AN INVESTIGATION OF THE TOXIC PROPERTIES OF THE BACILLUS DYSENTERIAE SHIGA AND EXPERIMENTS WITH A VIEW TO THE PRODUCTION OF A NON-TOXIC DYSENTERY VACCINE.

BY

ROBERT CECIL ROBERTSON, M.C. late Capt. R.A.M.C.

M.B.,Ch.B. Glasgow.

M.R.C.P. Edinburgh.

F.R.F.P.& S.G.

D.P.H.

- - - - - o O o - - - - -

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

C O N T E N T S.

-----oOo-----

I N T R O D U C T I O N.

Dysentery is a very ancient disease, our knowledge of it dating back to the time of Hippocrates. It has played a great part in most of the campaigns of history, and in the recent Great War it was extremely rife in the near Eastern theatre, particularly in the Dardanelles.

The present thesis deals only with Bacillary Dysentery and gives an account of a series of experiments undertaken by the author with the object of investigating the toxic properties of the Bacillus of Shiga and producing a prophylactic vaccine against infection by this organism. These experiments were performed in the vaccine department of the Royal Army Medical College.

A vaccine of this nature would be of considerable value in countries where bacillary dysentery is endemic and if a state of active immunity against this disease can be produced, would provide a means of protecting European troops prior to their entering a zone where the condition is prevalent or the sanitary condition such that infection is probable.

HISTORICAL OUTLINE.

During the last few years many attempts have been made to produce a vaccine of this nature, the most successful being Gibson's sero-vaccine. This vaccine has not however come into extensive use owing to its undertain keeping qualities and the fact that the local and constitutional reaction following inoculation is frequently severe.

A review of the extensive literature dealing with the toxic action and other antigenic properties of the Bacillus dysenteriae Shiga reveals the fact, that Bacteriologists are not by any means in agreement as to the method in which the organism produces its toxins and its pathological effects

The earlier works including Todd, Doerr, Conradi, Vaillard, and Dopter seem to favour the view that the soluble toxin of the Shiga bacillus is of the nature of an exo-toxin.

The latest research on the subject which has been published in January 1920 by Olitsky and Kligler indicates that two distinct toxins are produced by the organism, an exo-toxin and an endo-toxin which differ in their pathological action.

In 1903 Todd proved conclusively the fact that the injection of the killed organism produced the same pathological effects as a living culture would do. He further isolated the toxins by filtration of alkaline peptone broth cultures

and found that this filtrate produced diarrhoea and paralysis in a similar manner to the living or killed bacillus. He considered the toxin to be a true extra bacillary poison excreted by the organism and not an intra-bacillary substance extracted from the bodies of the bacilli by the action of the alkaline broth. In confirmation of this theory he observed that the anti-toxin only protects against the toxin and not against the living or dead bacilli.

In 1907 Doerr (Das Dysenterie toxin, Jena 1907) observed that the toxins may be obtained in the dry state by precipitation with ammonium sulphate and resolution of the precipitate in water.

Olitsky and Kligler in their paper on the Toxins and anti-toxins of Bacillus dysenteriae Shiga, in the Journal of Experimental Medicine. Jan.1st.1920 repeat Todds work and confirm his observations to a certain extent but produce experimental evidence of the existence of an endotoxin depending on the principles of autolysis or dissolution of the bacterial cell with liberation of its intracellular components.

This endo-toxin they found, on injecting sub-lethal doses in the rabbit, to produce mucoid blood-tinged stools

but no disturbance of the sensory or motor function. It was found to be thermostable in comparison with the exo-toxin, requiring 85' to 90' C. exposure for one hour to destroy its properties. The method of production of the endo-toxin is to wash off a 24 hours' growth of Shiga bacilli on solid media with saline, then to incubate this suspension for two days at 37'C. A subsequent filtration is carried out through a Berkfield N.candle and in order to separate it from the exo-toxin the filtrate is heated to 80'c. for one hour. With reference to the exo-toxin the authorsfind that there is no toxic production in the acid phase in the broth medium but that it appears in the alkaline phase and increases thereafter. Aeration favours the yield, and the removal of muscle sugar does not influence the toxicity.

The intestinal symptoms produced by the exo-toxin are inconspicious. The central nervous system being chiefly affected and the postmortem findings are described as visible hemorrhages into the grey matter of the medulla and cervical cord. Microscopically the appearances are described as showing a noticeable infiltration of small round cells about the arterioles and capillaries. There being also granular degeneration or caryorrhexis of the cells of the gray matter.

In an article on the Pathogenesis of experimental colitis and the relation of colitis in animals and man, S.Flexner states that the Shiga bacilli readily produce a soluble toxin also that they readily undergo lysis and liberate a toxin. He does not further differentiate between these toxins but contrasts these properties with certain observations on the Flexner group of dysentery bacilli. The experimental work findings are interesting in the light of the theories of other observers.

Flexner states that in rabbits and probably in man the toxin is excreted chiefly by the large intestine which being injured by the act of elimination reacts by the development of inflammation. This characteristic action of the toxin depends on the integrity of the biliary secretion into the intestine because when bile is prevented from entering the bowel by artificial means such as the establishment of a biliary fistula, no lesions of the large intestine appear. This however, does not prevent lethal effects which are caused by the toxin as a nerve poison. Dysentery toxin is destroyed by peptic digestion and more slowly by tryptic digestion.

The above notes from recent literature on the subject summarises the main views that are held concerning the toxins of the Shiga bacillus.

- - - - - - - - - - - - - - - - - - - -

E X P E R I M E N T A L W O R K

P E R F O R M E D B Y A U T H O R.

EXPERIMENTAL WORK.

In order to find out if the observations of Olitsky and Kliger would be confirmed on using the available laboratory strains of B.dysenteriae Shiga, a well known toxic strain was selected for the following experiments. The strain chosen was B.Dysenteriae Shiga Dean I and for the sake of uniformity this strain was employed throughout the subsequent experimental work.

EXPERIMENTS.A. Filtrates from alkaline broth cultures were prepared in accordance with Olitsky and Kligler's methods containing the exo-toxins and 1cc and 2cc amounts were injected intravenously into Rabbits. (for details see Appendix)

EXPERIMENTS. B. Twenty four hours growth from Roux bottles were washed off with saline and the suspensions incubated for two days prior to filtration. The filtrate was heated to 80'C for one hour. According to Olitsky and Kligler this filtrate contains chiefly the endo-toxins. 1cc and 2cc amounts were inoculated in a similar manner to Experiment.A.

Observations;- The animals injected with exotoxins all died within four days and showed symptoms of paralysis but no diarrhoea. The post-mortem findings were those noted by Olitsky and Kligler but in one rabbit there was some haemorrhage into the mucosa of the caecum. Each animal had a very

much distended bladder. Apparently paralysis of the sphincters was a prominent symptom. The series inoculated with endo-toxin died with one exception within four days. No marked diarrhoea was noted but the post-mortem revealed extensive intestinal congestion affecting principally the caecum and ascending colon. The rabbit which survived was subsequently inoculated with filtrate which had only been subjected to 70'C. for IO minutes and it died within 24 hours of well marked diarrhoea and paralytic symptoms as well.

- - - - - - - - - - - -

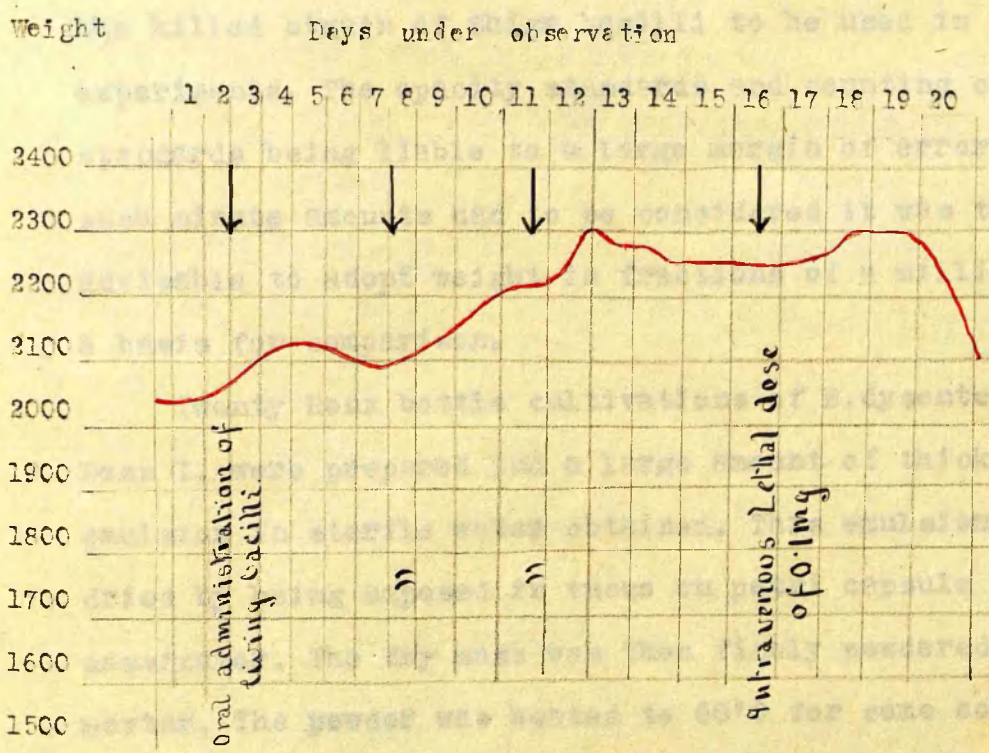
Full details are given
in Appendix pages. 27-32

EXPERIMENTS.C.

Three Rabbits of 1000 grammes, 1500 grammes and 2050 gramms were fed by the mouth with living cultures of B.dysenteriae Shiga. The bacilli were suspended in gelatine and eaten on cabbage leaves at least twice a week for over a month. No pathegenic effect was observed although the doses were estimated to be 20,000 millions. The animals were bled and their sera tested for agglutinins but the result was absolutely negative in each case. The rabbits after receiving this oral administration of the living bacilli were given 0.1 milligrammes of dried Shiga bacilli intravenously with lethal effect within 4 days. Table I illustrates the weight chart of an animal of this series.

- - - - - - - - - -

TABLE 1.


Weight
Days under observation
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
Oral administration of living bacilli
"
"
Intravenous Lethal dose of 0·1mg

EXPERIMENTS.D.

The object of this experiment was to arrive at an accurate means of estimating the Minimal Lethal Dose of the killed strain of Shiga bacilli to be used in subsequent experiments. The opacity standards and counting chamber standards being liable to a large margin of error when such minute amounts had to be considered it was thought advisable to adopt weight in fractions of a milligramme as a basis for comparison.

Twenty Roux bottle cultivations of B.dysenteriae Shiga Dean I. were prepared and a large amount of thick bacterial emulsion in sterile water obtained. This emulsion was then dried by being exposed in vacuo on petri capsule lids in a dessicator. The dry mass was then finely powdered in an agate mortar. The powder was heated to 60°C for some hours to ensure sterility and to drive off any remaining moisture. IO milligramme amounts were carefully weighed in the chemical balance and stored in sealed glass tubes for further use. When fractions of a milligramme were required for injections etc. well shaken suspensions of IO milligrammes of powder were made in IOO ccs of sterile normal saline and proportionate quantities used.

M.L.D. for a rabbit of approximately I500 grammes weight

was next determined. The inoculations were intravenous and the lethal effects determined within 5 days.

Results..... .5,. .25, .I,.05 milligramme and .OImg.doses were lethal after a dose of .005 milligrammes the animal survived.

The M.L.D. was taken to be .OI milligramme.

- - - - - - - - - - - - - - - - - -

EXPERIMENT.E.

The dried powder obtained in the manner described in the preceeding experiment was utilised in this case, IO milligrammes were suspended in sterile water and the suspension boiled at IOO°C for 20 minutes. A quantity representing .I mg. was then injected intravenously in a rabbit of I650 grammes. This animal died within three days. The neurotoxin had apparently not been destroyed at this temperature as the symptoms were similar to those observed on utilising the Shiga exo-toxin.

- - - - - - - - - - - - - - - -

EXPERIMENT F.

The object of this experiment was to investigate the action of acetone on Shiga bacilli.

A thick emulsion of the organism was prepared from the product of ten Roux bottle cultivations on agar, the growth being washed off in acetone. The emulsion was then transferred to a fat free extraction thimble and this was placed in a Soxhlet apparatus and extracted with acetone for 30 hours. This experiment had to be repeated several times as the acetone used at first was found to be impure. A chemically pure acetone was finally obtained and distilled prior to use. The extracted bacilli were examined as follows

I. Microscopically. They were found to have retained their morphological appearance and characteristics. They lost none of the staining reactions of untreated Shiga bacilli.

2. Serologically. They were agglutinated to a similar controlled titre by specific anti-sera.

3. Toxicity. The extracted bacilli were powdered and weighed amounts inoculated into rabbits intravenously in exactly the same way as was carried out in estimating the M.L.D. of dried bacilli.

Result .OI and .I milligrammes were lethal.

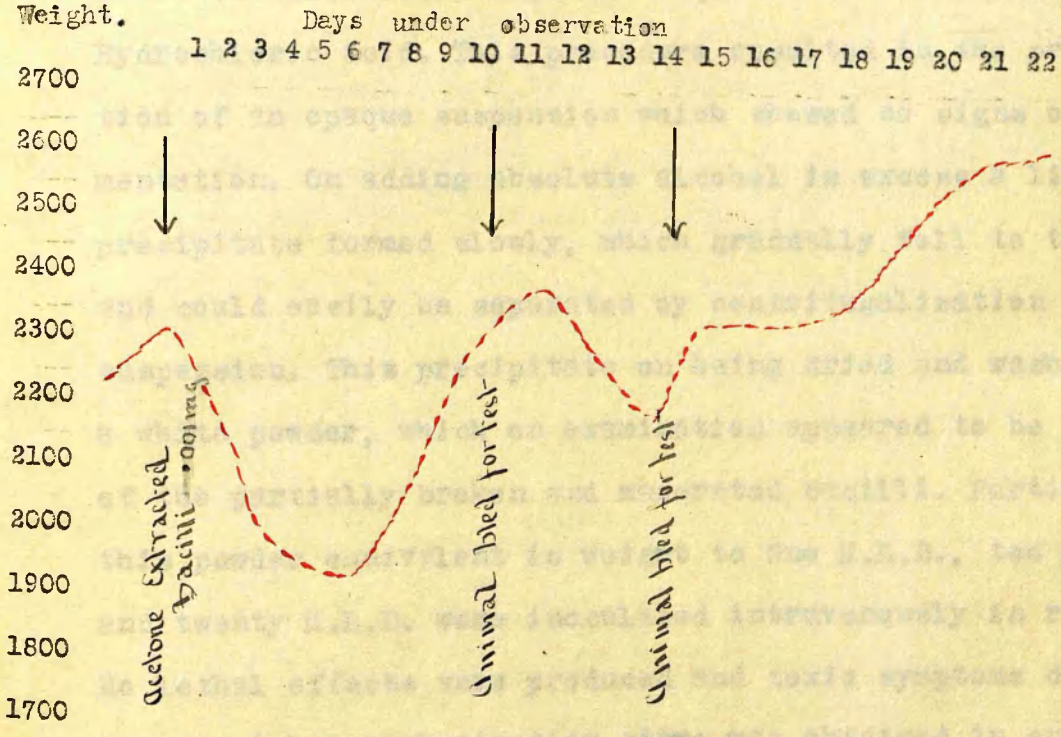
Conclusion. No detoxication had occurred.

A sub-lethal dose of .001 milligramme was then administered to a rabbit and the rabbit's serum tested periodically for the presence of agglutinins. A titre of I/256 was obtained by testing the serum eight days after inoculation. Table 2 illustrates the weight chart of this experiment.

In order to find out if the acetone had dissolved out any toxic fraction of the bacterial content, the acetone used in the Soxhlet apparatus was poured off and exaporated in a clean sterile porcelain basin. No visible residue was observed. However, the basin was rinsed out with sterile water and the fluid injected into a rabbit. No symptoms were observed and the animal was not protected from one M.L.D. subsequently injected.

- - - - - - - - - - - - - - - - - - -

TABLE 2.



One of the Acetone extracted bacilli animal inoculation experiments.

EXPERIMENTS.G. The action of alkali and acid.

In the first place an emulsion of Shiga bacilli was treated with Normal Caustic soda solution and allowed to remain in contact with the alkali for an hour. The emulsion was then neutralised with the requisite amount of Normal Hydrochloric acid. This procedure resulted in the production of an opaque suspension which showed no signs of sedimentation. On adding absolute alcohol in excess a light flaky precipitate formed slowly, which gradually fell to the bottom and could easily be separated by centrifugalisation of the suspension. This precipitate on being dried and washed formed a white powder, which on examination appeared to be formed of the partially broken and macerated bacilli. Portions of this powder equivalent in weight to One M.L.D., ten M.L.D. and twenty M.L.D. were inoculated intravenously in rabbits. No lethal effects were produced and toxic symptoms did not appear. A low agglutination titre was obtained in one animal representing I/I00 after a course of three inoculations of .I mg at weekly intervals. This animal was protected against a dose of ten times the M.L.D. of dried Shiga, (that is .I.Mg)

This alkali acid and alcohol method of producing a detoxicated antigen did not appear to be sufficiently satisfactory for several reasons. The immunity produced was not very

great and as at this stage of the investigation the author wished to produce a serum from the rabbits which would act as a high titre specific agglutinating serum the method was only useful as a preliminary means of protecting rabbits against large subsequent inoculations of killed B.dysenteria Shiga cultures. In addition the amount of absolute alcohol employed was found to be excessive and it was troublesome to recover this by distillation.

- -

EXPERIMENTS.H.

Emulsions of B.dysenteriae Shiga were poured into excess quantities of strong Ammonium hydrate (The Liq.Ammon. fortis.B.P.) and allowed to remain in contact for periods up to two hours. The solution was then transferred to a tall glass cylinder and bubbles of air passedthrough for some hours with the object of driving off a considerable proportion of the ammonia gas.

The solution was then neutralised with 20 0/0 Sulphuric acid. A precipitate was immediately produced. The concentration of Ammonium sulphate in the supernatant fluid was estimated to be approximately half saturation.

- - - - - - - - - - - - - - - - -

I. EXPERIMENTS WITH THE SUPERNATANT FLUID.

The precipitate was thrown down by centrifugalisation and the Ammonium Sulphate solution poured off. Icc was inoculated intravenously in a rabbit of I400 grammes. No toxic effects were observed although the animal appeared to lose weight for three days after the injection, then steadily put on weight.

A control rabbit inoculated with I cc of a similar strength of Ammonium Sulphate solution behaved in similar manner.

It may therefore be concluded that the supernatant fluid is not toxic to rabbits.

- - - - - - - - - - - - - - - - - -

2. EXPERIMENTS WITH THE PRECIPITATE.

The Ammonium Sulphate was got rid of by washing and the precipitate dried in the dessicator. The method employed in washing was to thoroughly centrifugalise the deposit and pour off the supernatant fluid, then to replace this with sterile normal saline. For the purpose of animal experimentation known weight suspensions were prepared as was done in the method described for the dried Shiga bacilli. The morphological characteristics of the precipitate were studied microscopically and the time of exposure to the Ammonium hydrate noted in each case. It was found that the longer the bacilli were treated the more macerated they became and the less toxic their effects on rabbits.

- - - - - - - - - - - - - - - - - - - -

Rabbits inoculated. intravenously.	Milligrammes. Doses at 7 day intervals			Titre of Sera on 24th day.	Lethal. doses of Shiga bac.28th day.	Result
Rabbit 351.	0.1.	0.2	0.5.	1 in 500.	10.M.L.D.	Survived.
" 352.	0.1.	0.2	1.0	1-1000	20 M.L.D.	Survived.
" 353.	0.25	----	----	----	----	Died.
" 354.	0.5	----	----	----	----	Died.
" 355.	1.0.	----	----	----	----	Died.
Subcutaneously.						
" 346.	0.5	1.0	5.0	1-150	10.M.L.D.	Survived.
" 347.	0.5	1.0.	5.0	1-50	20.M.L.D.	Survived.
" 348.	1.0.	2.0	7.5	1-150	50.M.L.D.	Survived
" 349.	1.0.	2.5.	10.0.	1- 60	50 M.L.D.	Survived
" 350	2.0	5.0	10.0	1-150.	100.M.L.D.	Survived
Subcutaneous Control.						
367	----	----	----	----	20.M.L.D.	Died

The detoxicated precipitate which was employed in the experiments noted in this table had been produced from bacilli treated with Ammonium hydrate for two hours.

- -

On reviewing the observations made on this series of animal experiments the following conclusions can be made.

The Ammonia method of treating B.dysenteriae Shiga has considerably lessened the toxicity of the bacterial substance towards rabbits. Animals may be immunised by utilising these treated bacilli as a vaccine.

Agglutinins appear in the serum of these immunised animals which are still specific for the unaltered B.dysenteriae Shiga but the titres obtained are comparatively low.

There is no local or general reaction of a severe nature on inoculating subcutaneously, quantities which if they consisted of the ordinary dried killed bacilli would represent 200 times the lethal dose for subcutaneous injection.

After a course of three doses of the vaccine rabbits are found to be protected against many times the lethal dose of B.dysenteriae Shiga.

- - - - - - - - - - - - - - - - -

EXPERIMENTS.II.

In order to estimate the degree of immunity conferred on the animals utilised in the last experiment the series of rabbits were bled prior to their receiving the Lethal doses of ordinary dried bacilli.

The serum thus obtained was pooled and preserved in 0.5 %.Phenol saline the dilution being I in 2.

To this pooled serum was added varying amounts of ordinary untreated dried Shiga bacilli in saline suspension The mixture was then incubated at 37'C. and intravenous inoculations carried out in rabbits as undernoted.

Rabbit.	Inoculum & Dosage.	Observations.
364.	Icc pooled serum and .4mg. of Shiga bacilli = 200 M.L.D.	Survived but was slightly paralysed on 4th day.
365.	Icc pooled serum and .4mg of Shiga bacilli - 400 M.L.D.	Died
366.	Icc pooled serum and I.8 mg of Shiga bacilli-800 M.L.D.	Died.

Remarks:- The above test was probably too severe and the anti-toxic value of the serum although high was about sufficient to neutralise 100 M.L.D.

- - - - - - - - - - - - - - - - - -

Experiments J.

The Antiformin method of treating B.dysenteriae Shiga

Since performing the experiments already described a reference to a non-toxic dysentery antigen was noted in a paper by Jotten, Zeitschr. fur Immunitatsforsch,.1920, 29,

This worker detoxicates Shiga bacilli by treating them with antiformin solution in dilute concentrations, He states that if a 24 hrs. growth of B,dys Shiga on an agar plate be treated with 2ccs of a 4./. antiformin solution the bacilli are killed and detoxicated in about sixty seconds. The procedure subsequent to the antiformin treatment is to neutralise by the addition of I0 per cent Sulphuric acid and then decholorinate the resultant solution with ten per cent Sodium sulphite.

The bacilli treated in this manner produce a solution which may be injected in I cc. doses with out producing any toxic effect in rabbits.?

In order to compare the properties of the antigen produced in this manner Jottens methods were followed as described above.

The antiformin solution used was freshly prepared according to Rosenau as equal parts of Liq. Sod. chlorinatae and a I5 ./. solution of Caustic soda (vide. Hise and Zinsser . Text book of Bacteriology) . The bacilli were allowed to

remain in contact with a 4'/. solution for five minutes. The solution was then neutralised with the IO'/. Sulphuric acid to neutral point for litmus. Dechlorination was effected with IO'/.Sodium sulphite until no free Chlorine was demonstrable by the starch-iodine test. Icc of this solution was given to a rabbit intravenously with a fatal result in three days.

For the purpose of comparison with the detoxicated Shiga antigen produced by the methods given in experiments G and H. The antiformin bacilli were made up in opacity suspensions corresponding to O.I milligramme per cc and O.2 milligramme per cc.

Rabbit 359 received .Img intravenously.

Rabbit 360 received .2mg.intravenously.

both rabbits died within four days after rapid loss of weight. The symptoms were similar to those produced by the ordinary untreated bacilli. The post-mortem findings were, considerable congestion of the gut around the ileo-caecal valve and the presence of minute haemorrages. The contents of the bowel were liquid. The liver was congested. The bladder was not distended. The general appearances were those noticed in the animals killed by the so called endo-toxin.

The conclusion that may be made from this experiment seems to be that the antiformin is not such a powerful detoxicating agent as either normal Caustic soda or Ammonia.

Its action as used by Jotten apparently depends simply on the strength of alkali present and this is not strong enough to act in the short time he describes using a 4'/. solution. The antiformin method does not offer any advantage over the simple alkali acid procedure

- - - - - - - - - - - - - - - -

SUMMARY

AND

GENERAL CONCLUSIONS.

SUMMARY & GENERAL CONCLUSIONS.

I. The observations of Olitsky and Kligler have been in the main confirmed, although it has been found almost impossible to produce "endo-toxin" without at same time having present a considerable quantity of the neuro-toxin or "exo-toxin".

2. Although two definite toxins for animals may be produced by Shiga bacilli. There is considerable evidence that the neuro-toxin or "exo-toxin" has a secondary action on the intestine through the initial inhibition of peristalsis prior to the passing of mucus or desquamated epithelial debris. The animal usually dies before the effect on the intestine becomes characteristic.

3. The oral administration of living Shiga bacilli to rabbits does not produce dysentery or any demonstrable serological reaction.

4. The toxicity of the bacterial substance of dried Shiga bacilli is not lessened by the treatment with Acetone or the subjection to 100°C. for 20 minutes.

5. The various means of producing a detoxicated antigen from the bacterial substance appear to depend on the principle of macerating the bacillus with a strong alkali then to neutralise the solution and to utilise the resultant

precipitate of less toxic bacterial substance.

6. The detoxication is only relative but it affords a means of producing a vaccine which is useful in commencing the active immunisation of animals. The intradermal reaction with this vaccine is not severe and large subcutaneous doses can be given without local reaction.

- - - - - - - - - - - - - - - - - -

BIBLIOGRAPHY.

Doerr - Das Dysenterie Toxin. Jena.1907.

Boenche and Elkeles- Zeits.f. Med.Beamte.Berlin 1918.xxxi.20

Hoffmann- Deutsche Mil.Artzl.Berlin 1918.

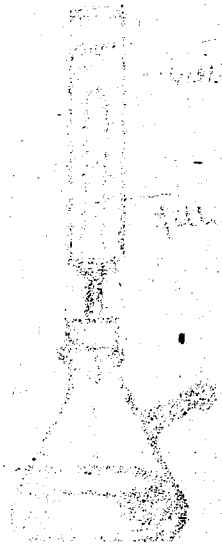
Olitsky and Kligler.) Journal.A.M.A.1918 Dec 28.p.2126.
) Journal of Experimental Med.Jan.I.1920.

Zotten- Zeitschrift fur Immunitätsforschung - 1920.29.

Hiss and Zinsser.- Text Book of Bacteriology.

- - - - - - - - - - - - - - - -

A P P E N D I X.

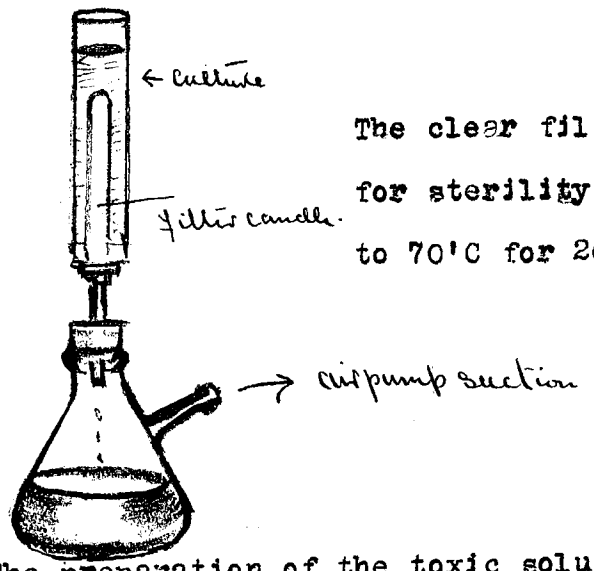


The clear filtrate was then tested for sterility and successfully heated to 70° for 20 minutes.

APPENDIX.

A more detailed account of the preparation of the toxic solution derived from B.dysenteriae Shiga which is regarded as an exo-toxin by Olitsky and Kligler.

A nutrient peptone broth was prepared and the reaction adjusted to P.h.7.8 on the Hydrogenion scale by the colorimetric method using Sorensens tubes. A 500 cc amount of this broth was sterilised and inoculated with B.dysenteriae Shiga Dean.I.. The cultivation was carried out for ten days at 37'C The flask being well shaken from time to time. At the end of the incubation period the culture was filtered through a Berkfeld candle by means of the apparatus illustrated in Fig.



The clear filtrate was then tested for sterility and subsequently heated to 70'C for 20 minutes.

The preparation of the toxic solution which is regarded by Olitsky and Kligler as consisting chiefly of endo-toxin.

Four Roux bottles containing sterilised nutrient agar agar were inoculated with minimal quantities of a broth culture of B. dysenteriæ Shiga Deam I and incubated in a flask at 37'C. for 2 days. The resultant "emulsion" the bacilli presumably having undergone some degree of autolysis, was then filtered through a Berkfield N.candle in a similar manner to the procedure for the broth filtrate.

The filtrate from this experiment was heated to 80'C. for one hour as "endo-toxin" is supposed to be more thermo stable than "exo-toxin" and it is quite possible that there is a large fraction of "exo-toxin" present in this latter filtrate as well as "endo-toxin" (always allowing that it be presumed that two separable toxins are elaborated by Shiga bacilli)

- - - - - - - - - - - - - - -

Animal Experiments illustrating the action of the two Filtrates the preparation of which has been described.

I. The Broth culture filtrate."exo-toxin"

Rabbit 326. Weight 2000 grammes.

Method. Intravenous.

Amount. 2ccs of filtrate (heated 70°C. for 20 min.)

Observations. Animal rapidly lost weight 200 grammes in two days. Paralysis was of rapid onset on the third day after inoculation and the animal died suddenly. There were no symptoms of diarrhoea.

Autopsy. No distension of abdomen, the intestines appeared unaffected except for a very few patches of slight haemorrhage into the mucosa of the large bowel. The intestinal contents were well formed The bladder was much distended with urine.The liver and other abdominal organs appeared healthy. The suprarenals were examined histologically but no pathological change was noted. The spinal cord was exposed and found to be hyperaemic and somewhat oedematous particularly in the lumbar region.

Rabbit.328. Weight 2450 grammes.

Method. Intravenous.

Amount. Icc of filtrate as above.

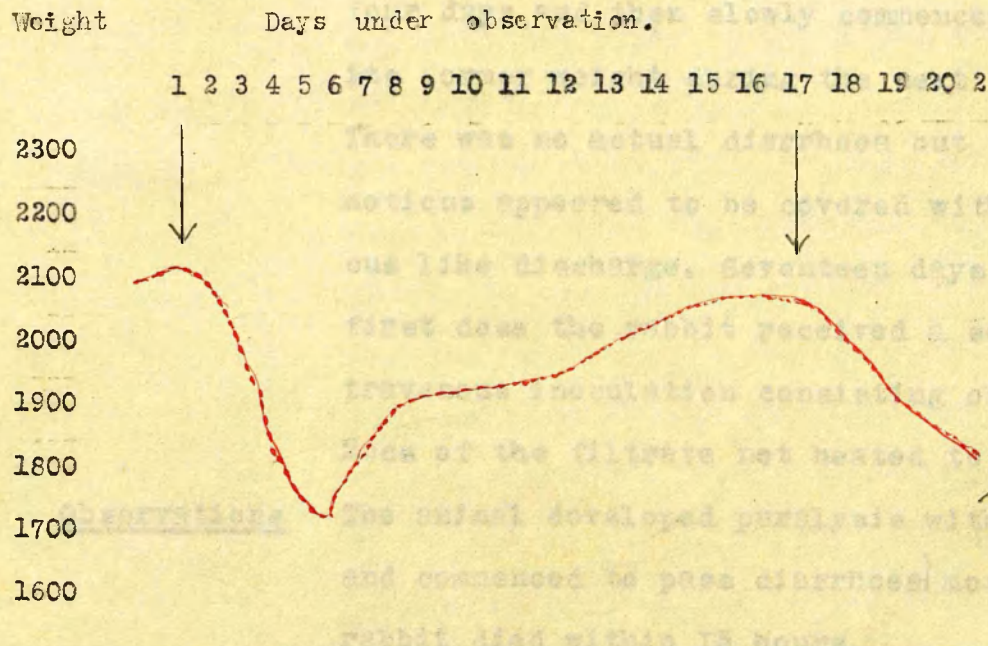
Observations. The weight was not affected till two days after inoculation when there was a drop of 50 grammes and the onset of paralysis. Death took place three days after the inoculation No diarrhoea or signs of loose motions were noted.

Autopsy. The appearances are those noted for Rabbit 326 But the intestines in the neighbourhood of the caecum are more injected in appearance. There is no definite lesion of the mucosa or marked haemorrhage. The suprarenal glands appear to be slightly enlarged. The urinary bladder is distended with urine. The brain is softened and friable. There are also visible haemorrhages into the gray matter of the spinal cord.

- - - - - - - - - - - - - - - - -

TABLE 3.

Weight Chart of Rabbit No. 327.



Description on following page.

2. The filtered incubated saline suspension "endo-toxin"

Rabbit 327. Weight 2I00 grammes.

Method. Intravenously.

Amount 2ccs of this filtrate heated to 80'C for I.hr.

Observations The animal rapidly lost weight for the first four days and then slowly commenced to regain its former weight during the next ten days. There was no actual diarrhoea but the rabbit's motions appeared to be covered with a thin mucus like discharge. Seventeen days after the first dose the rabbit received a second intravenous inoculation consisting of --------
2ccs of the filtrate not heated to 80'C.

Observations The animal developed paralysis within I2 hours and commenced to pass diarrhoeal motions. The rabbit died within I5 hours.

Autopsy. The large intestine and caecum exhibited extensive lesions of mucosa. The congestion and haemorrhage were very marked all over the caecum and for a distance of six inches above the ileocaecal valve. The urinary bladder was not distended. The small bowel appeared distended with gas. The spinal cord and brain did not appear to be extensively affected.

2. The "endo-toxin" experiments continued.

Rabbit. 329. Weight 2000 grammes.

Method Intravenously.

Amount. Icc of the filtrate heated to 80'C. for 20 min.

Observations. Rapid loss of weight. Animal died within twoddys. No diarrhoea observed. Prior to death the animal lost the power of its limbs and simply collapsed.

Autopsy. The caecum shows signs of desquamation of the mucosa and slight haemorrhage. On washing the bowel the desquamated portion is washed off and the wall appears thinned. The urinary bladder is distended and injected with distended veins. The walls appear inflamed. There is no pus in the urine. The central nervous system is affected as in the rabbits inoculated with the broth filtrate.

- - - - - - - - - - - -oOo- - - - - - - - - - - - - - -