in 3 rapid cases: in 2 out of 5 in gall bladder.
Doyen. Cultd. CB. from liver. & Venous blood.
Rasstoschewsky — From. liver.
Tizzoni & Cantani. in gall bladder & blood. Once from Sub arach: fluid.
Babes. in blood & spleen of animals dead after being infected with CB.
Watson Cheyne — ditto in blood of Heart.
Van Ermengem. in blood of G.Ps after duodenal inoc.n Doyen. Hueppe. Tizzoni & Cantani

Lustig. 2 cases pulp from spleen. No CB altho CB in stools.
2 — blood from finger do do.
From p.m. blood, liver, spleen, no results.
Seven foetuses exam.d from Chol. dead — no CB found.

XXVI Tizzoni et Cantani. Gall. Exam.d in 3 cases. Fd in all. Twice among other bac: once alm. pure.
Cerebro-spinal fluid — 2 cases — Fd. pure culture in both. Describes path: appearances in Cranium.
Blood. 2 cases. No result. Foetus: 5 months. Fd in blood & intestine.
In Liver & Kid.s Sections. No result. ~~Brain~~ In brain thought they saw CBs.
31 G.Ps. infected by stomach. 15 died. in 14 CB ~~fd.~~ [together] in blood. & found in 9 — In 3 looked for in periton fluid, fd. in 3.
In 9 G.P.s of 11 which died after sub.cut. injection + Opium or Alcs: CB fd. in blood.

Animals G.Ps. died after intraven: inject. of CB. + intraperiton: opium.
In 2 G.P. — between 6 & 18 hrs after infect. dead — In Blood — pure culture: R.K.
" 3 No result.
Intes: contents of these — 3 cases negative — 1 case positive: but not certain.
Urine & Gall — No results

Pieces of liver, Kidney, spleen, placed in test-tubes with blood serum 18 to 30 hrs. After this procedure CBs were fd. Also in brain without previous growing in Bd. serum.

Presence in Vomit.

Klein. Exam^d six cases, found only in one, & in small nos: II^o. I p 38.
Koch. II 344 - Fd. twice.
XXI Tizzoni et Cantani. 5 cases. Contents of Stomach. Present in 3. in large quantity.

Morphology

Klein: Length 0·6 and 1·2 μ. Thickness about 0·2 μ. N°1 p41.
" Great difference exists as regards amount of curvature.
" Describes circular & oval forms, which he states divide longitud.ly after vacuolation N°7 p60.
Koch. ¶ 332.

XXVI Izzoni et Cantani: Talking of shape of CB. ps in blood of I. B. "Several times found characteristic CBs. in various stages of development." But after stained as new repractile balls, which appeared somewhat larger than C. Bs. & which stain paler with fuchsin.!

XXVIII. Dowdeswell. Describes various cycles of development. vide paper.

Staining.

Klein. One peculiarity is that the stain is easily taken out with alcohol. No 1 p 44

Growth in Broth.

Klein. 35° & 37°C. a pellicle forms after 36-48 hrs. made up almost entirely of long or short spirals
Koch #338 - Do not grow in diluted broth, vide paper.

Vitality in artificial Cultiv: media.

Klein. Many tubes were barren of all life before the end of 12 months or earlier. In Gel: tubes death of the growth sets in in many instances after 4 or 5 mths, in others after 6-8. N:1 p 52

Koch ff 384. Nicati & Riesch. CB alive in harbour water 81 days-

" Agar 144 days -

Growth in milk cu page 79.

Klein. Grow well, but not so luxur. as in broth. Casein not ppt. Appearances unaltered. Mr Warrington has noticed curdling of milk to take place at 30°C.

Inoculation Experiments

Klein. No true Chol: infection, as is understood in Path, has as yet been produced K. No/p 114
Koch - "/ 350 -
"/ 15 - Inoculation Experiments in Spain show that CB. may be largely introduced into the
of Report of Commission - system without causing Cholera -

Colour test.

Klein: Bujwid shows that the reaction is the more pronounced the purer the culture is v. c. N° 1 p 118

" 5 to 10% HCl. (also NH_3. H_2SO_4) pink reaction: (Dunham shows peptone necessary. H_2SO_4 best) previous addition of a drop of HNO_3 enhances the reaction, which under this condition can be obtained also with Finklers & Deneke's CB. Salkowski. Indol & nitrite N° 1 p 111.

Lewis' C Bacillus

Klein. No 1. p 112. Thinks has cultivd - & are strikingly similar to Kochs. p 113. It does not follow that they are identical -

Diagnosis - Presence of CB.

Klein - p 115 No 1 - If pd. in any case of diarrh: most prob.y the: not necessarily the disease is Cholera.
-do- p 116 " - Experiments on mixing CB with faecal mixture - showing difficulty of finding them on pl. cultus - if few in numbers -

XVIII In Trieste Epidemic Lustig confirmed P.t case adm. to hosp.l by means of plate cultivations

Vitality in stools

Klein - Nº 1 p117. Quotes Kitasato to show that CB. kept in mixture of fecal matter die.

XIX Hueppe. CB after anaerobiosis in fresh stools are at first easier to destroy than in every other condition.

Occurrence in outbreaks of Choleraic disease.

Klein No. 1 p 118.

Feeding Experiments.

Klein. Vol I p 119:-

Koch II 356 Dogs, cholera dejecta nil. Pigs, died in 2½ hrs - (Richards).

" II 381. Bochefontaine pills. nil. Macnamara's case. Case during course!

Klein III p 27 Mice &c.

Injection Experiments – Blood stream of rabbits & Guinea Pigs

Klein – No 1 p 120 – ~~No result~~ Rabbits seemed very ill, but recovd in a few days (Koch). II 351.

XXVI. Izzoni et Cantani (A) ·7 to $2\frac{3}{4}$ ccm. Broth: culture into G. Ps. Intravenous: Animals remained healthy.
B. First, intraperiton. inject. of Opium, then $\frac{2}{5}$ of a drop to 1·5 ccm. Broth. Intraven. 5 animals died.
" do 1 got $\frac{3}{5}$ other $\frac{5}{7}$ of a drop 2 " remained alive

Injection Experiments - Into peritoneal cavity of mice.

Klein. No 1 p 120 - Died in 24 to 48 hrs - PCB were found in their blood - (Koch) II 351.

Similar disease among animals.

Klein. N°1 p 120. No case known to have occurred (Koch).

Artificial Cholera. Nicati & Rieschs Experiments.

Klein. No 1 p 122. Bile duct lig.d peritn cavity & intestine exposed, CB injected. animals died. Klein ventures to say that these symptoms can be as readily produced without the CB.

do do p 123. Koch repeated these exper. without ligaturing the bile duct.

do do p 124. When injected into first coil of intestine which appd. 1 out of 6 guinea pigs died.

do p 124 Van Ermengem. Guinea pigs inocd per duodenum & died in 2 to 18 hours.

Koch ff p 370.

10 GPs. ligature of bile duct 6 died.
18 animals without lig. 13 " Control Exps. none died.
6 GPs into first loop 1 "
4 Rabbits do 0 "

Injection Experiments of Sterilized Cholera Cultiv: fluids. See p. 31.

Klein. p 126 No 1 _ Van Ermengem: Chamberland filter. (Serum chiefly) produced death _

See page 31.

III Rep 20 _ Aitken _ Alkaloid pd by M. Villiers _

Ptomaine production. Toxines. (see page 30)

Klein. No 1 p 126. Nicati Klebs & others state there is present in cultiv. fluids a chemical poison whether when CB introduced into the intestine they produce the same poison is open to objection &c

do do p 127 - Hueppe grows CB in hens egg - No doubt about the presence of a chemical ferment in artificial cultures of CB (espec in Serum).

do - Cultures, Gelatine, duodenum, dogs & guinea pigs - No result - Klein - p 128.
- Agar Agar - do do do.

do - Fresh Chol: material { Small intestine. D. McCat & R: - do.
Jugular vein -
Peritoneum -

V 99. Cantani. steril: cultures periton cav. small Dogs - Became ill -
Brieger - A diamine - and another.

XVII. Woodhead &c In some cases the toxines have been separated, from Animals or Cultivations, for Cholera - Nicati & Riesch. Brieger. Hueppe. (Papers named).

XX. Wood. CB produces its poison in gt. quantity when grown anaerobiotically.

XXXI. Pizzoni & Cantani. 2 to 4 day old broth cultures, ster.d 1 hour at 80°C.
6 g B.S. this fluid injected into stomach, + Hocks Opium & Soda, 2 died.
Sub. cut. injection - slight fall of temp - No result -
do + Sub. cut. inject of opium - remained alive -
do + 40% alcohol in Stomach or intraperiton: opium 8 out of 12 died -

XXXII. N. Ireland writes there is an article on Infantile Cholera in which it is said a toxic resinous substance has been isolated which is common to all kinds of Cholera.

Growth in egg.

Klein. N.1 p127. Heuppe grow CB in egg.

Injection Experiments. Subcutaneous.

Klein. No. 1 p 128. Guinea Pigs. Broth culture, 3 days old, 37°C. 2 G.Ps. 4 ccms No result.
do. Gelatine old. 4 " Death.
do p 129.

XXVI Izzone et Cantani: 5 G.Ps. ½ drop to 7.5 ccm. Broth culture: Slight fall of temp: Animals lived.
11. Sub. cutaneous injection + intraperitoneal injection of Tr. Opii (Koch). 8 died.
5. do + [illegible] 40% Alco. into the Stomach: 3 died
Experiments with Sub. cut. injection of C.B. & at the same time sub. cut. injection of Opium — No result.

Attenuation of Bacilli

Klein No. 1 p 129 - Agar blanks.

Artificial Cholera. Kochs Exper^t.

Klein. p135. No.1. — Klein injected opium subcut^y. & got no results – Was his cultures still virulent?

do — Kleins opinion is that the intestine must be diseased before the CB. develops in it – p136 & 137.

do p179. — Macleod & Millers Experiments – Means used cannot cause death of animals – vide paper –

Koch II p372. 4	7 G.Ps.	5ccm 5% Soda.	10ccm Broth CB.		No result – Killed, in 6 CB fd in sm. intestine
	2 –	2% –	–		do.
	6 –	5% –	–		do.
	4 –	5%	–		do one died. (Had aborted).
	35 –	5%	–	+ Tc opii	30 died.
	14 –	5%	⅓ drop.		7 –
	24 –	5%	still less.		7 –
In all	80 – have taken Cholera.				
Finkler – Control	15 –				5 –
Deneke do	15 –				3 –
Millers do	21 –				4 –

Anthrax sporeless + – Briegers B + – Fowl Chol. – Osteomyelitis – rabbit septic – Erysipelas – Typhoid – doubtful –

Croton oil – Castor oil – turpentine, tincture of iodine – glycer. alcohol. successful results.

XVI Macleod – p468. Method. ① No.3 Catheter, no stilette. ② Wooden gag with hole in it & notched to receive upper & lower incisors ③ Dose of opium. Repeated doses of 1ccm or less were injected till the animal was suff^y stupified to lie on side or back for at least 10 minutes when placed in that position. Several times the dose recommended by Koch had to be given but usually a smaller amount sufficed. Sterilise needle of Kochs syringe in flame & glass in hot box. – ④ Hold G.P. in palm left hand, the front abd. wall being projected forward and made tense by the thumb & fingers on each side when it is easy to just enter the periton. cavity with the needle point. An assistant should hold the fore and hind quarters – All vessels should be sterilised –

Organism used. taken from stools of Chol. case in Shanghai in Oct./87 pure cultiv^s. being made from plates & fresh gelatine tubes being inoc^d. at intervals of 3 to 6 weeks. The organism retained its virulence for G.Ps till following Oct^r.

① No prelim^y. starvation. ② 5ccms of 5% Soda Solution ③ 10 to 20 mins. later a quantity of Chol. matter ④ Immed. thereafter opium introduced. It is necess: to introduce sufficient opium to cause animal to lie on side – Of 9 G.Ps which did not receive suff. only one died
54 " " rec^d enough " 38 (some 2^nd, 11 other 3rd & 4th day)

Injections with bowel contents of G.Ps dead of Chol. were f^d to be very fatal. 21 – 19 died.
– – coecum – – – – – – harmless, altho fluid cont^d many CBs

Doses of Cultiv. and Effects p470. –

Experiments wanted.

If one had a fresh cultivation from Cholera case, would Klein's modification of Koch's Experiments on G.Ps be successful.

vide No 1 page 136

Does injection of Tc opium into peritoneal cavity of G.P. relax the intestine & arrest peristaltic movement No 1 p 136
XVI Macleod p469. 4 G.P. - 2 spirit - 2 Tc of Opium - Killed 6 to 9 hrs afterward and showed distension and arrest of peristal. movement.

With what other species of bacteria can you get positive results by Koch's method of inducing Chol. in G.Ps.
Klein says Finkler & Deneke.

Can you exalt virulence by growing CB anaerobically in intestine of guinea pig through several generations. —
By doing this the virulence is exalted for guinea pigs: Macleod. Pfeiffer.

Gamaleia _ White rats. 61 –
Lowenthal _ Recul: Cult. Med. 62.

Exaltation of Virulence + Gamaleia _ G.P. then pigeons p 37.

Klein. No 1 p 139 – Gamaleia & Lowenthal:

VI p 473. IV 20: 8. 88. Gamaleia. ~~& Lowenthal~~ — G. states easy to heighten virulence of CB by inoc.^g pigeons after passage th. G.P. Chol. symptoms. CB fd in blood. After several passages it becomes so poisonous that 1–2 drops of pigeons blood killed Ps. in 8–10 hrs & still smaller doses G.Ps.

in circulating blood. ××

VII p 39. Pfeiffer & Nocht – Undertook reexam. Exper. on pigeons. they used the contents of the small intestine of G.Ps after 6 to 8 generations. Intramus – Intraperiton – per os – The pigeons died rapidly but not of Chol – of Septic.
Next pure cultures were used – Pigeons bear relat. lg quan.^y if injected slowly & carefully. Fd. that the CB are killed rapidly in blood of pigeons, organ free even after having passed many times th G.P. & having become very virulent for this species Only by injection into the pleural or periton cavity could the ~~animals~~ birds be killed with any certainty – In these cases the CB was fd in the blood but in very small nos. Pigeons require a consid. quantity 3 to 5 ccm of a fresh broth culture — a rising virulence was never seen, but always the contrary – A direct carrying of the Chol from pigeon to pigeon was never successful. — pigeons.

~~XX~~ p.

~~XI~~V p 196. Gamaleia: Demonstration of above method of exalting virulence "ihm schwierkeiten bereitete Has: ∴ simplified process (See Wht. Rats p 61).

XXV Baslein – Author thinks this the surest method of exalting virulence

X Low 1888.

Immunity. _ Vaccination for Cholera _

Klein. p 139. No I _ Injected into pectoral muscle several ccs of recent broth culture, after 24 hrs no trace

Gamaleia. VI p 473. A Pigeon inoc^d with ordinary culture of CB was refractory to virulent:_
20.8.88. Virulent Culture in broth heated 120°C: contains ptomaine which kills pigeons & GPs. 4 ccm.
If small quantities of this were injected over several days animals became proof against a fatal dose:

Semaine Medic. Lowenthal. Mice which recovered from 1st weaker injection were proof against strong later
1888. No 35. 1st mouse. 2 injections of sterilized broth. proof against two injections of virulent _ but succumbed
30 August? to a 3rd (5th in all) which followed 14 days later. the 1st injections followed 2 & 4 days after ster^d injection
2nd mouse. Refractory to injections, succumbed to an injection into the intestine _
A. How long is immunity conferred. B. Is it of use against intestinal inoculation.

XIV Gamaleia: In preceding year had announced a process for the protective inoc: of cholera.
His process consists essentially in the inoculation in the first place of G.Ps, then
30.11.89. transmitting the disease to pigeons, & after its passage through several pigeons to come
thereby into the possession of a strain of virulent CBs. _ Heat cultures of these to 120°C
small quantities of this injected into pigeons render them immune to the virulent
material itself.

In this paper G. modifies his process for exalt^n of virulence (see Exalt of Virulence).

Preparation of Vaccine:
- Sow CB in broth (Calf foot): (no matter what culture) 35° to 38°C
- Shake once a day to detach pellicle.
- In 14 days decant & heat "Ruckstand" (remainder)? 20 min. _ 120°C.
- By standing 14 days at temp of room the power is heightened.

Power of Vaccine on GPs.:
- Fresh vaccine (intramus.) G. Ps., 4 – 8 ccm kills accord^g to strength of orig: culture
- after 14 days strength of the poison in vaccine 2 to 3 times as great.

Amount to confer immunity:
- The fatal dose injected into G. Ps. in several doses, on diff. days makes them immune.
- For detailed experiment see paper.

XXV Baslein: Exalted virulence _ 1½ – 2½ drops into periton: cavity without opium = Vaccine.
The same amount + 1 cc Tc opii, 15 minutes later proved fatal _
27 non-protected G.Ps _ all died :_ 16 protected _ 1 died.

Injection Experiments - Pigeons.

Klein Nr. 1. p. 139.

Arguments against -

Klein: 1st p. 140. Lg. nos of CB can pass thr: healthy intestine without producing serious results.
He thinks this fact disposes of the idea that they can be the cause of Cholera.
These experiments of Koch were on G.B. No proof that same obtains in man -
Klein + Cunningham finding CB in tanks - the description is not definite Enough -
do do p. 147. Certain localities enjoy immunity — Season -

Occurrences of Koch's CB in other situations than intestines of Chol cases.

Klein. No 1. p 164. 3d. in intestines of Monkey.

XVI Macleod p 417. Exam? cases of diarrh. &c. but did not fd the CB. Criticism of Klein's position

Pathological appearances in Chol: cases

Koch No 2. p 329.

Roy IV p176. —— 177. "Epithel: less firmly attached, not detached." – Chytridiaceae

XVI. Macleod: p. 417. Sm. intes: distended & ext[l] rose-red: Case 10 hrs duration
" more deeply congested with haemorr. in M. M. Case 30 hours duration
" Congestion & swelling of M. M. with much stripping of Epithel – 32 hrs.
" {St congestion, haemorr. ulceration. strip of Epi. —— 4 days.
{stools not char: no CB — CB fd. during life

Path: appear. in G.P. p470.

XXVI Tizzoni et Cantani – Give path: appear: in cranial cav: In liver & Kid[s] freq[t] F.D & W.D. also necrotic patches

Growth in Milk. See p 79.

Koch x 330. Do not cause the milk to curdle, nor do they separate the casein.

Substances which inhibit growth.

Koch II 340.

Infection through lesions.

Koch II 352 — "as the CB is the only organisms in question".

" II 380 - Dead after 3 or 4 days.

Klein III 31. Explanation by V. pettenkoffer. (p 33 Drasche).

Macleod XVI 471. Infection th: custom of washing the dead.

Occurrence in drinking water. see page 51

Koch II 354.. See also Klein. I p156.

XXVIII. Hesse: "That sterilised tap-water for example in other cases a very suitable soil for the growth of CB can be, is by the careful investigation of Wolffhügel and Riedel proved—

XXVII Cunningham.

Periods of Survival of Commas in Water.

No. of Exp.	Quality of Water.	Period.
1	Tanty clear. unboiled.	Disapp. in 4 days.
2	" "	" " 5 "
3	Fond. "	" " 4 "
4	" boiled	" " 25 "
5	" unboiled	" " 9 "

Periods of Survival of Commas in Soil.

No. of Exp.	Quality of Soil.	Period.
1	Garden Earth.	Absent after 26 days.
2	" "	" " 14 "
3	" "	" " 10 "
4	Gard. Earth & faeces	" " 6 "
5	- do - boiled	Present " 47 "
6.	- & faeces unboiled	Absent " 9 "

Action on Bd. Corpuscles

Koch II 355. RBC. in plate culture were destroyed round colonies.

XXV. Jaslein. In protected animals No. of RBC. ~~fell~~ diminished from 4 to 3 millions: the WBC remained the same. In the non-protected animals both the RBC + WBC. showed a considerable increase - most marked among the WBC.

Cause of death in Cholera.

Koch ii 356 "Sh. be considered poisoning" 1st by CO. 2nd Results of decompos. of alb. fluid in intestine

Roy iv 177. due to escape of fluids from the vessels into the intestine

~~xtx~~ Hueppe. Due to formation of toxines; Escape of fluids, desquam. of Epi: merely 2ary

Infection through air.

Koch II 358. Cannot take place.

XXVI. Tizzoni et Cantani: Flies in the hosp.l were placed in fluid blood serum, CBs devel.d among other bac.
various kinds of putr. also given as means

Infection by human intercourse.—
Koch II 358: Cholera has alw. come to us by mankind himself.

Growth in water: See page 46. N.B.

Koch # 359.

" # 380. Spring water 30 days. # 384. Harbour water 81 days.
Berlin Sewer water 6 or 7 days.
Excrement 27 hours.
Cesspool water 24 hrs.

Spontaneous origin.
Koch ii 360.

Home of Cholera.

Koch II 361. "Delta of Ganges is only place".

Improved Water Supply.

Koch II 364. Calcutta. Fort William (see Klein) Pondicherry.

Diagnosis - As aid to.
Koch. ¶ 368.

Theory of Epidemic diffusion.

Klein III 33. A living organism, from a Chol. locality into a new & suitable soil (transferred) therein multiplies & gives rise to a chem: ferment which gaining access to the body of a person, sets up the disease.

XIX - 3 - 90. Hueppe. The Cholera process runs its course only in the intestine — It must be looked on as a specific putrefaction (Darmfäulniss) in the intestine with the formation of specific toxines. The loss of fluid, the denuding of the villi: muc: mem: of its epithel however weighty these processes may be in the Symptomatology, they must be considered as merely subordinate ätiologically — The localisation of the Cholera process in the Ali: canal follows from our researches, corresponding with (anatomical) results got by Brohmer & Cunningham, not only by infection per os: but also as Secondary localisation in this "locus minoris resistentiae, when the infection took place by other paths. (Vide p 71)

do — Enigmatical fact that CB very seldom contagions direct from Sick to healthy receives an Explanation in fact of Super-sensitiveness of CB after Anaerobic growth in intestine — Require to grow Aerobically in order to become resistant enough to pass acid of stomach.

Contrary to Pettenkoffers idea: the CB are very infectious & can best form toxins immed: on leaving the intestine, but they are Super. sensitive to ext: agencies

do Chol. Asiat. is a miasmatic-contagions disease, with proof on Epidem. grounds of it's strong dependence on External conditions, which can also be explained On bacteriological grounds.

Infection th.: water.

Klein III p 34:

Nerve Cutting
IV 177.

Lowenthal. X p583. A substance sought for, harmless to man, but stop.s the devel.t of CB in duodenum.
Thought to have found this in Salol. which is decomposed by pancreatic juice.
The Salol being added to the Pancreas Broth & then mod.n with CB, kept broth sterile.
also on addition to the Salol + Broth of 3 com of a pure culture of CB. in bouillon
This action took place with the employment of 2 grm Salol 10 grms Broth, but the greatest part of the cultures remained sterile when the Salol was reduced to .10 grms.

Stuppel: XI. p583. Claims priority to Lowenthal in regard to Salol. (10 ap 488) Tribromophenol, Salycilate of Bismuth + Salol.

Therapeutics.

Antiseptics: Salol.

Hueppe IX. 1889. Salol: Not first recog? by Lowenthal, but by Sahli & Hueppe himself. H. thinks that it must be tried thoroughly in case of human cholera, before any assertion can be made in regard to its practical use. H. thinks the result of Lowenthal's experiments on G.P. as doubtful. Lowenthal at any rate made the error of supposing that the Salol became decomposed into ~~Carbol~~ Phenol & Salycilic acid by the pancreatic juice, since the active substances of the same had been destroyed by the sterilization.

Md. 1890.

XIX Hueppe. Mentions that the experiments made up to the present in India are very satisf.y and thinks that perhaps Salol has a future in Cholera as therap: means.

XX Wood. Thus if the organisms experimented with are a brood of very young cells obtained by incubation for 18 or 24 hrs. at 37°C. the action of the anti-septic is much more marked than on an older culture.

XXX Stevenson. Gives 4 cases, Salol tried little effect.

XXXI Nicolson. Gives report of 18 cases, Salol tried, all recovered.

Therapeutics.

Loewenthal: X Salol: see p 59 of notes.

XXVI. Tizzoni et Cattani. Calomel & Thymol by the mouth, no influence on No of CB in stools. Only in one case, an hour after administration of a large dose of Calomel, the CB cd. no longer be cultivated from the intestine at the p.m.

XXX. Stevenson. I. Gives 4 cases where this drug tried. Little or no effect.

Exaltation of Virulence — White rats. Jamaleia.

Jamaleia XIII. White rats die readily - injection of CB. through thoracic walls into lungs, and there is found after successive inoculations an exaltation of Virulence — Even may be said to bring about a Chol. Septicaemia; with numerous Vibrios in blood, & often with no local symptoms in lungs & pleura —

Also in this case the most Vir. Vib. are fd. in pleuritic exudation — on being cultiv. artific^{y} the CB's lose their Virulence, but they show higher energy of growth & are more fitted for anaerobiosis than formerly.

Also in CB. as in Vib. Metsch: the isolated poison in combin^{n}. with ordinary CBs gives the phenomena of the Vir: CBs.
∴ G. concludes that it is possible to obtain exaltation of Virulence in bodies of refractory animals.

Jam: XIV. 30.11.89 G. has modified his proces for exalt. of Virulence, & as he states because the demonstration of the exalt. of Virulence before the Commission of the Academy of Sciences (prepared difficulties to him). He has ∴ simplified the proces:

States that any culture will do.

Experiment showing Exaltation of Virulence & immunity after Vaccination:-

1st day { 1 ccm. Emulsion of CB from Agar Culture, in ster: water into right lung of white rat — Rat died in 24 hrs with pleur: Effus: a pure Cult^{n}. of CBs

2nd " { Exudat + water into 2nd " Rat:-

3rd " Exudat + water " G.P. 300 grms :-
This exudat: from pleural cavity was tested on 3 Vaccinated & 3 non-vaccin G.P. — 2 Intraperiton^{l}. 1 ccm — 2 per trachea ½ ccm — 2 through thoracic wall. ½ ccm
Foll^{g} day all control animals dead — Vacc^{d} lived.

Exaltation of Virulence. Lowenthals by growth on partic. medium.

VI p473. 30-8-88. Lowenthal. Can restore virulence by growing on a partic. nourish.g med. made of beef, pancreas juice, leguminos. juice. Not known to which of these ingredients the power belongs, what the proper proportions, how much time &c. He thinks certainly that the poisonousness of the 1st generation augment after 24 hrs - & also with the duration of the cultivating & the No of the generations - Ref: of Pancreas &c: Central V p582: Method of experiment given.

IX p502. 15-8-89? Hueppe thinks that Lowenthals opinion, of his "pancreas" nourish.g medium giving the CB. the same conditions as they have in the human intestine, is founded on error.
① L. has left out of consideration the fact that the CB. grow in the intestine without Oxygen, often gas present
② By his boiling he undoubtedly destroyed the Pancreas Enzyme.
L. idea that the CB grow in ordinary cultiv. media with forming poison is a fallacy.
Certainly in Broth, Agar, Gel. they steadily lose virulence.
By growing on raw albumen, Chopped up meat, in the absence of air they reach in a short time the highest grade of virulence.
H. :. thinks that the exalt. of V. in L's exper: depended on the use of some albuminate favor-ble to the growth of the CB. & 2nd on the growth being to some extent Anaerobic - in depth.

X Lowenthal: 1888. As the result of many exper: L. thinks that the Pancreas juice, in the presence of albumen & Pepton: substances, occasions the generation of the poison by the Bacillus:
L is of opinion that the action of the Pancreat juice explains the clinical picture in human Cholera.
The CB. in the human intestine give rise to the same poison with the help of the pancreatic juice, as they do in the Pancreas Broth which is a rough imitation of the duodenal Contents.
The experiments are in harmony with the Anat: path: conditions, the CB are limited to the intestine, also explains sudden cases of Chol. also the exper. of Ricali & Riech & Koch on animals.

Hueppe: XI p583: 1889. p105 States that CB. grown on a suitable medium, as Anaerob. E.g. in hens eggs take on toxic qualities. He thinks the cause to lie in the fact that the Ptomaines and basic products form in Anaerobic culture, are not further decomposed - whereas in aerobic life they are oxydised, just as Fatty acids are in Fermentation of Carbohydrates.
Thinks Gamaleia & L: Exalt: of Virul: may depend on this Anaerobiosis, the one in the body of pigeons, the other in depth of broth.

Lowenthal. XII. 1889. Thinks His opinion that the toxicity of CB. rests on Anaerob: is unfounded - & Bouillon The cultures in a N. M. not containing pancreas show no toxic action, while in that with pancreas do possess it, although cultures in ordinary bouillon do not act poisonously.

XIX Hueppe. Pancreatin has no influence &c &c

XXV Basler Lowenthal's process of little use. B. succeeded by growing on alkal: nutr. Pancreas juice of cattle.

Exaltation of Virulence. By Anaerobiosis.

XV Hueppe. June 1888. Eggs. - CB. grow luxuriantly: in a few days form toxines which would take weeks to accum: in presence of air.

XII Low u. Ithab. 1889. Thinks Hueppe's opinion that the raised toxicity of CB. rest on anaerobiosis is unfounded.

Passing through G.P.

Exaltation of Virulence. Macleod.

		G.P.S.	Dose
XVI p470.	Broth culture taken from Gel: tubes 1 year from Cholera Patient killed	5 out of 7	5 ccs.
" " "	2nd ~~generation~~	0 - 4	1 "
		1 " 3	1 "
" " "	" Mice: contents of G.P.s — 3rd " generation, i.e. after passing th. 3 G.P.s.	4 " 4	3
/		1 " 2	2.5
		0 " 2	2
" " "	6th generation.	3 " 5	2 ccs

Theories to account for immunity.

XVII Woodhead Hº "Immunity is an acquired tolerance of the specific poison"

Saprophytes as vaccines.

XVII Woodhead. Putrefactive organisms produce minimal quantities of the same specific poison as the parasitic form.

Antidotal Action.

XVII p 394 Woodhead &c = Culture of B. pyocyan: sterilized: 1cc-safe =

Rabbit mixed - Anthrax - same time 2 & 4ccs Ster: Pyo: Cult. 2cc daily, 5 days.

- - - 3ccs - - - - 1cc - 7 -

These rabbits afterwards remained refractory to Anthrax (both remained alive).

Summation actiñ

+VII p395. Woodhead &c. M. Prodig. is able to convert Charbon Symptematique, which is innocuous for rabbits, into a fatal affection.

Tubercle Bacilli + S.p. aureus & albus — Disease more acute. 16th day Died
" + B. pyocyaneus. 12th day. "
" + S pyogenes. 52nd day "

Epidemics:

XVIII. Trieste 1886. 5½ months duration. 1st case 7 June 86

Injection Experiments: Into periton: cavity of G. Ps. Ruppe.

XVIII. G. Ps. with or without opium rec'd broth culture of CB. into periton: cavity: CBs f'd affer death in gall, blood &c. This is to show that whatever the place or manner of infection the small intestine is the Chief place of development—

XXV. Vaslein. 1½ – 2½ drops virulent culture + 1cc 3c opii, 15 min. later, into peritoneal cavity. Some CBs in duodenum & in blood.

Paths of infection.

+viii Lustig. Exp. on G.P.s prove that whatever the place or manner of infection the small intestine is the chief place of development. also that the changes in the gut do not prove that the infection is by way of the intestine, but that the Sm. intestine being the place of least resistance (L. minimae resistentiae) is often the victim of 2ary infection. (vide p 56)

+ix Hueppe. The naturbl ~~path~~ manner of infection in man ~~must~~ is probably in most cases, if not always, that the parasites pass the stomach and then ~~pass~~ immediately ~~into~~ the intestine.
It is an open question whether the Chol: bacilli can only reach the stomach by eating or drinking or whether they may be taken up in the breath from the atmosphere, be swallowed in the saliva into gan' stomach. Or may be inhaled into lungs.
Not much prob.y of man being infected by way of lungs and blood. —
When dried but still capable of growing, can pass into air, breath, into lungs or swallowed in saliva.

Experiments. Artefic Chol: Kochs Method. Control Exp. continued.

Date	No.	Animal	Time	Weight	Method	Result.	Result Dec.	
6 IV 91.		G.P.		500	Under Sus. form Cartilage 2·5 ccs	On back 25 mins.	R.	
6 IV 91.		G.P.		515.	-do- 2·0 ccs.	" 25 "	R.	
6 IV 91.		G.P.		510.	-do- 2·5 ccs.	Refused to lie on back	R.	
25 IV 91.	95.	G.P.		420	-do- 2·1 ccs.		R.	
25 IV 91	96.	G.P.		445.	-do- 2·2 ccs.	Died straightaway		D
25 IV 91	97	G.P.		525.	-do- 3·1 ccs.		R.	
25 IV 91	98	G.P.		490.	-do- 2·5 ccs.	Opium Passed into sub. cut. tissue	R.	

Disinfection.

×Tx Hueppe: The great sensitiveness of CB as present in fresh Chol. stools. very important
-3-90. & imposes doubly upon us the duty & destroy them at once when it is possible,
since the CB after this take on greater resistance to ext: agencies.

Growth in Gelatine.

XIX Hueppe. Wood was able at pleasure to influence the growth of Cols: as descr^d by Koch – so that they liq^d as quickly as Finklers, or to make them so that they no longer liq^f the gelatine. –
Anaerobic growth in cultures or in intestine tends to this so that the failure in finding Kochs "funnel" has lead to many CBs being overlooked.

XX Wood. Addition of ~~gelatin~~ glycerine retards liq. W. thinks by affording more easily got-at food to the M.O. — Carbolic Acid also retards – reason not given.

do. p262. W. observed complete loss of power in liq^g gelatine in an old gel: culture No sign of liq^n of gelatine – No separation of casein of milk – No growth or much impaired on potatoes – showing great sensitiveness to acids. This variety retained its char^s for long time when grown on Agar Agar. When freq^y inoc^d into fresh broth & grown at 25°C. it recovered its power of liq^g gelatine in about 3 weeks. Reason given is ① that if a culture is left for a long time undisturbed a membrane forms on the surface which effectually prevents the entrance of Oxygen to M.Os at bottom of tube, & the habit of taking its food otherwise than by enzymes may perhaps persist for some time after it is again grown under ordinary conditions ② Metabolic products formed which accumulate in old cultures – Must look upon it as the production of some special substance, which acts upon this function, as organisms which were unaffected by their own products suffered this loss when grown upon sterilised cholera cultures – Attention directed to Indol – This too expensive & difficult to prepare so recourse was had to phenol – Experiment Tubes cont^g 10ccs Ster: Broth received drops of 1 in 20 Carb: Sol. 1 to 2 drops was sufficient to cause CB to lose its enzyme function – By this means varieties of CB were got in 6 weeks which grew on gelatine without any signs of liq^n (p264). Precip of casein in milk disap^d sooner than liq^n of gelatine.

Groundwater.

Fick Hueppe. Pettenkoffer's epidem. C. observation – Lowering of G.W. increases the Cholera – Rising of G.W. decreases – Must find bacteriological proof for this. Cholera germs reach the soil in very sensitive condition – If there is in the soil much moisture and little air, then the CB simply perish. If air is present, i.e. if the soil is only moist, then the CB can multiply aerobically. This is the prelim: condition for a miasmatic diffusion of the C. Epidemic.

If the G.W. rises when there are germs in a resistant stat in the soil they cannot multiply on acc. of the excess of water + absence of air, so the epidemic ceases from want of suitable infection-material, or it can eventually later break out again.

Enzyme of Cholera Bac. vide also p 75.

XY. Wood: p 254. H. Bitter in 1887 first afforded rigorous proof that Bacteria can produce enzymes separable from the organisms which form them. At killed the organism at 60°C. Enzymes was able apart from the organism to liquify the gelatine. Chamberlands filter.
Wood investg^s CB. Denke. Junkles & Miller: Chol: Enzyme was found to be most sensitive to acid reaction. &c. &c.
CB. appears to digest its proteids by means of enzymes, but the faculty of digesting the Carbohydrates appears still to reside in the protoplasm.

p 261. Flügge has observed that organisms when grown without the presence of oxygen, under conditions of Anaerobiosis appear then to lose their enzyme function, inasmuch as they then cease to liquify the gelatine.

266. The devel^t of Enzymes, by which the complex indiffusible Compounds which supply it with nourishment are rendered diffus: (by enzymes) outside the organism, would allow of the protoplasm being invested by a firmer, resisting, bounding membrane.

Wood. All Cholera group liq.ᵈ serums with formation of H_2S. – This is retarded by adding glycerine.

Milk. Butter. Cheese. Whey.

xxii Heim. In non-ster. milk longest vitality 6 days, earliest non-vitality 24 hrs..

– In common butter, slightly acid, bacilli dead in 24 hrs.

" best " " lived 32 days.

The author ascribes difference to difference in reaction

Cheese 24 hrs — 2nd Exp. 24 less th 48 hrs:–

Whey: after 48 hrs: not more than 3 days still vital:

xxv. Kitasato. Milk. Non-steril: 36°C. died in 14 hrs –

" " 8–12°C. latest 3½ days:–

" Sterilised. 36°C. 2 weeks. –

" " 22° & 35°C. after 3 weeks still found –

xxxiii Cunningham. Exp. of non-ster- partially ster & ster: milk as mediums for growth of CB.

Klein – See p18 of these notes. Grow well but not so luxuriantly as in broth. Casein not ppt. appreciable unaltered. Mr Warrington has noticed Curding of milk to take place at 30°C.

" Koch. Do not cause the milk to curdle, nor do they separate the casein.

Growth in Gelatine coloured by lachmoid :—

13. 5. 90. CB. grows well in Gl. co. deep blue by lacmoid. No change in colour on 6.6.90 when the growth was advanced.

Experiments. Exaltation of Virulence. White rats:

Injection into Rg: pleural cavity of W. rats. By growth in raw Egg.

Date	No.	Time.	Amount injected	Virus used.	R.	D.	Remarks
6 VI 90	16	2 pm.	½ cc.	A. Egg. 3 days growth of CB 379. + ½ cc Stir. Salt. Sol.	R.		A. Egg. Pure culture. - By plate — Alive + well 16 VI 90
6 VI 90	17	2·30	½ cc	A. Egg. — do —	R.		do do.

Guinea pigs:—

Experiments. Exact: of Virulence.

Injection into ng. pleur: cavity.

By growth in raw egg.

Date	No	Time	amt. injected	Virus used.	R.	Died	Remarks.
7 VI 90.	19.	453 gm 12½ oz.	1 c.c.	Egg 4 days at 37°C:	16 VI 90		Control G.P. 1 cc Salt Sol: into ng. pl: cavity Plates made fr. Egg. Showed Pure Culture:
18 VI 90.	32.		2 c.c	No 15 " " " + 1 cc Salt Sol.		19 VI 90	No p.m: Egg slightly acid:

Experiments. Exact of Ver: Injection of C.B. grow^{g} in raw egg. into stomach of G.Ps. + Opium & Soda. (or without)

Date	No. of Exp	Egg	No. of days growing	Weight	Rec^{d}	Died	Remarks
18 vi 90.	33.	C. 5ccs Sal. Sol.	15 days.	744 grms. (5ccs Soda 5% Opium, 3 " Opium)	3ccs.	19 vi 90.	Periton: cavity contained much stinking fluid, evid^{t} perforation;
22 vi 90.	34.	F. 5ccs.	11. "	520 "	0 30 vi 90.		No opium No ill effects.
"	35	" 5ccs.	11 "	715 –	2ccs.	23 vi 90:	+ 2ccs Opium. No perforation found.

Plate Cultivation: No. I. From artificial Cultivations.

Date.	No.	Source.	After 3 days.	6 days	9 days	12 days.	18 days.	Thrown out.
17 VI/90	31.	Berlin. Agar.	Minute Non-liq.d	No blisters. 4 ..	Small blisters Non-liq. Col.s still minute	do – do – No good for demons.	Few gas blisters Mostly minute	5 VII/90
11 VI/90	25	Broth.	Minute cols. Non-liq.	Non-liq.d	—	—	—	21 VI/90.
23 VI/90	42	B. Agar. 31 V/90. Petri.	Numerous. minute. 2 & 3. No blisters.	Numerous. minute. a few dimples.				27? VI/90.
23 VI/90	41	" " " Koch's.	Num. minute Covd. with mould	—	—	—	—	27 VI/90
30 VI/90.	44	London Agar 20 times into broth daily.	No blistering Covered minute cols.	—	—	—	—	5 VII/90

Plate Cultivation. Nº II From Artific Cultivations

Date	Nº of Cult.	Source	After 3 days	6 days	9 days	12 days	18 days	Thrown out.
17 VI 90	31	Berlin Agar.		VII. No blisters.	25. Blisters. Non-liq. Shallow.	do — do — No good.	Cov.d mould	5 VII 90.
11 VI 90	25	Broth.	Gas blisters. Non-liq. many small cols	Non-liq.d many small cols 1 or 2 dimples.		Non-liq. X. Gas blisters	Dimpled. Non-liq. Minute. Partly good for Dem.	5 VII 90.
23 VI 90	42	B. Agar. 31 V 90. Petri	5 to 8. No blisters					
28 VI 90.	41	. _ . Kochs	5 — No blisters Cols. numerous blistering	Cov.d with Mould	—	—	—	30 VI 90.
30 VI 90	44	vide Nº I		—	—	—	—	5 VII 90.

Plate Cultivation. No. III From Artificial Cultivations.

Date.	Source	After 3 days	6 days	9 days	12 days	18 days.	Thrown out.
17 VI/90. No. 8 Exp. 31	Berlin. Agar.		XXII. Nobleister	35. Slight dimpl.	50. Dimple non-liq.	⊙ Single curl liq.	5 VII/90.
11 VI/90. 25	Broth.		Non-liq.	—	—	—	21 VI/90.
23 VI/90. 42	B agar. 31 VI/90 Petri.	very few. 5 & 8 Nobleisters	Covered with Moulds.	—	—	—	30 VI/90
23 VI/90. 41	B agar 31 VI/90 Koch.	very few 8 or 10 Nobleisters	Cov^d with moulds	—	—	—	30 VI/90.
30 VI/90 44.	vide No. I	Few colonies, Well marked bubbles. 4 VII/90. Good for dem.	20 & 30 interval	Placed in ice-chest.			5 VII/90

Plate Cultivation. No. I After growing C.B. in egg.

Date.		Source.			After 3 days	6 days.	9 days	12 days	18 days	Thrown out.
4 6/90.	15	A egg: C.B. growing 1 day in egg.			Plates all liq.d "					6 6/90.
7 6/90.	15.	B egg:	4 days.		"		Begun to liquefy the Col.s still small.			Thrown out.
9 VI/90.	15	C. "	6	"		.10 int. Non-liq.		Col.s minute; Gas blisters, Non-liq.	Cols: largest 4 1 5 mm. Liq.d very many minute still.	27 VI/90.
16 VI/90.	24A	E "	13	" 4 in B egg.	liq.d, evidently contam					21 VI/90.
15 VI/90	25	F "	4	" "		Minute cols. Gas blisters. Non-liq.d	Cols still minute. Gas blisters. Nonliq	Still minute Gas. blisters.	Still minute G. blis: Non liq	5 VII/90.
21 VI/90	25	F "	10	" "	Innum. very minute No blisters. Non liq 3 to 7 internals.	Innum. minute Tiny dimpling	—	Cols. minute. Sl. dimpling. Nonliq.		5 VII/90
14 VI/90	29.	C "	14	" "	Innumerable minute cols: Non-liq.	Minute gas blisters. 5 int Nonliq.	Gas blisters. 4 & 20 Non liq.	—	Gas blisters. Non liq. Pretty good for dem	5 VII/90
18 VI/90.	30	G "	1	" "	Innum. minute col. Non-liq.	Minute: N. liq. No G.B. 6 — a few 25.	Mostly minute biggest have G.B.		Many minute Many liq.d 5 liq. (·8·) Good for demonst.	5 VII/90.
18 VII/90	43	G "	13.		Nil					

Plate Cultivation. No. II After growing in Egg.

Date.		Source.	After 3 days	6 days	9 days.	12 days	18 days.	Thrown out.
4 VI/90	15	A. Egg: 1 days growth.	Liquefied	Gas Blister Liq^d.	—	—	—	6 VI/90.
7 VI/90	15	B. " 4 " "	—	—	Gas blisters.	—	—	Thrown out.
9 VI/90	15	C. 6 " "	—	Larger than No 1. Non-liq	—	—	Nothing noteworthy	27 VI/90
16 VI/90	24A	E. " 13 " 4th in Egg.	—	Liq^d. Evid: contamination.	—	—	—	21 VI/90.
15 VI/90	25	F. " 4 "	—	—	—	—	—	20 VI/90?
21 VI/90	25	" 10 "	Gas Blisters. 10 to 15.	Very characteristic bubbles 10.15	—	Placed in ice chest for demonstration Char. bubble. Nonliq	—	5 VII/90.
17 VI/90.	29	C. 14 "	Few minute cols Non-liq?	Slight blisters app. 25 pd Nonliq	Many minute & no blister 60 (one). Bubble..	No good.	—	5 VII/90
18 VI/90	30.	G. 1 "	Minute. Non-liq?	20×30. — No G.B. Nonliq.	Very sl. depression. No G.B. Nonliq.	—	Gas blisters. Non liq.	5 VII/90.
18 VII/90	43	G. 13 .	nil.					

Plate Cultivation. 17°. III. After growing in egg:—

Date.	Source	After 3 days.	6 days	9 days.	12 days.	18 days.	Thrown out.
4 VI 90. 15	A. egg. 1 day's growth in egg.	Liq.d	——	——	——	——	6 VI 90.
7 VI 90 16	B. " 4 " "	—	——	Gas blisters.	——	——	Thrown out.
9 VI 90 15	C " 6 " "	——	——	——	——	nil.	27 VI 90
16 VI 90 24	C " 13 " 4 days in egg.	——	14 colo: large liq.d Evid: contam:	Plate almost all liquified:			
15 VI 90 25	J. 4 "	——	——	——	——	——	20 VI 90 ?
21 VI 90 25	J. 10 "	Gas blisters. 25 & 30.	About 20 colo. Some liquifaction 35. Good for demonst.		Cov.d with Moulds		5 VII 90.
17 VI 90 29	C. 14 "	Nothing precipitable	nil.	nil.	Nil.		27 VI 90
18 VI 90 30	J. 1 "	Few mould. Non-liq.	20 × 35. No S.B. Non liq.	35. No S.B. Non liq.	——	Doubtful looking sl. hollow liq.	5 VII 90.
18 VII 90 43	J. 13.	nil.					

Species of Choleraic Comma Bacilli.

XXXIV Cunningham: Describes 10 different species - divided into 2 classes.

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1. Sherrington. On varieties of Leucocytes. Deuxieme Congrès Internat: de Physiol. Liège 1892.
2. Kanthack & Hardy. On the characters and behaviours of the wandering Cells of the Frog especially in relation to micro-organisms. Proceedings of Royal Society. Vol 52. N°317. Nov^r. 17th 1892.

Varieties of Leucocytes in dog.

1. Sherrington. I Finely Granular. II Coarsely Granular III Lymphocytes.

I Finely Granular. About 80% – Amoeboid & phagocytic. Nucleus invisible during life & when fixed during the activity of the cell and stained is found to consist of pieces always united into a chain by nuclear bonds (of chromatin). This irregular form of nucleus is not a sign of degeneration, nor of reproduction but of amoeboid activity, the nucleus is never multiple, it is single & by a method described by S it can be reconverted into a simple spherical nucleus, after slowly quieting the cell. Increased by feeding. Not so numerous in lymph as in blood. Almost absent from lymph glands. Forms 70% of pus cells & in pus is more granular & often truly multinuclear owing to breaking of the nuclear bonds (fragmentation). It is amoeboid between 17°C and 41° C – Can be kept alive 3 weeks in sterile tubes of 'oxalated blood'. Can be fed with carmine or bacteria in test tubes. Often contains vacuoles.

II Coarsely Granular. About 3% – Amoeboid, not phagocytic. Never contains vacuoles. Position of nucleus is seen during life by the absence of the granules there – The granules are round in the dog but ovoid in guinea-pig and cat. Every one of these cells is an eosinophile of Ehrlich and no other true eosinophile besides these are present in normal blood. Doubtful if Eosin: cell of bone marrow is the same as that of blood. Sparse in spleen and lymph glands from which the blood has been washed. It occurs in lymph but L contains few granules – In pus it occurs in small number, less than in blood – It is less actively amoeboid at low temps. than Cell I. but even more active at about 38° C. Can be kept alive three weeks outside body – When blood clots Cell II has nothing to do with the formation of fibrin filaments. Its granules give the Eosin reaction for weeks after the cell has been dead, after 5 weeks in stomach of leech.

III Lymphocytes. About 20% – Cell Substance clear, nucleus regular & visible in life, & stains at once in Meth: blue.

Two Kinds A large – Amoeboid or phagocytic never contains vacuoles,
B. Small – " " numerous in lymph glands – absent in pus.

Starvation reduces ratio of II to I – From 4% to 1% & later to .1%. On feeding after starvation proportion at first falls lower – indeed Cell II may be absent from the blood (thymph not from bone marrow) but in 24 hrs. rises – This increase in Cell II also occurs in the blood in vitro.

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2. Morphology. (a) Curved rods (b) Spirillar forms.
3. Spore-formation?
4. Conditions essential for life of Bacillus—
a Temperature. B. Oxygen.
16°-40°.
5. Effect of drying.
6. " " growth of other bacteria
7 " " acids.
8 Occurrence in cases of Cholera.
9. Presence in blood & organs.
10. " in Vomit.
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14. Cause of death in Cholera—

Laboratory Army Medical School,
Royal Victoria Hospital.
Netley.

21st June 1890.

Graxia. Cholera Bac: in the soil. Central p 269. VIII Bd.

Annales de micrographie: 1890.

This paper confirms the opinion that C: B: are able to exist only for a very short time in the soil: & that the ground-water theory of infection-diseases can not be applied to Cholera.

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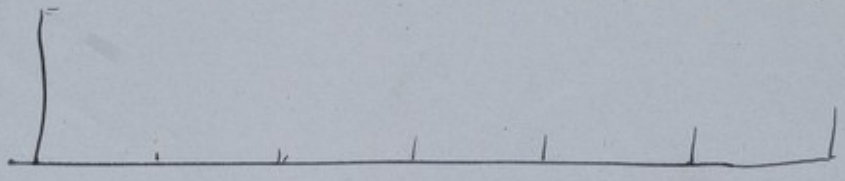

Bibliography. 2. WB.C.92.

Air. Carbonic Acid Gas. 4.

Acid. Colour test 20.

Arti Cause of death in Cholera. 48.

Atten

Actio

Arg.

Anti

Anti

Drying 7.

Diagnosis. As aids to .22.

Disinfection: 74.

Experiments Wanted. 36.

Exaltation of Virulence (Gam. Sam. Low. Anaerob. Medium) 37: 61; 62. 63. 64:

wing Epidemics. 69.

Atten Experiments: Artif: Chol: Koch's method. Control: 72.

Actio " Exalt: of Vir: Wht. Rats 73: Injecting cultures –

Arg Enzyme of CB 77.

Ante Experiments. Exalt: of Virulence – By injecting growth of Koch's CB. (after growth in egg.) into pleur: cav W. Rats 81

Ante do. do. Growth of CB. in raw egg: Into pleur: cav: G. Pigs 82

do do: Growth of CB. in raw eggs. 83.

do do. Injecting raw egg in wh. CB: into stom: of G. Ps + Soda + Opium 84

do Artific: Cholera. Koch's. 91.

Fudüng Experiments 25.

Growth among other Bacteria 5.

" in Broth 16. in egg 32. [illegible]. Water 51. Gelatine. 75. Bl. Serum 78. Lachmoid 80

Groundwater 76.

Arg

Aute

Aute

Heighton virulence 37.

Inoculation Experiment 19.

Infection Experiments. Blood stream of rabbits 26 - periton cav^{y} of mice 27 - of sterilized Chol. cultures 30.

do. Sub cutaneous 33. Pigeons 39. Periton. cav^{y} of G.P. 70.

Immunity 38.

Infection through Liver 45. Air 49. human intercourse 50 Water 57. Flies 49 Food 49.

Improved water Supply 54.

Lewis' CB. 21.
Liner 45.

Nerve-cutting. 58.

5

of Koch CB.
Occurrence, in Cholera 9.

" outbreaks of Choleraic diseasi 24.

" in other situations than intestine of Chol. cases 41.

" drinking waters 46.

Presence in Muc. Memb. of intestine 11.

" " blood & organs 12 28.

" " vomit. 13.

Ptomaine production 36.

Pathological appearances 42.

Paths of infection. 71.

Plate cultivations 85. 86. 87.

ds. After growing in Egg 88-9-90.

at which growth take place.

Temperature 1.

Theories to account for infection 56. immunity 65. Groundwater. 76.

Therapeutics. 60. 59.

Toxemia 31.

Temperature. 1.

Vitality in artific. Cultiv. media. 17. in stools 23. Water 51.

Virulence 57. 61. 62. 63

Vaccination 38. Saprophytes as Vaccines 66.

Water 46.