

A CONSIDERATION OF THE
EFFECTS OF VACCINE THERAPY
IN CASES OF CHRONIC INFECTION
WITH ORGANISMS OF THE
COLON-TYPHOID GROUP

BY

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T H E S I S

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I N D E X.

1. Introduction.
2. Methods. (a) Preparation of Media
 (b) Bacteriolytic and opsonic methods, etc.
 (c) Preparation of vaccines.
3. Description of Cases. - I to XIX.
4. Review of Colon cases.
5. Summary (a) Typhoid results
 (b) Colon results
6. Conclusions.

INTRODUCTION.

Introduction.

Owing to the contradictory opinions appearing in various books on the value of inoculation with vaccines ^{in infections} of the colon typhoid group, ~~infections~~, especially those of a chronic nature resulting in bacilluria, an attempt has been made to select a series of cases and to treat them considering at the same time their bacteriolytic, opsonic and agglutinating reactions before and after inoculation.

A number of cases have been included in which the post mortem changes throw light on the actual lesions present in kindred cases to those which have been treated. All the cases considered are those of certified lunatics at the London County Asylum, Bexley, Kent.

Originally a case, Augustus G. was investigated a considerable time before the other cases were taken up, and the favourable results obtained in this case induced the writer to follow out the investigation of this subject. About the time when the biological characters of the organisms causing the colon bacilluria in these cases were being investigated one of the cases of ^a the typhoid epidemic showed signs of chronic infection with the B. Typhosus, and this case was included in the series.

Selection of Cases, and general methods employed.

Points considered. I. An acid bacilluria of at least 1 million per c.c., of urine removed by catheter into a sterile plugged tube. In most cases the bacilluria reached fifty to a hundred million.

II. The isolation of a motile acid and gas forming bacillus which was either the sole causal organism or greatly

predominated in the plated out specimens.

The routine of isolation consisted in taking ¹100 cc of urine ^{100cc} of urine diluted 1 in 10,000 with broth, spreading each on malachite green agar plates.

In 24 to thirty-six hours a discrete separate colony which had a characteristic blue-black appearance was transferred to an agar slope and grown for 24 hours. From the agar slope all the media, liquid and solid, were, including malachite green agar, inoculated and observed. If all were satisfactory agar slopes or plates were inoculated for the preparation of a vaccine. As far as possible a complete table of the various organisms has been prepared and this is sent with the thesis with as many preparations of the actual specimens as possible.

Attention was drawn to the various cases owing to the patients complaining of one or more of the various symptoms characteristic of the condition. The cases naturally fall into groups

- (a) the two typhoid cases I and II
- (b) a case of acute colon infection III
- (c) two cases associated with symptoms of cholecystitis IV & V
- (d) two cases of chronic cystitis, pyelonephritis becoming acute VI and VII
- (e) a case of colon infection associated with tubercular infection of the kidney and bladder. VIII.
- (f) six fairly typical cases of chronic colon bacilluria which showed occasional exacerbations. IX, X, XI & XII
- (g) a case of chronic colon bacilluria with an atypical organism associated with chronic colitis XVI
- (h) three cases of chronic bacilluria associated with masturbation (female cases). XVII, XVIII and XIX
- (i) Two cases of infection with a variety of ? B. Proteus
XIV & XV
- (j) A case of colon bacilluria in a terminal general paralysis of the insane - Case XIII.

Case I. Patient Elizabeth D.

A case of chronic (?) typhoid bacilluria with a number of chronic periosteal abscesses which showed a pure culture of the same organism; these manifestations continuing to appear nearly a year after the original disease. This case was treated by inoculation.

Case II. Fanny K.

A case of typhoid bacilluria associated with the typhoid abscess affecting the dura mater, Death followed status epilepticus which occurred after the close of the ordinary course of the disease.

III. Patient Lily B.

A case of renal infection following a retroperitoneal abscess. Treated with vaccine with some relief. Later, however, the patient died.

IV. Patient Emma S.

A case of cholecystitis without definite evidence of the existence of gall stones, which was associated with renal pain and profuse colon bacilluria. Treated with vaccines with some improvement.

Case V. Susan G.

A similar case to No. IV with slight transient bacilluria. Case not treated.

Case VI. Joseph W.

A case of pyelohephritis and some cystitis with considerable irregular pyrexia. Treated with a vaccine, and cured.

Case VII. Patient Thomas C.

This was treated with urinary antiseptics, bladder washing, etc. The patient became progressively worse and died.

Case VIII. Stella D.

A case in which colon infection was superadded to tuberculosis of the kidney and bladder. Was treated for a colon infection.

Case IX. August G.

A case of chronic colon bacilluria, with some pus. Treated with vaccine, and improved.

Case X. Ann W -d.

A case of chronic colon bacilluria. Treated with a vaccine.

Case XI. Ann W - h - d.

A case of chronic colon bacilluria, treated with a vaccine.

Case XII. Margaret W.

A case of Chronic colon bacilluria. Treated with a vaccine

Case XIII. Annie Di.

A case of colon bacilluria in general paralysis of the insane with post mortem notes showing early pyelonephritis. The case was not treated.

Case XIV. Alice N.

A case of chronic ~~colon~~^{proteus} bacilluria showing pyelonephritis at the post mortem examination, and evidence of chronic colitis. This case shewed extreme emaciation.

Case XV. Anna Maria W.

A case of chronic ~~colon~~^{proteus} bacilluria with marked emaciation. Treated with vaccine.

XVI. Clara J.

A case of chronic colon bacilluria associated in a marked degree with chronic diarrhoea, blood and mucus frequently being present in the stools. Treated with vaccine.

XVII. Elizabeth L.

A case of chronic colon bacilluria associated with masturbation. Treated with vaccine.

XVIII. Emily A. Ch.

A case of chronic colon bacilluria associated with marked emaciation and masturbation. Case not treated.

XIX. Elizabeth J.

A case of chronic colon bacilluria associated with masturbation. Treated with vaccine.

The results of treatment have not ^{been} ~~be~~ quoted in a number of cases here as it is difficult to express a concise opinion on the results obtained. These cases are cases that have previously proved themselves unamenable to urinary antiseptics, alkalis, etc.

In considering the causal organism, it may be stated that coliform organisms were present in all cases. By this is meant organisms which ferment glucose and lactose form acid and clot in milk, and form indol and do not liquify gelatine. The variable results obtained with dulcitate hardly justify my placing great reliance on this sugar in my series. All the cases gave evidence of coliform bacilli, except one case. In this case it is most probable the real infection was with a variety of *B. proteus*. The results of inoculation generally suggested that the true causal organism had been injected

In case XIV a similar protein form of organism^{to XIV} was isolated. In case XVII a fluorescent type of organism was isolated, but this was inconstant and did not appear to be the causal organism.

On glucose free agar reddish or bluish spreading growths were obtained with the protein organisms. This blue colouration was also formed in peptone water, and when the pigment was separated by means of chloroform it was found that in alkaline solution the blue colouring was maintained, the addition of acid caused a pink colour to appear.

The general figures quoted by Brown (1) are for bacteriauria and a number of the organisms quoted do not appear in acid urine. Quotations from Albarran, Halle and Légrain and also from Melchier show the colon bacillus appears in about 50 per cent. of all bacteriauriae, but the figures when purely renal conditions are considered give the colon figures much higher, namely, about 80 per cent.

The following figures are quoted -

Lenhartz - 66 colon cases out of 80 cases of infection.

Von Albeck - 76 cases out of 92

Rovsing - 16 out of 18 in women and 5 out of 14 in men

Other organisms found include Staphylococci, streptococci, *B. paratyphosus*, *B. proteus vulgaris*, and the uro-bacillus liquificans. This last organism is described by Hiss and Zinsser (2) as a variety of *B. proteus*. These figures give a colon percentage of about 80. Four of the organisms out of 20

(1) Osler and Macrae, *System of Medicine*, vol. 6, p. 230 and 238.

(2) *Text Book of Bacteriology*, p. 452.

in my series are not coliform, two being non-lactose fermenters (cases VIII and XVI) and the third case (XV) being that of the proteus infection referred to above. It will therefore be seen that the proportion of coliform organisms in my series is fairly typical.

Estimation of the number of organisms present per c.c. in these cases.

Variation occurred in this as a result of treatment, but other factors certainly entered into this.

1. Natural variation in the patient's condition.
2. Variation on different media, non-lactose fermenters growing very sparsely on Conrad-Drigalski medium, while malachite green agar inhibited ordinary colon organisms more than the Conradi medium. Glucose agar grew very prolifically with certain organisms and proved favourable with types which did not ferment lactose.
3. Anomalies from imperfect mixing in dilution.

It was found that less uncertain results were obtained when the bacilluria was profuse by counting the organisms against blood corpuscles as in Wright's method for standardising a vaccine.

METHODS.

- (a) Preparation of Media.
- (b) Bacteriolytic and Opsonic Methods, etc.
- (c) Preparation of Vaccines.

MEDIA USED.

- I. Conradi-Drigalski Plates.
- II. Malachite Green Agar plates. (Dr.Houston)
- III. Agar Slopes.
- IV. Glucose Gelatine Slopes and Stabs.
- V. Sorbite Gelatine Slopes.
- VI. Glucose Broth.
- VII. Litmus Milk.
- VIII. Potato Slopes.
- IX. Peptone Water for Rosindol reactions.
- X. Glucose Neutral Red.Broth.
- XI. Proskauer and Capaldi No. 1, (modified by Houston)
- XII. Proskauer and Capaldi No 2, (~~modified by Houston~~)
- XIII. L.S.D.S. media (Houston)
- XIV and the following series of sugars

- | | |
|---------------|--------------|
| 1. Glucose | 7. Dulcitol |
| 2. Mannitol | 8. Salicin |
| 3. Galactose | 9. Raffinose |
| 4. Levulose | 10. Adonite |
| 5. Lactose | 11. Inositol |
| 6. Saccharose | 12. Maltose |

Durham's tubes were used with all these sugars.

SOURCE OF INFORMATION.

A personal visit to Dr.A.C.Houston supplied me with much valuable information as to his methods. A summary of these methods is contained in his sixth research report to the Metropolitan Water Board. All the media were prepared in accordance with his directions except the Conradi-Drigalski and Lactose Litmus Medium which was prepared in accordance with the directions given

in Muir and Ritchie's Bacteriology. Ordinary agar and broth were of course made in the usual manner.

The only modification made in the series recommended by Dr. Houston was the additional use of Conradi plates and the substitution of maltose for inulin in the sugars.

Dr. Houston laid down no standard of a hard and fast nature but simply told me to isolate an organism, state its biological and morphological character and, provided it conformed within certain limits, to call it coliform. This has been done, and a fairly complete table of the organisms dealt with has been made out. This table has been illustrated as far as possible by cultures which have been sent with the thesis.

Reference may be made to the characteristics which should be possessed by coliform organisms. In Vol. 3 of the fourth Report of the Sewage Commission, p. 106, Dr. Houston summarises the characters of a typical B. Coli -

1. Gas formation in ordinary glucose gelatine shake
2. Formation of indol
3. Formation of acid and clot in Litmus milk
4. Formation of fluorescence in neutral red broth
5. Acid gas formation in lactose, peptone broth
6. Acid, gas and clot formation in peptone lactose milk-cultures.
7. Acid and gas ⁱⁿ ~~and~~ glucose peptone broth
8. Nitrate reduced to nitrite in nitrate broth cultures
9. Acid and gas C and P 1.
10. No marked formation of acid in C and P 2.
11. Presence of motility.
12. Non-liquifaction of gelatine.
13. Acidity in litmus whey, varying from about 20 to 40 cc. $\frac{N}{10}$ Na_2CO_3 per 100 cc. of culture.

Tests VIII and XIII were not performed in my series, but with three exceptions the organisms isolated conformed to the above specification with the exception of three organisms, two of which were non-lactose fermenters, the third of which was a member of the proteus group. The formation of fluorescence in neutral red was not satisfactory, but this applied also to a stock organism of Escherich.

On p. 140 of the Second Sewage Commission Report appears a list of methods employed and here it is pointed out how closely the indol formation is related to typical B.Coli. It is noted however that indol formation is produced by other organisms, namely those liquifying gelatine and forming spores.

On p.190, Vol.4., part 3, of the Fourth Sewage Commission's Report appears an analysis of the characters of 229 coliform organisms investigated by Dr.Gordon working under Dr.Houston's direction. The charts illustrated in this shew the marked parallellism of the curves of colon and indol forming organisms.

These 229 organisms were tested as follows:-

1. Gas in gelatine shake
2. Acid and clot in litmus milk
3. Indol formation

130 of these conformed to all the tests, and 223 formed gas, but varied in other particulars. Only 34 failed to form indol and 35 failed to clot milk

PREPARATION OF MEDIA.

Materials were obtained from Baird & Tatlock, Ltd., London.

Ordinary Agar - Composition.

1. Powdered agar 2.5 grammes
2. Witte's peptone 1 gramme
3. Glucose 1 gramme
4. Sod. Chlor. .5 gramme
5. Lemco .5 ,,
6. Water distilled 100 cc.

These constituents are carefully dissolved in a porcelain dish, especial care being taken not to let the lemco burn. The resultant emulsion is formed into a flash and put in a ^{Koch} ~~Koch~~'s steriliser or an autoclave for half-an-hour at least. If an autoclave is used it must not be run at any appreciable pressure or the resultant medium is a rich burnt brown colour. It then has the reaction taken and is carefully neutralised and .5% of Na_2CO_3 added.

It is then cooled below 60°C . and a small portion removed and beaten up with the white of an egg and re-added to the medium. This is replaced in steriliser for from 2 - 3 hours until the white of egg has precipitated in large flakes and the supernatant fluid is nearly clear.

The medium is then filtered through muslin in a hot water funnel, and having been reheat^eed it is filtered through papier chardin, also in a hot water funnel, and filter^{ed}ed into sterile plugged tubes which were sterilised for half-an-hour and then sloped off by inclining the tubes on glass rods laid on a table.

It was found advisable to use media free from glucose, when the culture was used for inoculating the various sugars as bubbles of gas formed in sugars in which no acid or gas was found when glucose free media was used.

Broth .

Composition. Lemco .5 grammes,
 Peptone 1 gramme
 Glucose 1 gramme
 Sod. Chlor. 1 gramme
 Water 100 cc.

These constituents were dissolved, care being taken to prevent the lemco from burning. The lemco being weighed in a watch glass which was balanced against another of equal weight, the weights being made equal by placing small portions of paper underneath the lighter one. The watch glass was placed in the water and the whole of the lemco dissolved from off it. When all the ingredients were dissolved the fluid was poured into a flask and sterilised for twenty minutes, then neutralised with normal sodium hydrate or sodium carbonate solution, until it was very faintly ~~acidified~~ ^{alkaline}. It was then allowed to cool and filtered into tubes which were sterilised for thirty minutes on three successive days. Filtration while hot produced a ~~foamy~~ ^{fibrous} deposit which was liable to be confused with deposit resulting from bacterial growth.

Glucose gelatine.

Composition. Peptone 1 gramme
 Sod. Chlor .5 ,,
 Glucose 1 ,,
 Gelatine 12 grammes (Coignet et Cie
 gold label)
 Kubel Tiemann
 Litmus solution 15 cc. water ~~to~~ to 100cc.

The gelatine was cut into strips and placed in a flask. The other constituents were dissolved in water and added. The flask was then put into the steriliser and the whole of the contents thoroughly dissolved. White of egg added as for agar. The clearing and filtering were done as with agar, the filtering however being much more readily done with gelatine media. It was then tubed off and used either as slopes or stabs.

Sorbite Gelatine.

This was prepared in a similar manner to the above, .2 gramme of sorbite being substituted for 1 gramme of glucose in each 100 cc. of medium.

Malachite green agar media.

This was prepared according to Dr. Houston's ⁽¹⁾ directions It is called by him "Rass Rebepelage".

Constituents for preparing 250 cc. of media, sufficient for 12 to 16 4" plates.

Composition

- A. Agar 5 grammes
- Peptone 5 ,,
- Sodium Tauro cholate 1.5 grammes
- Distilled water 200 c.c.

These ingredients ~~were~~ dissolved in a large porcelain dish, gentle heat being applied. The contents were then poured into a flask and sterilised for 30 minutes and cleared with ~~albumin~~^{WHITE} of egg and filtered as for ordinary agar. Its reaction was not however interfered with.

The following solution of sugars, etc., was then prepared -

Composition.

- B. Lactose
- Sachharose
- Adonite .5 grammes
- Raffinose
- Salicin
- Distilled water 50 c.c.

1 c.c. of a 1 % solution of neutral red is then added to this solution which is itself added to the cleared agar, etc., and the whole sterilised for 30 minutes. When required for use five c.c. of a .5 % solution of malachite green is added to the medium, which is at once poured into plates.

Media not at once poured with malachite green in it tend to form flaky deposits of malachite green.

(1) Sixth Research Report to Metropolitan Water Board by Dr A.C. Houston

The completed medium is a dark yellowish green in colour with however a distinct tinge of red in it, best observed when the medium is viewed horizontally from the side.

If from any cause the medium becomes distinctly acid the malachite green tends to become bluish in colour. An excess of alkali results in the colour being discharged, the medium appearing like ordinary agar with however a tinge of red apparent. This medium required much time and care to produce a successful result, and as a large number of plates were required for the bacteriolytic experiments a simplified cheap unfiltered form, was evolved. The various organisms grew fairly characteristically on this and it proved thoroughly satisfactory for its purpose.

"Unfiltered malachite" green agar.

Composition for 250 c.c.

A.	Agar	5 grammes
	Peptone	5 ,,
	Sod. Tauro-	
	-Cholate	1.25 grammes
	Water	200 c.c.

This was dissolved as before and sterilised for fifteen minutes, filtered through muslin and again sterilised for 15 minutes.

B.	Saccharose	.5 grammes
	Lactose	.5 ,,
	Water	50 c.c.

was

This solution was boiled and added to the agar, etc., while hot and finally 1 c.c. of a 1 % solution of neutral red and 5 c.c. of a .5 % solution of malachite green were added. This medium was usually of a rather bluish tinge, and 2 to 2.5 c.c. of a normal solution of sodium hydrate being added rendered it a more typical green tinge. The reason of this being necessary is that

that probably the properly prepared medium derived some alkalinity from the mixture with white of egg. The medium was always poured at once. The whole process described can be completed on $1\frac{1}{2}$ hours, while the properly filtered medium required at least six hours or longer, the time being absorbed with the tedious processes of clearing and filtering.

Potato medium.

Large potatoes were selected, washed and peeled; cylindrical pieces were removed with a borer. Potato tubes were prepared with cotton wool plugs in the bottom below the constriction. The wedges of potato were soaked for 30 minutes in a 10% solution of sodium carbonate to counteract their normally acid reaction. They were then placed in the prepared plugged tubes and sterilised for half-an-hour on three successive days.

Litmus milk.

Fresh milk was taken and sterilised for an hour and cooled and then allowed to stand in a cool place for 24 hours. The fluid underneath the cream was then syphoned off into another sterile flask and litmus solution added until a good purple tint was obtained. ~~K~~ubel Tiemann litmus was not used in this medium as filtration was not necessary. The fluid was then poured into sterile plugged tubes and sterilised for ten minutes on three successive days. The reaction was always slightly alkaline, which was a matter of indifference in dealing with acid forming organisms.

Conradi & Drigalski medium.

Composition.

Agar	3 grammes
Peptone	1 gramme
Nutrose	1 ,,
Sod. Chloride	.5 ,,
Lemco	.75 ,,
Distilled water	100 c.c.

These ingredients were mixed and dissolved in a porcelain dish and sterilised for thirty minutes in the autoclave, and then alkalinised very slightly. It was then cleared with white of egg and filtered as for agar and hot sterile water added to bring it to the original bulk. It was then re-sterilised.

Other constituents:-

- 13 c.c. of ~~Kubel~~ Tiemann litmus solution
- 1.5 grammes lactose
- .4 c.c. of a 10% solution of sodium carbonate
- 2 c.c. of a 1% solution of crystal violet.

13 c.c. of K.T. litmus solution and 1.5 grammes of lactose were boiled and added to the agar solution and a slightly alkaline reaction restored if necessary. .4 c.c. of a 10% solution of sodium carbonate is boiled with 2 c.c. of a 1% solution of crystal violet, in a sterile test tube, and this is thoroughly mixed with the rest of the medium which had again been sterilised. The whole is then poured into sterile ~~Petri~~ dishes without further sterilisation.

Peptone Water.

<u>Composition.</u>	Peptone	grammes 1.5
	Sod. Chlor.	,, 5
	Water	100 c.c.

These were dissolved by heating in a porcelain dish filtered and tubed off into plugged sterile tubes and sterilised on three successive days for fifteen minutes.

The solutions added to this peptone water in doing the rosindol test were

I. Paradiamidobenzaldehyde	grammes	2
Hydrochloric acid		40 c.c.
Absolute alcohol		190 c.c.

This makes a golden coloured clear solution.

II. A saturated aqueous solution of potassium persulphate

The solution was supersaturated and the supernatant fluid used.

Glucose neutral red broths.

Composition	Peptone	grammes	1
	Glucose	,,	.5
	Sod. Chloride	,,	.5
	Lemco	,,	.5
	1% neutral red	,,	.5 cc.
	Water		100 c.c.

The ingredients were mixed dissolved and sterilised and made faintly alkaline. It is used with Durham's tubes. Houston recommends heating this medium to 100^C and cooling rapidly in water just before use. (1)

Sugar media.

These media were all prepared and filtered into sterile plugged tubes containing inverted Durham's tubes. Sufficient media was poured in to leave $\frac{1}{2}$ to $\frac{3}{4}$ inch of media at least around the base of the Durham's tube when the fluid had risen to the top of the Durham's tube. 15 c.c. of K T. solution was added to each 85 c.c. of distilled water. They were then placed either in the autoclave for 1 to 2 hours until on cooling slightly the fluid rose thoroughly to the top of the Durham's tubes or placed in a beaker of water and boiled for 15 to 20 minutes when on removing the fluid rose quickly to the top of the inverted tubes.

Proskauer and Capaldi No. 1, modified by Dr. Houston
(Chart abbreviation = "C & P I")

Composition

Sodium Chloride	grammes	·02
Calcium chloride	,,	·02
Magnesium sulphate	,,	·01
Asparagin	}	·2 of each
Galactose		
Glucose		
Mannite		
Pot. Monophosphate		
Levulose		
Maltose		
Water	100 c.c.	
Water tinted litmus	15 ,,	

The solid ingredients were dissolved in the water tinted litmus with gentle heat. They are placed in a flask and sterilised for 30 minutes and filtered in sterile plugged tubes containing Durham's tubes.

Proskauer & Capaldi's No II.

Composition.

Peptone	grammes	2
Mannite		·1
Water tinted litmus	100 c.c.	

prepared as above.

L.S.D.S. medium. - Dr. Houston's formula.

Composition.

Lactose		
Salicin		
Dulcite	grammes	·25 of each
Saccharose		
Peptone	grammes	2
Water tinted with litmus	100 c.c.	

prepared as above.

Series of Sugars. (The numbers correspond to those in chart)
(prepared as above)

No.	I	Glucose	}	·1 gramme of each sugar 2 grammes of peptone Water tinted with 15 % of K.T. litmus solution 100 c.c.
	II	Mannite (same as C & P II)		
	III	Galactose		
	IV	Levulose		
	V	Lactose		
	VI	Saccharose		
	VII	Dulcite		
	VIII	Salicien		
	IX	Raffinose		
	X	Adonite		
	XI	Inosite		
	XII	Maltose		

An ordinary autoclave has been used in preparing the media. This autoclave has been run at $2\frac{1}{2}$ atmospheres pressure, i.e., at 139°C ., in sterilising tubes, plates, etc., and in ~~the~~ *the* killing of old cultures, plates, etc.

In preparing media it was used at a very low temperature, the exhaust being left open rendering it practically equivalent to a Koch's steriliser. This was found essential in dealing with solid media such as agar, which became a rich brown colour if cooked at, say $2\frac{1}{2}$ atmospheres pressure. In dealing with media containing lactose great care was taken not to overheat it by raising pressure for fear of decomposing ^{the} lactose.

It was run at a temperature of rather over 100°C . in sterilising media such as broth and other liquids containing no substance easily broken up.

Plates were cleaned by boiling for an hour in a large iron saucepan in the following solution -

Potassium *Bichromate*
Sulphuric acid (pure) 60 parts
Water 1,000 c.c.

These were then thoroughly rinsed, dried and sterilised.

In forming media into plates the lids were left off until the media set; the lids were then replaced and the media was placed in a small tin oven which was run at a constant temperature of 55°C . They were left in this for about an hour. When most of the water of condensation was found to have risen into the lids which were dried with a clean cloth and flamed and replaced for another hour. In this way, while the surface of the media did not become unduly dried, the great bulk of the water of condensation was got rid of, and clean discrete colonies obtained.

The plates were then stored in long tins 18 inches high and 5 inches across containing a flat circular piece of tin with

three uprights rising from it curving over at the top. The plates were previously sterilised in these tins. Tubed media were placed in large tins containing cotton wool at the bottom. When filling solid tubed media care was exerted to prevent the media touching the tube around the neck, otherwise the plug of cotton wool could not be removed from the neck in a cleanly fashion.

Appearance and use of Media.

1. Malachite green and agar plates.

These plates appear green with a faint barely perceptible reddish tinge. These plates as prepared ^{at} by the Metropolitan Water Board Laboratory under Dr. Houston's directions have no alteration made artificially in their reaction to litmus and other indicators. Occasionally, as prepared by me at Bexley, a degree of alkalinity has been induced, with the result that the malachite green colour has been discharged. This has in some instances undoubtedly been due to the "new laid" egg used for clearing not having been absolutely fresh.

The appearance of typhoid colonies on this medium is that of a small discrete colony with a definite circular margin and a translucent appearance. This refers to a 24 to 36 hours growth. When killed by heat this translucent appearance tends to become more opaque and the growth resembles more a certain type of colon organism. Certain types of organism which liquify gelatine also resemble typhoid colonies, but their more vigorous growth, tending to produce a rapid coalescence of the colonies, enables them to be readily distinguished.

Conradi - Drigalski Medium.

On this medium the typhoid formed small blue colonies, while the colon organisms formed larger colonies which were distinctly red in colour. Coliform organisms which did not form acid in the lactose very closely resembled the typhoid. Various organisms liquifying gelatine also formed blue colonies with the same translucent appearance characteristic of the typhoid organism, but their more vigorous luxuriant growth enabled them to be easily differentiated. These remarks apply to 24 to 36 hours growths.

Litmus Milk.

The organisms were inoculated and allowed to grow for 18 days. The typhoid organism formed acid and no clot. This acidity was less marked towards the end of the period of culture. Most of the colon organisms showed acid and clot. In about half the cases this appeared in 24 hours, and in all cases but two it occurred in two days. In two cases although marked acidity was present no appreciable clot was formed. Negative results were obtained in the case of non-lactose fermenters. Various liquifying organisms rapidly liquified the clot formed, generally decolourising the medium.

Potato medium.

The typhoid bacillus grew a filmy white growth, while the colon organisms grew a thicker brownish growth. In one case the organism - one of those isolated from Case XIII - grew a thin whitish growth. This organism formed acid and gas in the usual sugars. The liquifying organisms produced luxuriant brown growths. In the case of the organism producing green pigment referred to before the wedge of potato was found to be distinctly greenish all over.

Glucose neutral red.

The results with this medium were disappointing, distinct fluorescence not being obtained with any organism, even with the stock organism of ~~Escherich~~; at the same time there was distinct difference between the growth obtained with the typhoid organism and that occurring in the various colon organisms.

Peptone water.

Indol was readily obtained with all organisms, ^{Coliform.} except the typhoid bacillus and the pigment forming organism previously mentioned. The Rosindol reaction of Ehrlich was the method employed. In most instances the reaction appeared in a few moments with the addition of the paradimeth. solution, but in some cases it was delayed, even after the addition of both solutions. In all cases the colouring matter formed was readily dissolved in amylalcohol.

Gelatin media.1. Sorbite gelatine slopes.

On this medium the colon organisms all formed bluish white growths, while the typhoid organism formed a thin translucent red growth. In this connection it may be mentioned the organism isolated from case I was not so characteristic as that obtained from a stock organism from Guy's ^{Hospital}. One of the colon organisms isolated rather resembled typhoid in its growth (Case XVI).

Glucose gelatin slopes.

Typhoid appeared as a thin translucent reddish growth, while the colon organisms showed a much more vigorous luxuriant growth.

Glucose gelatin stabs.

In stab culture the typhoid organism produced acidity but no gas. All the other ^{COLIFORM} organisms in this series produced acid and gas formation with splitting up of the media. The method of inoculation was as follows:-

A quantity of culture was taken on a stiff platinum needle and stabbed into the medium. The inoculated tube was then placed in an ordinary Harkon's incubator for three hours and then removed and allowed to solidify. This procedure was recommended to me by Dr. Houston to eliminate errors through splitting of the media in inoculation. All gelatin cultures were allowed to grow for three weeks so that a slowly liquifying organism should not be overlooked. The liquifying organisms met with varied greatly in the rapidity of their liquifying action, some taking 24 hours, others two to three days, and some a week. Experience with liquifying organisms was mainly gained in endeavours to isolate the typhoid ~~from~~ from the faeces in various cases..

Growth in broth.

In 24 hours little or no deposit occurred in any of the colon organisms in this series nor with the typhoid bacillus. Scum and free deposit was only met with in dealing with liquifying organisms.

Sugar media.

With the series of sugars employed the typhoid organism produced acid and no gas in 48 hours on glucose, mannite, galactose, levulose and maltose. No change was obtained in L.S.D.S. medium C. & P.No.1, but marked acid occurred in C. and P No. 2. No other sugars were changed. These remarks apply both to a stock organism from Guy's and to the organism isolated from case No. 1. The colon bacillus formed acid and gas in the above mentioned sugars, and upon lactose. Two organisms did not ferment lactose, viz., case No.VIII which fermented saccharose however, and Case XVI also failed to ferment lactose. A colon bacillus of Escherich was obtained from Dr. Houston and the media tested with this organism. Disappointingly few organisms fermented dulcete. Very slight acid and gas was obtained with most of the colon organisms on mannite. In the case of maltose the fermentation was in several cases delayed beyond 48 hours.

In conclusion great precaution must be taken to exclude ~~an~~ error in working with these media in Durham's tubes. The solution may appear to be tight at the top of the inverted tube but on incubation a small bubble of gas may form without inoculation. This is especially liable to occur when the media have been kept for a long period.

In this connection it may be mentioned that some slight variability in results have occurred, and these have been indicated on the table of organisms by brackets placed round results that were obtained on one occasion and not on another. This may be due to four causes -

1. Failure to inoculate
2. Delayed fermentation: this especially occurred with maltose.
3. Contamination of the culture.
4. Variation in biological cultures on repeated sub-cultivation.

This last is referred to by Revis in reference to B.Coli in the Centralblatt fur Bakteriologie 2^{te} alt vol.26,1910, p.161. (reference from Hewlett's bacteriology not confirmed)

Another reference to variation on sub-cultivation on agar with streptococci occurs in work by Walker (1) and this article cultivation shewed variation on milk, saccharose, lactose, salacin, mannite and nutrose. Contamination of the organisms was here absolutely excluded

(1) Proceedings of the Royal Society, Biological series, B.85,
No. B.581.

Methods (b) and (c)

Bacteriolytic and Opsonic Methods,
Preparation of Vaccines, etc.

Estimation of the Bacteriolytic Power.

Method employed.

The general idea was to test the sera of the various patients against the organisms obtained from the respective patients, using as far as possible undilute serum. Controls were done with normal serum in every instance

1. One unit of serum was mixed with one unit of broth culture of organisms, 24 hours growth.
2. Ten units of serum were mixed with one unit of broth culture of organism.
3. Twenty-five units of serum were mixed with one unit of broth culture
4. Fifty units of serum were mixed with half a unit of broth culture.
5. One unit of serum was mixed with one unit of broth culture diluted to 1 in 100 .
6. One unit of serum was mixed with one unit of broth culture diluted 1 in 1000.

There were drawn into capillary pipettes and incubated for three hours and blown out on to plates. The colonies were counted in 24 to 36 hours, varying with the organism.

Requisites.

- 1 Serum of patient, used freshly drawn.
2. Control serum from a normal person, also freshly drawn.
3. 24 hours culture of the organism.
4. Sterile broth and flamed (or otherwise sterilised) watch glasses for making dilutions of the broth culture.

5. Sterile and standardised pipettes for making culture dilutions.

6. Sterilised and (as far as possible) standardised capillary pipettes. These were sealed and incubated as described above.

7. A number of flamed drop slides for blowing serum and organisms out of the capillary tube and mixing them on.

8. Yellow grease pencils, small sticky labels and platinum needles.

9. Plates of culture media. The unfiltered malachite green agar medium described elsewhere was used.

1. The sera required were withdrawn from one of the big veins of the arm in 5 c.c. glass syringes in the usual manner. The blood was transferred from the syringe to sterile centrifuge tubes. After centrifugalisation the serum was transferred to capsules similar to those in which serum was retained.



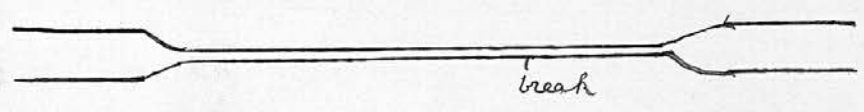
These were made from No.9 thick glass tubing and were readily sealed and reopened.

5. Standardised pipettes. These were made for me by Messrs Down, graduated as illustrated, and it will be readily understood how the various dilutions were easily made with these. As a rule however the 1 in 1000 dilution was made on a smaller scale from the 1 in 100 dilution with smaller standard pipettes.

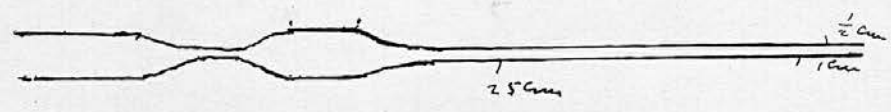


6. Preparation and standardisation of capillary pipettes.

These were drawn from No.4 thick glass tubing, and were drawn either in an ordinary Bunsen flame or in a mechanically worked blow-pipe flame. The glass was cut into 4 to 5 inch lengths and drawn in the usual manner into an even capillary tube about 40 to 50 centemetres in length.



It was broken so as to form lengths of 30 cm. and also lengths of 10 to 20 cm. of capillary tubing with a piece of glass tubing attached. The unit referred to above adopted, was 1 cm of this capillary tubing and it was found in properly drawn pipettes to contain .002 of a c.c. and to weigh .002 of a gramme when water was the medium used. On weighing water contained in 25 cm. of this capillary tubing it was found very closely to weigh .05 gramme. Experience was soon gained in selecting capillaries which conformed very closely to these conditions. The pipettes were marked by placing them along the edge of the bench, which consisted of a hard dark wood in which lines were scored thus - $\frac{1}{2} \text{ cm}$ $\frac{1}{10} \text{ cm}$ and the graduations were carefully marked with a grease pencil. A convenient modification for the pipettes, 25 cm. long was found in drawing pipettes as below



Mode of Work.

1. A set of labels required was written with the name of the patient, and the proportion of sera and the word "control" added where necessary. These were performed with the pencil used for writing them.
2. The marked pipettes were flamed until the yellow grease mark turned dark coloured, an efficient test of sterility according to Wright. These pipettes were laid out in their different classes with the ends protruding over the edge of the bench to avoid contamination.
3. The flamed watch glasses were laid out in a row on a white background with the initials of the patient and the dilution of the broth to be made chalked on the under surface in "looking-glass" writing.
4. The broth cultures to be used were placed in a test tube rack.
5. A beaker was kept boiling containing the standardised pipettes.
6. Capsules of sera plainly labelled were laid out in a row.

The serum was drawn out in a pipette, a small bubble of air being allowed to enter and the unit of organism drawn up. The whole was ^{blown} ~~drawn~~ out on to one of the drop slides, carefully mixed, and the whole drawn up again into the capillary tubes, care being taken to avoid air bubbles. The end of the capillary tube was sealed, the fluid being kept an inch away from the end while this was being done. The suitable prepared label was slipped on to each and the pipette placed in a Pearson's incubator. At the end of three hours the contents were blown

out on to a malachite agar plate and spread carefully over the surface, care having previously been taken to remove the water of condensation without unduly drying the plate. When pipettes with reservoirs were used the mixture was drawn up into the reservoir and the superfluous glass broken off. The mixtures were blown out on to the plates either by the mouth or with the aid of a teat.

Counting of plates.

Small numbers of colonies of a discrete organism were of course easily counted. Larger numbers could however only be estimated. Numbers over 800 or 900 are hardly countable on a small plate. When dealing with numbers much over a 1000 it is obvious that many organisms failed to found colonies by being crowded out, so when this occurred the result was marked "unlimited".

Large numbers such as 400 to 700 were hard to estimate, but a comparison of two plates often shewed which grew the more freely. Every effort was made by dividing the plate with a knife into small sections to estimate the number growing as accurately as possible. In this connection it may be asked why a greater dilution of the broth was not used, but it was found that with many organisms the control serum as well as the patient's serum completely sterilised the emulsion in these dilutions..

Reasons for Bacteriolytic Methods adopted.

The method had to be in vitro as no vivisection license was obtainable.

1. Capillary tubes were used because they were easily made and manipulated and also easily sterilised. There was little risk of contamination and practically no loss of the mixture of broth and serum. Reasonably accurate standardisation was found practicable.

2. A fixed unit of broth culture was adopted. This was adopted so that with the majority of organisms a reasonable range of bacteriolysis was obtained in the proportions used. The organisms varied greatly of course and with some of the more prolific ones a greater dilution would have served better. But for the average number of organisms investigated it was found very suitable for use. Of course in using undilute broth cultures the controls grow an unlimited number of organisms on four inch plates, but when mixed with control serum and still more with patient's immune serum the number of colonies appear in countable numbers in many instances.

3. An undiluted serum in different proportions was used as far as possible because the results were less variable with this method. Definite proportions of mixture of broth culture and serum were adopted owing to the impracticability in a series of organisms in doing an indefinite number of dilutions and controls.

5. Incubation for three hours was adopted as bacteriolysis occurred in this time. Incubation for 24 hours occasionally producing more colonies than after three hours incubation, especially with freely growing organisms mixed with a serum of low bacteriolytic power.

6. Malachite green plates were used owing to their inhibiting power on contaminating organisms. *Inserts of Plates 4"*

The methods considered were

A. 1 - those of Wright

B. 2 - those of Neisser and Wechberg (1)

(1. Muir & Ritchie, 5th ed. p. 127.)

A 1. In one of his many methods Wright takes a fixed quantity of serum and dilutes the broth culture. He then finds the lowest dilution which proves sterile. The disadvantages of this are the clumps occur in the broth cultures and are liable, even with great care, to get into the higher dilutions and cause anomalous results.

2. Difference in the virulence of different organisms of the same group and of different subcultures of the same organism. *This applies to 4 as to be observed in all methods*

3. The enormous number of dilutions and controls necessary to do this in a series of twelve organisms

B. 1. Neisser and Wechberg's methods.

1. A fixed unit of broth is taken.

2. Variable proportions of sera are used.

3. Dilution with saline.

4. Placed in plugged sterile tubes.

5. Incubation for three hours.

This method requires more apparatus than the capillary tube and there is greater risk of contamination. It will be seen that some of the features of each method were adopted. Since this was evolved in July, 1912, Wright's book on the technique of the capillary tube was obtained. In this he sanctions every mixture of dilute and undilute serum and broth in the series of principles at the end of his article on bacteriology

bacteriolysis (p.113)

On page 135 of the same book Wright emphasises the importance in opsonic and bacteriolytic work in having a sufficient number of organisms of organisms to differentiate between serum with some immune power and normal serum. While this was certainly so with the unit adopted by me when using colon organisms, in the case of an attenuated typhoid culture it was hardly large enough.

(1)

Allen states 10 million B.Typhosus in emulsion are killed when mixed with equal quantities of normal serum. This however appears to vary somewhat in my experience with the virulence of the organism employed.

Other methods quoted by Emery (2) mainly consider testing the power of immune serum obtained from animals and heating this, bringing in the question of complement. This factor which was felt to be beyond the scope of the present paper was eliminated as far as possible by the use of fresh serum both in that obtained from the patient and with the control.

(1) Allen, Vaccine Therapy, 4th ed. p.19.

(2) Emery, Immune and Specific Therapy, p. 175, etc.

Opsonic Methods.

A number of opsonins were done by Wright's method but a complete series was impossible owing to some of the sera shewing bacteriolytic action and owing to some of the bacterial emulsions, especially those of the protein group, having haemolytic effects.

While a number of the opsonins done corresponded with the other results obtained, they can hardly be relied upon owing to these complications.

The dilution method of Klein was found impracticable in a series such as this owing to the large amount of time involved in their performance.

Methods.

Requisites

1. Patients' sera.
2. Control pool serum.
3. Washed leucocytes.
4. Capillary pipettes, marked off about $\frac{3}{4}$ inch from the end with grease pencil.
5. Twenty-four hours broth culture of organism.
6. Slides, etc.

The methods followed were those of Wright, as quoted in Allen's book on Vaccine Therapy, and only special points which occur in these cases need be mentioned. The incubation period taken was ten minutes as less bacteriolysis occurred in this time.

The staining methods adopted were

1 - the modification of Leishmann's method, viz., staining for five minutes with the undilute stain and fifteen minutes with the dilute stain.

2 - Staining for two minutes with undilute carbol thionin. This latter method was found the more satisfactory.

Counting.

The leucocytes were searched for along the edges of the films, and in many cases a hundred of these were counted, in some cases where the leucocytes were defective owing to leucolytic effects it was only found possible to enumerate fifty. In some cases this leucolytic effect prevented the performance of the test at all.

The usual spreader recommended by Wright was used, and a sample of one of these is enclosed.



THE Edge used being slightly concave.

Several other methods of performing the opsonic work were considered -

- 1 - ⁽¹⁾The dilution method of Klein referred to above.
- 2 - ⁽²⁾Heating the serum to 55°C. to prevent bacteriolysis.

This was rejected as such a large amount of the available opsonin was removed. Whether immune opsonin is regarded as thermo stable and ordinary opsonin as a thermo labile variety, or whether the thermo labile part is simply regarded as complement and the thermo stable part as agglutinins, bacteriolysins etc., it is obviously unfair to estimate a part of the opsonin against a control which has been depleted more in proportion.

1) Allen. Vaccines THERAPY 4th Ed. P 52.
2) Minin & Ritchie 5th Ed. P 126

3. Simon's method of counting leucocytes which contain no organisms against those containing organisms was rejected owing to the large number of leucocytes it was necessary to count, and also to the leucolytic effect of some of the broth cultures exhibited.

4. Leishman method. This was done in one case but the difficulty occurred when the patients (as was usually the case) were in woods a quarter of a mile from the laboratory.

Agglutination methods. The ordinary methods employed in doing Widal reactions for typhoid were employed. The minimum dilution employed was 1 in 20, and dilutions were done up to 1 in 1000 when necessary.

Preparation of Vaccine.

The isolated organism was taken and six tubes of agar were inoculated with a double *STROKE* on each and ~~removed from~~ ^{placed in} the incubator.

After growth The cotton wool plugs were removed and any adherent wool cleared from the tops of the tubes and the tops thoroughly flamed. When cool the tubes were filled one third full of sterile salt solution; .1 % this salt solution was prepared and placed in a flask and sterilised. It was then kept in a tin oven run at a constant temperature of 53°C.

In many cases shaking the tube shook of the cultures and formed an emulsion. In a few cases however a sterile needle had to be introduced and the culture scraped off .

Two-ounce glass stoppered bottles were used for containing the vaccine, and into these were placed about twenty glass beads. These were placed in an autoclave, with the stoppers out to obviate the cracking of the bottles or at the best to prevent the stoppers being blown out.

When sterilised the stoppers were replaced and the bottles were left in the tin oven at 53°C until required. The emulsion of vaccine prepared as above was then poured into the bottle, either directly or through a small sterile glass funnel.

Care had to be taken that the agar slope itself did not follow the emulsion into the bottle or worse still that small ^{pieces} fragments of detached agar did not peel off and fall into the bottle. When all the tubes had been emptied into the prepared bottle it was usually found to contain between 25 and 30 c.c. of emulsion.

2

The amount was estimated roughly by filling from a burette a similar bottle containing the same number of beads, up to the same level; 5 % pure carbolic was then added. This was conveniently done by adding 10 % of the amount of the emulsion of $\frac{1}{20}$ carbolic acid solution, thus 2.5 c.c. of $\frac{1}{20}$ carbolic were added to 25 c.c. bacterial emulsion.

It was then shaken for a few minutes to break off any clumps, the organisms dealt with being fortunately very easy to emulsify.

The vaccines were then placed in the iron oven at 53° C. for 1 hour, and later a c.c. was withdrawn in a sterile pipette or poured on to an agar slope or plate and incubated for 2 to 3 days. In one instance a *Sporing* type of organism, mesentericus vulgatus grew on the third day. The vaccine had been administered on the 2nd day but no untoward effects were observed.

Later, in view of Semple⁽¹⁾ and Harrison's work on typhoid vaccines and the great advantages of sterilisation by carbolic instead of heat, the time in the oven was reduced to half an hour at 53° C. and reliance^{which} was not misplaced placed in the carbolic. In a number of entirely satisfactory cases the vaccine was not heated at all.

On the third day the sterility of the vaccine was tested by pouring 1 c.c. on to an agar slope and culturing for three days. This last for fear of contamination with sporing organisms such as mesentericus.

The vaccines prepared in this way were found less toxic and quite as satisfactory from an immunity point of view.

Enumeration of vaccines. This was done by Wright's method.
Requirements.

1. A 2 % solution of Sodium Citrate. My own blood was used : it counts about 5,200,000 per c.m.m.
2. A capillary pipette drawn from No.4. thick glass tubing.

(1) Allen vaccine therapy 4th Ed 1908

A mark was made on the pipette with a glass pencil about $\frac{3}{4}$ inch from the end. A column of sodium citrate was drawn up, and then a column of blood exactly to the chalk mark, a small bubble of air being allowed to intervene the citrate and the blood.

Another bubble of air was admitted and the emulsion was drawn up to the chalk mark. This was blown out on to a slide and spread in the usual way after being thoroughly mixed. The slides were stained by Leishmann's method, the stain being allowed to act undiluted for five minutes and diluted for fifteen minutes. They were then mounted in the usual manner.

Most of the vaccine counting slides were prepared in this way, but later it was that staining with carbol thionin was just as effective and much easier.

Briefly, the method of counting was, - ~~the slides to be~~
~~slide~~ - to examine both slides to see if evenly spread

2 - to count thirty fields. These fields were limited by a circular chalk mark being drawn on the lower portion of the eye piece of the microscope.

The resulting count ^{with B. Coli} varied between 125 and 2000 millions per c.c.

The bottles containing the vaccine were labelled and sealed off ~~w~~ from contamination by having the necks dipped in ^{liquid} paraffin wax.

In two cases, one being that of the typhoid vaccine, Harrison's method was employed, viz., replacing the blood serum in a given quantity of blood by saline solution, this being done to obviate the bacteriolytic effect which the serum might exercise on the organism.

Administration was always by hypodermic injection. Necessary dilution for small doses was done with saline in a glass hypodermic syringe at the time of injection.

DESCRIPTION OF CASES

I to XIX.

CASE I.

Patient E. D., aged 27, admitted August, 1910, certified as chronic mania.

She was an excitable, irrational girl with many eccentric notions and many fleeting delusions. She frequently got into such an excited condition that great difficulty was sustained in taking blood and making clinical observations.

Clinical history. On September 20th, 1911, she became ill and on the 22nd her temperature rose to 101°; on the 30th it rose to 103, and to 104 on the 24th. She gave a negative Widal on the 23rd but was positive at 1 100 on the 27th. She maintained an irregular temperature of 103° to 100° until October 4th when it sank to 99°, but rose again to 102. It gradually sank from the 9th October in a 'staircase' manner until it became normal on October 13th. It continued normal until the 18th October when a slight rise occurred, and on the 19th it rose to 102 and on the 20th October to 103.4. She continued an irregular pyrexia of 102 to 97 to the 3rd November. From thence until November she had 17th an irregular rise extending to 100 in the afternoon and evening and sinking to 98 in the morning. On October 28th a periosteal abscess developed on the left side of the forehead above and external to the outer canthus of the eye. This was not opened and continued enlarging and retracting slightly until August of 1912, when it caused a slight adherence to the skin of the periosteum and bone.

From November 17th until the 27th the pyrexia varied from 104 to 100, and she was sponged at intervals when her temp. reached the higher figure.

On the 2nd November a swelling was noticed over the anterior surface of the left tibia: this increased in size and on

November 26th a periosteal abscess was opened under gas. This was found to contain some chocolate coloured pus which on culture proved to contain pure B. Typhosus.

After the 27th her pyrexia decreased somewhat until the 30th when the highest figure was 100°. From December 1st until December 15th she maintained an irregular pyrexia of 102° to 97° and it then slowly sank to normal on December 19th. It rose again to 103 on December 24th and sank to normal on the 28th December. She had a slight pyrexia to 100 from January 1st to January 6th, 1912, when it finally sank to normal.

No further pyrexia occurred except after the injection of typhoid vaccine.

On February 20th a periosteal abscess developed over the inner surface of her left tibia; this was opened and a culture of B. Typhosus obtained.

On April 29th a fourth abscess developed on the middle finger of the left hand and this was also incised and a culture of B. Typhosus obtained.

On May 30th a fifth abscess developed below and slightly external canthus to the outer side of the Δ on the left hand side of the forehead. This was opened and a culture of B. Typhosus obtained.

On the 5th June a culture of the urine showed B. Typhosus, the organism corresponding in all details with the organisms obtained from the abscesses and 28 colonies grew from $\frac{1}{100}$ cc. On June 22nd, 50 millions of a vaccine prepared from the organism were injected and patients' temperature rose to 102: it sank to just above normal next morning. On the following day it again rose to 100. It did not rise again.

On June 29th an injection of 100 millions was given, and the temp. rose to 100.8 on the following evening.

After the first injection some shrinking was observed in the chronic indurated patches which surrounded all the abscesses which had been incised.

On the 9th July an injection of **200** millions was given, and the temperature rose to 103.2 on the following evening, sank to normal in the morning to rise again to 99.8 at 2 p.m. on the following day. Great shrinking occurred on the chronic indurated patch on the left tibia, and the abscess on the forehead also shrank and discharged slightly. The middle finger of the left hand discharged an amount of serum and the finger which had been twice the normal thickness commenced to shrink rapidly. At this stage, failure to isolate B. Typhosus from the urine occurred for the first time in the patient's illness.

On August 9th 500 millions were injected: the temp. rose to 103, sinking gradually to normal on the 11th. Fresh discharge occurred from the middle finger, and some pus occurred which on culture produced staphylococcus albus. This was probably due to the patient's dirty habits: the patient continually rubbed her finger and annointed it with urine, believing this to be beneficial.

The effects on the blood are contained in the chart.

On the 10th September 1,000 millions were injected and this caused a short rise of temperature to 102. The various abscess areas after this shrank down to normal, and the patient continued in sound health with no further rise of temperature.

The bacteriol~~ytis~~ figures in this instance were very interesting, as a steady rise in bacteriolytic power was observed and once raised was well maintained. The bacteriolytic results are shown in the table.

Before injection her blood showed ~~more~~ ^{increased} bacteriolytic power towards her own organism. A mixture of $\frac{1}{1}$ of serum and broth culture showed an 'unlimited' growth with normal serum and also with the patient's serum, but a $\frac{10}{1}$ of serum and organism showed over a thousand colonies with the control serum but only about 480 with the patient's serum. $\frac{25}{1}$ of serum to organisms produced over a thousand colonies on the control and only 250 colonies with the patient's serum. 50 units of serum to half a unit of broth produced about a thousand colonies in the control and only 160 in the patient's serum. The ~~amount~~ ^{unit} of sera to ^{one unit of} $\frac{1}{100}$ dilution of broth culture and $\frac{1}{1000}$ dilution of culture were proved sterile while the control showed over 1000 ~~and~~ over 800 colonies respectively.

The patient's serum was *retested* after the 200 million injection and only 600 odd colonies were found with the $\frac{1}{1}$ test. 400 odd colonies were found with the $\frac{10}{1}$ test: 140 colonies with the $\frac{25}{1}$ test: 90 with the $\frac{25}{2}$ test. These showed a diminution compared with the previous series. 80 colonies however grew in the serum to $\frac{1}{100}$ of broth test, which showed a discrepancy. The 1 to $\frac{1}{1000}$ of broth proved sterile. The controls were similar to before, except that the $\frac{1}{1000}$ grew fewer colonies. The next series done after 500 millions were injected showed an *increased* bacteriolytic power: 600 odd colonies growing in the $\frac{1}{1}$ test, 300 odd in the $\frac{10}{1}$ test, and the rest proving sterile.

The controls were similar to the previous series. After 1000 millions had been injected great improvement resulted: all the tests proved sterile when the patient's serum was used, while the control showed over a thousand colonies except with the $\frac{1}{1000}$ which again showed about 800, distinctly less than the $\frac{1}{100}$ dilution.

At this stage the patient's serum killed a unit of typhoid organism obtained from Guy's Hospital Laboratory at a mixture of one unit of serum to one of broth culture, and also with one unit of serum to two of broth culture. After the injection of 500 millions, the patient's serum sterilised the typhoid culture from Guy's except at 2-1 where 24 colonies grew.

Bacteriolytic results done one month later also proved sterile in all series. They were again repeated after the agglutination results had disappeared, and large numbers of colonies grew in all dilutions, the records of which were mislaid.

Agglutination results.

Before injection the serum of the patient did not agglutinate a Guy's typhoid organism at a 1 in 500 dilution although it did agglutinate a Guy's organism at a 1 in 200 dilution.

A curious partial reaction only occurred with the patient's own organism - a number of clumps appearing in dilutions of $\frac{1}{20}$ $\frac{1}{200}$. After the injection of 200 millions of the *autogenous* vaccine agglutination occurred at $\frac{1}{1000}$ dilution with the Guy's organism, but only a partial result was obtained with the patient's organism. After the injection of 500 millions the Guy's organism continued to agglutinate, but the patient's organism while now definitely agglutinating at dilutions of $\frac{1}{40}$ and $\frac{1}{100}$ only partially agglutinated at $\frac{1}{200}$, very partially at $\frac{1}{500}$ and not at $\frac{1}{1000}$. After the injection of 1000 millions complete agglutination up to $\frac{1}{1000}$ dilution occurred with both organisms. The opsonic power of the patient's serum was found to be 1.7 before injection. It was again tested after the last injection (i.e. of 1000 millions) and was found on this occasion to be 2.3. The slides however were not very satisfactory, probably from bacteriolysis, and the results should probably have been higher..

After shewing a positive Widal for a year after the original infection, this ceased $3\frac{3}{4}$ months after her last injection.

Widals with a stock organism were done at intervals until after ceasing two months after her last injection to give positive reactions in the higher dilutions. On Jan. 6th, 1913, she gave a negative Widal in all dilutions. Her bacteriolytic power was also found diminished and she proved to have little more bacteriolytic power than the control.

Her urine was tested at frequent intervals, but no more B. Typhosus was isolated. The faeces were examined at intervals but B. Typhosus was not successfully isolated at any time.

Her urine on the last occasion on which the typhoid was isolated grew 28 colonies from $\frac{1}{100}$ of a c.c. of urine, shewing her to have 2,800 organisms per c.c., a small amount but ^{notable} ~~reliable~~ as they were isolated ^{6 m} ~~two~~ months after the original attack.

General Results following Vaccination.

1. Increase of agglutinative power both towards her own organism and towards the stock B. Typhosus.
2. Increase of bacteriolytic power towards her own organism and B. Typhosus.
3. Drying up of chronic abscesses containing pure cultures of her organism.
4. Cessation of Typhoid Bacilline after its continuance for nearly 10 months.
5. Loss of Widal reaction $3\frac{3}{4}$ months after test injection after preserving it for 10 months, prior to injection and altogether for over a year.

Throughout this description the organism isolated has been called B. Typhosus. Grave doubts must however be expressed on this point.

Actually it resembled various typhoid organisms obtained from Guy's Hospital Bacteriological Laboratory for Widal reactions. It formed acid from glucose, mannite, galactose *levulose* and maltose. It grew a faint red growth on sorbite, which later however had a slightly bluer tinge than stock B. Typhosus. It formed ^{small} ~~such~~ translucent colonies on malachite green and small blue colonies on Conradi Drigalski.

A specimen was taken up to the Metropolitan Water Board laboratory, and while I did not personally see Dr. Houston about it I was told that it conformed culturally to B. Typhosus.

An agglutinating serum ^{was} obtained from the Lister Institute ~~however~~ ^{which} agglutinated a stock B. Typhosus from Guy's in all dilutions. ^{to 1/1000} It did not however agglutinate this organism. Whether passage through animals would alter this it is impossible for me to say.

A number of strains were of course isolated from the urine and the various abscesses, and which was used for the vaccine I cannot say. The organism sent is the survivor on sorbite gelatine of very many growths. Possibly all these growths would not have been negative in their agglutination reactions to stock agglutinating serum.

The increase in agglutinating power of the patient's serum both to her own organism and to the stock B. Typhosus is however remarkable.

F.K., an epileptic imbecile who was admitted in May, 1902.

Her case presents no point of special interest until, on March 10th, 1912, she contracted typhoid fever. She gave a positive Widal, and the course of the disease was fairly characteristic. She had one relapse and on May 2nd, viewed from the clinical aspect, she had entirely recovered.

On March 28th B. Typhosus was isolated from the urine. This typhoid bacilluria continued until her death.

On May 20th, the 51st day from the onset of the disease, she had a series of fits and this passed into a condition of status epilepticus and she died in this condition on May 21st.

On post mortem examination her spleen was found characteristically enlarged, the bases of her lungs were congested. The brain was much congested, as is invariable in these fortunately somewhat rare cases of status epilepticus.

The notable feature of the autopsy was a subdural abscess ~~on~~ the right frontal region. This abscess on culture proved to contain pure B. Typhosus. Whether or not this excited the status cannot be confirmed.

This case cannot be quoted as a persistent bacilluria owing to its very short course, but two cases of periosteal abscesses in an epidemic of less than thirty cases is somewhat notable, and in this case the development of the abscess in such an inaccessible spot in so short a time after the onset of the original disease is rather exceptional.

CASE III.

Lily Mary B., aged 19, admitted 29th April, 1911.

She was an imbecile who gave evidence of hallucination on admission. The urine was acid and had a sp.gr. of 1010 with no deposit. Her general physical health was good.

On August 28th, 1912, she complained of pain in the right iliac region, and later of pain in the region of the kidneys. Her urine examination was acid: it contained a large quantity of B.Coli and much pus. On September 6th a small fluctuant swelling developed in the groin both above and below Poupart's ligament. There was oedema of the parieties, and as the abscess increased in size it was opened on September 10th. It was found to contain a large quantity of foul smelling pus. On examination of this pus it was found that the principal infecting organism was B.Coli which proved similar to the one isolated from the urine.

The track of the abscess cavity followed up upon the line of the psoas muscle. The patient continued with a high somewhat swinging temperature, but signs of general peritonitis did not develop. Despite irrigation of the abscess cavity the patient did not improve, and as the organisms isolated from the pus and urine corresponded it was decided to make a vaccine.

Characters of the organism.

It was a small freely motile gram - bacillus, 2 to $\frac{3}{4}$ in length formed acid and gas in the usual sugars and did not liquify gelatin.

Administration of the vaccine.

Fifty millions were given at first and the temperature dropped sharply and the patient rallied somewhat. As the temperature again rose in 36 hours another injection was given this time of 200 millions. It was again followed by a fall in the temperature and some improvement in the pulse. It was feared in an acute case such as this to follow up with a yet heavier injection. Five days later, on the 23rd, the patient sank and died.

Changes in the urine.

After the second injection there was marked diminution in the pus. This however passed off after forty-eight hours.

Blood changes were not estimated.

At the post mortem examination she was found to have cloudy swelling of the heart, liver, etc., with some oedema of the lower lobes of the lungs.

A retroperitoneal abscess cavity was found extending upwards along the psoas muscle and extending downwards and infecting the whole of the pelvic cellular tissue. In the abscess cavity was found a pin, On the posterior surface of the caecum near the origin of the appendix was found an ulcerated surface which while sealed was probably intermittently infecting the abscess cavity. The abscess had also infected a right-sided cystic ovarian tumour, and also the bladder. There was marked acute cystitis and pyelonephritis with early ascending nephritis.

Just before her death her mother mentioned that she had swallowed a pin prior to her admission. The probable sequence, ^{if correct} as far as could be judged was the lodgment of the pin at or near the base of the appendix which was followed by perforation and

a retro peritoneal abscess formed. While the pelvic peritoneum was considerably involved the principal infection seemed to be in the cellular tissue. Whether the bladder or kidney were infected from each other ~~as~~ ^{or} independently, by direct spread is rather doubtful.

CASE IV.

E.S., aged 63, admitted 21st April, 1899.

This old woman was certified as suffering from dementia following melancholia. She was very confused and depressed on admission, but settled down and developed on the lines of delusional insanity. She was originally agitated and much depressed, hearing voices saying she was going to be killed. She early developed a delusion that she was pregnant, and through her increasing dementia maintains this idea to this day.

Her dementia increased until 1906 when she was certified as suffering from secondary dementia. She continues to be so certified.

No history of bacilluria occurs in the notes, but on 12th, 1911, she was taken ill and had a temperature of 104.4; her pulse and respirations were rapid for the first 24 hours, but after this her pulse did not exceed 70 and her respirations 24. She had no physical signs in her heart and lungs, and a Widal done proved negative. She complained however of some pain over the region of the gall bladder and later of pain in the back, and on heavy percussion over the kidneys pain was elicited.

She continued to have an irregular temperature of 103 sinking to 99 and rising again, each cycle of rise and fall taking two days to complete.

Her urine shewed a profuse bacilluria from which an uncontaminated culture of ^a colon organism was isolated.

She had a slight recurrence of her renal pain in December 1911, but after spending a week in bed with slight pyrexia she had no further pains for a considerable time, and in August, 1912,

her urine was free from B.Coli.

On January 16th, 1913, she again had a rise of temperature to 101, with pain over the region of the gall bladder and with pronounced renal pain. An organism, identical with ~~her~~^{her} original organism on culture, was isolated from her profuse bacilluria. A vaccine was prepared and 200 millions of the organisms injected. Her temperature rapidly sank to normal and the patient ceased to complain of pain either in the gall bladder or renal region.

The organism in question produced acid and gas on glucose, mannite, galactose, levulose, lactose and maltose and did not grow on dulcitate, salicin, raffinose, adonite and inosite. It grew a bluish white growth on sorbite gelatin; a reddish growth on glucose gelatin; formed blue black colonies on malachite, agar and red colonies on Conradi Drigalski medium. Morphologically they were short, stout, ^{mobile} organisms

Effects of vaccine.

There was some local reaction lasting for 3 or 4 days after each injection. The injections were made in the interscapular region. Her temperature rose to about 100°F at each injection. *but sank to normal within 36 hours*

Effects on urine.

Just before injection the specific gravity was 1018, the urea was .0009. She passed 62 ozs in the 24 hours. There was a little pus and very many organisms.

After the injection of 200 millions her urine concentrated slightly and the sp.gr. rose to 1022. The urea rose to .0015 and there was some increase in pus. The amount excreted dropped to 53 ozs. however. This shews nevertheless a distinct increase in urea.

On the 5th day after injection an increased amount of urine was passed - 69 ozs. The sp.gr. dropped to 1012 and the urea sank to .001, an absolute increase in urea however over the original amount. The organisms per c.c. sank to 15 millions.

On the 28th January an increase in the organisms having occurred, an injection of 1000 millions was given. This was followed by an increase in the pus organisms and a rise in the sp.gr. to 1015; the urea diminished to .0008. An improvement occurred on the 8th day, but this lasted only two days, so an injection of 2000 millions was given. This caused a pronounced negative phase, and this lasted for fourteen days, during which time the sp gr. varied between 1014 and 1010. The urea sank to .0007 varied between this and .001.

At the end of this time the urine became clear and contained no organisms on microscopic examination. There were a few pus cells excreted and some mucus. The sp. gr. was 1008 and the urea .0006. The amount excreted however was 74 ozs.

This continued for 2 days and then slowly the amount of organisms in the urine increased and although not reaching their full amount before injection they nevertheless reappeared and the patient continues to excrete a varying amount at present.

Effects on blood. (See Table)

The opsonic index was done prior to inoculation and came to 1.2. Later after inoculation during the positive phase it reached 1.9

General conclusions.

Effects on Blood.

(1) The bacteriolytic power was greater than normal blood prior to injection and greatly raised during injection. It sank however nearly to its earlier condition later after injection.

(2) Osmotic power was raised during injection.

(3) Agglutination results negative.

Effects on Urine.

(1) Increase in urea both relative and absolute, especially after first injection.

(2) Slight concentration of urine and increase in solid material, especially mucus and pus during negative phase.

(3) Some increased secretion during positive phase of both water and solids.

(General Effects.

(1) Relief of pain with small doses, but disappointing bacteriolytic effects, and also disappointing effects on urine.

(2) Large doses produced a very long negative phase but tended to produce more ultimate results on the urine; this result however produced the effect of large doses generally viz., a prolonged negative phase and a short positive phase.

Mental effects.

The patient was generally more active and more ready to tell without stimulation about her delusions and also to converse on general matters. This was hardly due to leucocytosis as the highest count during the temperature after injection was under 9000.

General Physical effects.

There was some pain on large doses which however passed off in a day or so. Less pain was caused and far less temperature occurred than during an exacerbation of her chronic bacilluria which had occurred from time to time previously. Her general condition seemed distinctly better during her positive phases.

The notable feature of this case was the way the urine cleared up completely after her original attack and the way it recurred with similar symptoms to those of her earlier attacks, i.e., renal pain associated with pain over the region of the gall bladder.

CASE V.

Susan G., aged 53, admitted 26th May, 1899.

She was admitted suffering from systematised delusional insanity, being very exalted, believing that she was "queen of the earth," etc.

She had typhoid fever in May, 1910, the disease running a normal course. In June 1912 she had a temperature of 103, which sank to 100 and thence to normal in three days. She had marked tenderness over the region of the gall bladder, but no jaundice. The Widal being done, she gave some clumps ~~to~~ the 1 in 40 dilution. Later in repeating the Widal considerable loss of motility of the organisms was observed in dilutions of 1 in 20 and 1 in 100. This did not occur on doing another Widal still later. All attempts to isolate any organism resembling typhoid from either the urine or the faeces failed, although a coliform organism was found to be present in small quantities and was isolated.

In January 1913 she again had a similar attack and an organism culturally identical with the previous organism was isolated. The organism shewed fairly characteristic colon characters.

The interest in this case lies in the fact that some time after enteric fever she had pain and pyrexia suggestive of cholecystitis associated with a curious partial Widal reaction which disappeared later. In the second pyrexial attack no agglutinating organism was available at the moment, and the Widal was not done. Possibly a more thorough examination of the faeces would have revealed the presence of a typhoid organism. The urine though very carefully examined did not grow any organism

resembling typhoid.

Clinically the case resembled case IV (Emma S.) except that the number of organisms in the urine was very slight indeed and intermittent in appearance, while in Case IV there was constant profuse bacilluria.

Joseph W., aged 65, admitted 24th April, 1905.

Mental state on admission.

He was suffering from senile dementia and was partly disorientated. He made numerous accusations against all with whom he came in contact. He believed he was kidnapped for his money. Actually he is a pauper. Physically he was in poor health and had considerable arterio sclerosis.

History. From September, 1912, he commenced to have an irregular pyrexia - 100° to 101° - and on examination he was found to have an acid bacilluria with considerable pus. He became much feebler as a result of this chronic cystitis^{et}, and the irregular pyrexia developed with a nightly temperature.

November 9th, 1912. The urine was found to show a considerable amount of pus, and an organism was isolated.

Characters of organism. It was a freely motile organism consisting of short stout rods. It formed acid and gas in glucose, mannite, galactose, levulose, lactose and maltose. It did not liquify gelatin. Indol was formed.

A vaccine was prepared and 50 millions administered on the 14th November, 1912: but slight improvement was noted although a slight diminution of the pus occurred.

On the 23rd November 100 millions was injected and this was followed in 24 hours by a diminution of the organisms and a lessening of the pus.

On November 28th an injection of 600 millions was given, and this was in 24 hours followed by a diminution of the pus which disappeared in three days time and a total disappearance of the organisms.

These have not reappeared.

He has had no pyrexia since the disappearance of his cystitis.

Urine (See table)

Bacteriolytic changes (see table)

Opsonic changes.

The opsonic index prior to injection was $\cdot 38$, it possibly being so low on account of the asthenic, feeble condition. The index was not done immediately the urine cleared and on waiting a few days it was found to be only $\cdot 65$, which was disappointing.

Agglutination. Changes were negative.

Conclusions.

Dosage of vaccine and effects on urine.

The vaccine was never pushed beyond 600 millions, this causing a disappearance of the cystitis and bacilluria. The injection of 100 millions caused some slight improvement.

It will be noticed that the urea was markedly increased relatively. The amount of urine passed in the 24 hours was not able to be measured owing to the patient's incontinence. The specific gravity fell with the disappearance of the pus, probably owing to *increased* secretion. Some mucus appeared during the injections. Later the amount of urea sank considerably, though not to so low a level as formerly.

Bacteriolytic changes.

Before injection the blood shewed bacteriolytic ^{power} greater than the control although ~~not much greater~~ only to a slight degree

After the 600 million injection the figures were greatly improved and some considerable bacteriolysis was shown.

Five days later the blood shewed no inhibiting effect at all, being remarkably deficient. This may be partly explained by the fact that he was such an *asthenic* old man and partly by the fact that no stimulation to form *antibodies* existed after the

clearance of his urine. This emphasises a point to which all the conclusions tend, namely the transient effect of the ^{vaccines} ~~cystitis~~.

Effects on mental state.

These were difficult to estimate in such an advanced case of dementia, but generally it was noticed that the patient was less dully resistive and more inclined to answer when spoken to.

Effects on physical health.

The nightly pyrexia ceased and has not returned, and the patient's general health is markedly improved, although he remains an exceedingly feeble old man.

(11)

CASE VII.

Thomas C. , admitted 19th July, 1909. 1188

This man was the subject of delusional insanity. He was admitted actively hallucinated and deluded. He believed he was going to be burnt and that "electricity was being put on him;" also that he was interfered with sexually.

The urine had a sp. gravity of 1008 and was acid: he passed 73 ozs in 24 hours: there was no deposit.

For many months during 1911 he was observed to have profuse colon bacilluria with considerable pus in his urine, but the resulting growths were very slight

In ~~January~~ 1913 he developed retention of urine, and this was relieved by catheterisation. Bladder washings were employed and urinary antiseptics given. Ten days later he developed a high irregular pyrexia which lasted for about ten days. It then fell to normal and slight improvement in his general condition was observed. He became however slowly and progressively weaker and latterly became comatose. He passed a decreased amount of urine latterly, but with less difficulty than in the earlier stages.

This case was not treated by vaccines, as it was desired to observe the effect of other remedies. Finally as these appeared to make no improvement the organism was isolated, but before administration could be effected he became moribund and died, two months after his first pyrexia.

Post mortem changes.

He had a dilated and fatty heart, marked chronic cystitis, and pyelo-nephritis with ^{the} slight chronic ascending nephritis characteristic of this condition. From the ^{PELVIS} ~~pelvis~~ of the

V

kidney an organism was isolated similar in its cultural characteristics to the one previously isolated from the urine. This was obtained in pure culture.

In this case the disease was probably a secondary infection from the kidney into the bladder. It is of interest that the renal infection was purely colon. It was bilateral, both sides being equally infected.

This case is included as being similar to Case VI., although it must be admitted that the whole trend of the disease was much more acute. There are reasonable grounds for supposing that Case VI (Joseph W.) would have followed similar lines if vaccine treatment had not been adopted.

CASE VIII.

Stella D. aged 46, admitted 23 December, 1909.

On admission she was suffering from melancholia with suicidal tendencies. She had some visual hallucinations but gave little evidence of these.

Physical condition. The urine shewed some bacilluria on admission, and she complained of various pains in the renal region, with pain especially over the region of the left kidney, and also marked supra pubic pain. Her renal condition became worse, and in July, 1911, she had an attack of haematuria and complained greatly of both renal and supra pubic pain. On August 24th she had much pain and some vomiting. She had an irregular pyrexia, the temperature frequently rising to 100. The temp. charts were however not maintained at all regularly as she frequently refused to have her temperature taken. She continued with much pain during the latter part of 1911 and throughout 1912. No definite evidence of renal calculus was obtainable and the pain was usually worse at night.

On examination the urine was found to contain a considerable quantity of pus and a fairly large number of actively motile coliform organisms. Tubercle bacillus was isolated from the urine by a colleague in July, 1912. This makes it probable that she is a case of tubercular kidney greatly accentuated and aggravated by colon bacilluria. This complicating factor makes accurate estimation of the amount of damage which each of the organisms causes, ^{doubtful} ~~typical~~, but the great amelioration after a successful injection of colon vaccine suggests the colon condition considerably accentuates the other infection.

Isolation of the organism.

The organism isolated was a freely motile gram - short stout bacillus. It fermented glucose, mannite, galactose, lavulose and saccharose. Lactose was not fermented and no liquifaction of gelatin occurred. On milk ~~rapid~~^{inconsistent} results were obtained. On three occasions no change occurred in^{an} apparently thoroughly inoculated specimen, while in two occasions acid and clot with some bleaching and no peptonisation occurred on the third day.

Administration of the vaccine.

The vaccine was prepared and 30 millions administered, but beyond a slight pyrexia and some local reaction there was ~~little~~ little result. Some days later a 100 million injection was administered with little effect on the urine. Nine days later 200 millions were given with some improvement after a short negative phase. Seven days later 800 millions were given, and after a very short negative phase the improvement noted was remarkable. There was a marked diminution of the pus and decrease in the bacilluria. The patient was obviously in much improved health, and the pain, both renal and supra pubic, was practically absent. She had not been so free from pain during the whole course of her disease. This continued for ten days when in the belief that further improvement might be effected with a heavier dosage 1600 millions were administered. This was promptly followed by marked increase of the pus and a return of the pain and other symptoms.

The case was left for over three weeks and then an injection of 600 millions was given. This produced a pronouncedly negative phase, and the pus did not clear for fourteen days. At present the pus is greatly diminished, but she has considerable

pain, most marked however in the bladder.

Bacteriolytic changes (see chart)

The original figures, although not consistent, show that the patient's serum possessed less than normal bacteriolytic power, and though improved by the 200 million injection they did not reach to normal until the 800 million injection was given. In the positive phase following this injection the bacteriolytic power possessed by the patient's serum rose very markedly above that possessed by the control serum. When taken at a later date they were not so good, but showed a great improvement over the original estimation.

Opsonic results.

The opsonic index was .87 before injection and it rose to 2.13 after the administration of 800 million, and sank to .48 after the injection of 1600 millions. This confirms the bacteriolytic results, except that the opsonic fall during a negative phase was much more pronounced than the fall in the bacteriolytic power.

The agglutinating results were negative.

General conclusions.

The general health of the patient was greatly improved after the successful injection, but later effects were distinctly unsatisfactory.

Mentally the patient remains an uncertain querulous individual, and no effects were produced by the vaccine treatment.

In a case associated with tubercle there is undoubtedly a great risk of an exacerbation of the tubercular condition occurring during the colon treatment and unfortunate results are then liable to occur. At the same time the clearing of

the colon condition undoubtedly helps to secure relief to the patient.

With regard to the clearing of the uring without relief to pain, the well known intermittency of pyuria in tubercular infection of the urinary tract must be taken into account.

Case IX

August G. aged 65, admitted 17 September, 1910.

He was admitted suffering from melancholia. He was hallucinated, hearing voices abusing him and threatening him. He was ~~very~~^{very} agitated and depressed and intensely hypochondriacal. Physically he was in fair health. The urine had a sp.gr. of 1024, and was acid.

In January, 1911, he complained of frequency of micturition, pain in the renal region and also supra-pubic pain on micturition. He was found to have a profuse bacilluria, some pus, and a colon organism was isolated.

An exact record of his fermentation reactions has been lost but the results were quite characteristic.

In January, 1911, he was injected with two millions with no result except some local reaction. Six days later five millions were injected, and this was followed two days later by eight millions, and eight days later by 20 millions. A slight improvement occurred in the urine after this, but it was followed by a very prompt relapse. Injections of 50 and 100 millions were given and this was followed with 600 millions. This produced a ~~marked~~^{MARKED} reaction and the urine cleared very appreciably, and to such an extent that only on careful microscopical examination were any motile organisms detected. The pus was reduced to a most imperceptible amount. The urine remained practically clear for about eight months, and after that it was not examined until February, 1913, when some pus and a distinct bacilluria was found to be present. The patient became acutely agitated during the course of the injections and remained so for two months afterwards, when his agitation gradually settled down.

He remains a miserable, hypochondriacal, agitated melancholic in indifferent health. His general health was certainly much improved during the time his urine was clearer.

CASE X.

Ann W., aged 56, admitted 30th November, 1908.

Mental condition on admission. She was admitted suffering from melancholia. She was depressed and surly and had numerous delusions of persecution dependent on aural hallucinations. She believed her food was poisoned and as a consequence at first required nasal feeding. She remained depressed and developed numerous delusions consequent on her hallucinations. She complained of electricity being passed through her body, and of people being under her bed at night.

The physical condition on admission was fair. She passed 42 ozs of urine on admission and there was slight pus present.

History.

On November 3rd, 1911, she went to bed with a slight pyrexia, with increased pus in her urine and a marked acid bacilluria. She had a slight pyrexia for several days, and complained of pain in the renal region. The pain and pyrexia continued until the end of November when although the pus and bacilluria continued the pain became better and the pyrexia ceased. She continued through 1912 until November with bacilluria, despite the administration of urinary antiseptics throughout a considerable portion of this time. In October 1912 an organism was isolated and a vaccine prepared and administered.

Characters of the organism.

The organism was freely motile and fermented glucose, lactose galactose, mannite, levulose and maltose, and (?) dulcific, but did not liquify gelatin.

(See table)

Administration of vaccines and changes in the urine.

200 millions were first injected, and the patient experienced some local reaction with a temperature of 99. Little improvement was noted in the urine. Five days later this slight improvement disappeared, and on the 6th day an injection of 1000 millions was given. This caused a marked increase in the pus and organisms followed by a distinct improvement on the seventh day. On the following day another 1000 million injection was given, and four days later marked improvement occurred, and on the fifth day the urine was nearly clear, 31 colonies appearing on a plate spread with $\frac{1}{100}$ c c. of urine, and on the eighth day cultivation of $\frac{1}{100}$ c.c. proved sterile. During the following month however the pus and organisms steadily recurred.

Bacteriolytic table.

The control blood unfortunately sterilised this organism in all proportions except $\frac{1}{100}$. Originally the bacteriolytic power of the patient's serum was less than that of the control. This was greatly improved after injection and had not sunk to its former level one month later.

The opsonic index was originally 1.87. After injection it sank to 1.36, but as it was taken some days after the most favourable injection it is possible that the decreased organisms in the urine accounted for this fall.

The agglutination results were negative.

Summary.

While only a narrow margin represents the bacteriolytic power it certainly started below normal and concluded distinctly above normal. The patient became brighter and rather better physically but as in most of these cases the improvement was very temporary.

CASE XI.

Ann W - h - d, aged 67, admitted 27th May, 1908.

She was admitted suffering from senile melancholia, and was actively hallucinated and much agitated.

On admission she was passing 80 ozs of urine with a sp.gr. of 1004. She was in very fair health.

History.

She was noted to be in indifferent health in November, 1909, and she spent some days in bed with a slight pyrexia and complained of pains in her back, and a little later was placed on urotropin etc., the results of which were not noted.

During this time her agitation gradually became less and she settled into a condition of dementia, and she was certified as such. In February 1912 she again developed renal pain, and on examination was found to have a profuse bacilluria.

Description of organism.

The organism isolated proved to be an actively motile gram - bacillus which fermented the usual sugars and did not liquify gelatin.

Administration of vaccine.

A vaccine was prepared and an injection of 30 millions was administered. This was followed 3 days later with an injection of 100 million with however little effect; four days later again by an injection of 200 millions. This last produced marked relief from pain, although there was a negative phase extending over two days, during which time there was increase of pus in the urine. But there was later a diminution of both pus and organisms.

The patient was now left alone for over a month, and at the end of this time pus was found to have increased slightly, and the bacilluria to be as profuse as formerly. Reinjection was therefore started on November 26th. An injection of 200 millions being given with little result, was followed four days later with an injection of 1000 millions. This caused increase in the pus and some pyrexia, but on the fourth day the pus was distinctly diminished and the organisms somewhat less. On the 7th day a further injection of 2000 millions was given, and this was followed by a marked negative phase lasting seven days, which was succeeded by the best positive phase so far produced, the urine becoming clear and only a few pus cells persisting. This condition only lasted three days, then the bacilluria increased in amount and the pus began to return, the final condition of the patient being a very slight amount of pus in the urine and a diminished bacilluria.

See Urine and Blood Charts.

There was very slight agglutination produced at 1 in 20 dilution.

Summary.

There was no effect produced except for very slight pyrexia after the 30 and 100 million injections and some local reaction. The 200 million injections produced increased renal activity and increase in urea which persisted after the urine had become clearer. There was an absolute return to the condition before inoculation after leaving the case for one month at this stage.

There was only a slight effect on repetition of a dose of 200 millions, and a marked effect by the injection of 1000 millions. There was a very pronounced negative phase lasting seven days after the injection of 2000 millions, this being

followed by a very good positive phase which practically cleared the urine. This however only lasted three days and there was a steady relapse during the next three weeks, the final state of affairs being better than the condition prior to inoculation.

Effects on the Bacteriolytic power of the blood.

1. Some bacteriolytic power above the normal before injection.
2. Very slight improvement after the injection of 200 millions.
3. Distinct improvement after the injection of 200 millions
4. Less bacteriolysis after injection than before injection possibly due to a decrease in the organisms in her body.

Physical condition.

The patient was much more brisk and active in her habits after injection and appeared in better health.

Effects on mental condition.

These were remarkable. The patient who was admitted in an agitated melancholic condition had for over two years sunk into a condition of dementia and was certified as such. After injection she became excited and much hallucinated and deluded. Her hallucinations were those of sensation and she believed that pigs and insects were inside her. She was much agitated at the idea that her nose was going to be cut off by the medical officer. She still remains somewhat over active, but her hallucinations and delusions are fading again and she is much less agitated.

Margaret W., aged 61, admitted 1st December, 1911.

Mental state on admission. She was markedly demented with a grossly impaired memory. She had hallucinations, believing people were under her bed at night. She was too demented to show much agitation, although she was depressed and liable to become emotional.

Physical state. She had considerable arterio-sclerosis, and her urine showed profuse bacilluria. The sp. gr. was 1006, the reaction acid, and very slight pus was present. A coliform organism was isolated in June, 1912, and again at the end of October.

Isolation of organism and characters of organism. (see table)

The organism was freely motile. gram - and formed acid and gas in glucose, mannite, galactose, levulose, lactose and maltose no liquifaction of gelatine occurred and indol was formed.

Administration of vaccine.

Ten millions was administered on October 3rd, 1912, with no pyrexia and little local reaction. 30 millions was injected six days later also with little effect. Eight days later a dose of ²⁰⁰ millions was injected. There was slight pyrexia and a fair local reaction. Five days later ~~another~~ ^{another} dose of 200 millions was injected. There was some pyrexia and local reaction and slight change in the urine.. Six days later a dose of 600 millions was injected; this produced moderate pyrexia and fair local reaction with ~~some~~ ^{marked} improvement in the urine. A month was now allowed to elapse. ^{then} The urine was found to have returned to its original condition. A new vaccine was now prepared and a dose of 1000 millions injected. This produced considerable pyrexia and marked local reaction. The urine showed increased

pus and organisms for five days. Following this marked improvement occurred. On the third day this improvement ceased to be maintained. Eight days later another injection of 1000 millions was given. After a negative phase of four days some considerable improvement was noted in the urine. This was not so well marked as after the first injection of 1000 millions. (See urine and bacteriolytic tables.)

Opsonic index. The index was 1.57 prior to the injection and later rose to 1.83 after the first injection of 1000 millions.

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Agglutination results. These were negative although carried out after each injection. No trace of agglutination occurred in any dilution (1 in 20 upwards) at any time.

Conclusions. - Dosage and effects on urine.

The effects were negligible with injections of less than 200 million and slight at that figure, although an increase in urea occurred. The first injection of 1000 millions caused after a negative phase a very distinct clearing of the urine, which however contained clumps of bacilli among the deposit.

Bacteriolytic results.

Originally the blood showed more bacteriolytic power than the control. After the 200 million injection some improvement was noted, and after the 1000 million very marked improvement. When tested one month later the figures were a little improved on the original estimation, but while some figures were improved others were worse than before injection.

The opsonic index. - This was originally positive and improved somewhat after injection.

The general physical condition was somewhat improved.

Case XII 3.

The mental condition was hard to judge on account of the patient being an amnesic dement.

CASE XIII.

Annie Di., age 45, admitted 15th December, 1908.

She was admitted suffering from general paralysis of the insane. This ran a typical though somewhat slow course, and she died on the 8th January, 1913. The urine was clear on admission. On January, 1911, she developed haematemesis, and this continued intermittently until the end.

She was found in September, 1912, to have marked bacilluria. This was not treated on account of her general paralysis.

Two very similar organisms were isolated from her urine. Both were gram \pm motile organisms. -

1. A longer form 2μ to 3μ in length by $\frac{1}{2}$ broad. It was freely motile, did not liquify gelatin, and formed slight acid and gas in the usual sugars; it also formed indol.

2. A short, stout, freely motile organism. It formed acid and gas freely on the usual sugars, and did not liquify gelatin.

Post mortem examination.

The brain and general organs corresponded closely to the usual findings in cases of general paralysis. No evidences of tubercle or dysentery were found. Haematemesis was found to be due to dilated oesophageal veins.

The bladder shewed no cystitis. The kidneys were in a condition of granular nephritis with a large excess of perinephric fat. The capsule was thin and stripped in layers. The stellate veins were engorged, and the cortex was narrow. The pyramids were slightly distorted. The mucosa of the renal pelvis was markedly congested.

Summary.

The interesting point in this case ~~was~~ that the bladder showed no signs of infection, while the mucosa of the pelves of the both kidneys was congested, and suggested a low chronic inflammatory change. While the possibility of the bladder infection clearing up after the kidneys had become infected was not lost sight of this case suggests a primary infection of the renal pelvis rather than an infection secondary to cystitis, although its bilateral distribution is against this.

A large number of general paralytics have been found to have bacilluria, probably owing to their generally lowered resistance

CASE XIV.

Alice N. aged 58, admitted 10th September, 1912.

On admission she was very agitated and depressed and required nasal feeding at times. She believed her food was poisoned and heard voices telling her night and day that she was to be burnt. She was very resistive to all attentions and had but irregular control of her organic sphincters,

Physically she was very emaciated and had considerable arterio sclerosis. Her urine was acid in reaction and contained some pus. The urea was 0.0014. The amount of urine was not ascertainable.

History.

She was too dull to complain of pain, and although pus increased in the urine and a marked bacilluria was found, no evidence of other symptoms could be obtained. On January 1st she had an attack of diarrhoea with blood and mucus in the stools. She continued in a very asthenic condition throughout January. Towards the latter end of January her urine was plated out, and two organisms were discovered. -

1. A greatly predominant protein bacillus, which was actively motile. It showed chromogenesis on agar, and fermented glucose, mannite, galactose, maltose and lactose. It produced greenish pigment in alkaline peptone broth. Generally it resembled closely the organisms isolated in case XV.

2. A coliform bacillus.

While the question of inoculation was being considered the patient had an attack of heart failure and died in a few minutes.

Post mortem examination.

1. The heart shewed marked fibroid degeneration
2. The lungs were congested and oedematous
3. The bladder shewed slight cystitis
4. The kidney was horse-shoe shaped, and shewed marked pyelo-nephritis and early ascending nephritis.
5. There was congestion of the lower part of the small intestine and upper part of the large intestine.

The post mortem was interesting in shewing the marked pyelo nephritis which was present compared to the inconsiderable cystitis. The rapid downward course of the disease was probably due to the patient possessing only one kidney. It is also of interest that direct evidence of colitis associated with the other condition was shewn.

This case resembled case XV in two points. -

- 1 - the marked emaciation, which was very extreme.
- 2 - The similarity of the liquifying organisms isolated.

CASE XV.

ANNA MARIA W., aged 51, admitted 18 April, 1912.

She was admitted in a state of acute mania and required feeding and every other attention. She was in indifferent general health.

History.

She settled down into a depressed condition and gave evidence of and expressed delusions of poisoning. These were based on hallucinations. She was actively hallucinated and heard the devil calling her "an old cat", etc. She believed also that everyone was dead. At first she had long periods of agitation and was very restless. Later she settled into a depressed, lethargic and almost stuporose condition.

Three organisms were isolated, the first of which was greatly predominant, -

1. A variety of *B. proteus* which fermented glucose, mannite, galactose, levulose, lactose and maltose. It formed reddish, greenish colouration on agar, a blue colouration in peptone water. This dissolved in chloroform and became pink on the addition of acid. It was very actively motile.

2. A coliform organism.

A vaccine was prepared of the proteus and also of the coliform organism.

Inoculation was commenced with the proteus organism and five injections were given, i.e., 200 millions, 600 millions, 1000 millions, 2000 millions and 2000 millions, at intervals of 3, 5, 7, 10 and 10 days respectively.

The bacteriolytic results were with the methods employed unascertainable as the organism was so prolific that unlimited

growths were obtained in all the various proportions, also the coalescence of the colonies rendered even approximate counting almost abortive.

Effects on Urine.

The results of the proteus injections showed some decrease of this organism in the urine, — with the result that the plating after the 2000 million injection showed a marked diminution in the proteus organism but an increase in the coliform bacilli.

At this stage a vaccine was prepared of the coliform organism, and this was administered in injections of 200, 1000 and 2000 millions.

The effects on the urine are difficult to gauge as the colon injections were given almost concurrently with the protean inoculations. Certainly marked diminution occurred after ten days from the administration of the 2000 million injection, which points to a colon infection being a real part of the patient's disease.

Later a marked recurrence of her bacilluria with marked diminution of the pus however compared to her former condition, at the last moment shows a large amount of proteus colonies with however a few coliform colonies.

Bacteriolytic results towards the coliform organism showed that the blood originally possessed ~~some~~ ^{very slight} increased power towards the colon organism compared with the control blood. ^{improved distinctly better after injection.}

Opsonic reactions.

A opsonic count taken before the injection of the coliform organism showed an index of .94; taken after injection it showed 1.0 exactly. This of course gives no definite information.

Satisfactory slides were not obtained with the proteus.

Agglutination results. These were negative with the coliform organisms. In a freely growing scum forming organism such as proteus it is, even with a centrifuge broth, impossible to obtain any results owing to the large number of clumps.

Summary and conclusions.

Slight improvement occurred in the urine after heavy dosage with B. proteus, and more marked improvement occurred very temporarily after the colon injections, but relapse occurred in a few days. It must however be admitted that the pus was distinctly diminished. A noticeable point was that the urine was acid.

Brown states these cases are usually acid but occasionally alkaline in reaction. (1)

Effects on mental and general physical condition.

She became distinctly brighter after the protein injection and spoke when addressed, which she cannot be induced to do at present. Physically she remains feeble, thin and lethargic. The general results must be noted as unsatisfactory.

The general opinion I have formed of this case is that the protein infection is the real cause of the disease, and that the colon infection is quite subsidiary.

(1) Osler and Macrae, vol., vi. p, 230.

CASE XVI.

Clara J. 57, admitted 29th January, 1900.

She was admitted suffering from alcoholic dementia. She had various delusions including those of poisoning.

Physically she was in feeble health. The urine shewed a sp.gr. of 1012 and was acid, with a deposit of mucus.

She had a marked history of recurrent diarrhoea, very rarely however with blood and mucus in the stools, but always associated with some pyrexia. She had sixteen distinct attacks of this each lasting from five to twelve days. These attacks were spread over a period from 1901 to the end of 1907, ^{but} and in only one of these was blood and mucus present in the stool. In one of these attacks it was noted that she had distinct pain over the region of left kidney. In addition to these attacks she had diarrhoea throughout this period on innumerable occasions. From 1908 to 1912 she had no pyrexial attacks although diarrhoea was frequent. In April 1912 she had another attack of diarrhoea associated with pyrexia, and at this time it was noted that she had pronounced acid bacilluria. She was now in very poor physical health and had advanced cardio vascular degeneration.

An organism was isolated from her urine and found to be a short motile gram - bacillus. It produced a growth on the various solid media employed resembling the B. Typhosus. It however fermented, ^{is formed acid & gas} glucose, mannite, lactose and levulose, but did not ferment lactose, although it formed acid in milk with slight clot after three days. It did not liquify gelatin, and indol was formed. An organism similar in its fermentation tests

although not forming indol occurs in Dr. Houston's report on the bacteriological examination of the Penryn River oysters (1).

In December, 1912, a 200 million dose of vaccine was administered, and this produced a rise in temp. to 103 with marked local reaction. The temp. sank to normal in two days, and five days later the urine cleared slightly. This was followed seven days later by an injection of 800 millions; this produced a temp. of 102, and less local reaction than had occurred before. The urine cleared appreciably on the seventh day, and ten days later an injection of 1600 millions was given. This produced a very marked general reaction, and there is no doubt that the dose was excessive. Pus increased and also the organisms. This lasted for fourteen days when improvement occurred.

Twenty days later an injection of 1000 millions was given, and this after four days produced a pronounced improvement in the urine. It was followed by another of 1000 millions with similar results. The urine improved after three days but again relapsed after being nearly clear for seven days.

The bacilluria never entirely cleared up, although very markedly diminished. There are at present distinctly less organisms than formerly.

(See Urine Chart)

Bacteriolytic results.

These were disappointing owing to lack of vitality of the organism. When mixed in equal parts with serum slight results were obtained, but both the patient's blood and the control blood proved sterile in all other proportions. In the I proportion a thousand colonies developed with the patient's serum while the control serum grew even more prolifically. After the 1000 million injection the patient's serum grew only seven colonies in

(1) No. 13, p. 150, vol. iii, Fourth Report Royal Sewage Commission

proportions of $\frac{1}{1}$ while the control shewed nearly 1000 colonies. When this was repeated later the patient's serum grew 130 colonies on and an increase in the condition after injection and a decrease on the condition prior to injection.

Summary.

The patient was much demented, and it is difficult to say whether she was brighter during the injections. Physically she is in better health than she has been for some time. She has had no diarrhoea for five months, which is a condition she has not been in for over ten years. The possibility exists that this is a coincidence, although it is curious that this should occur. It must be mentioned she was clear of diarrhoea before injection.

Effects on Urine.

During her positive phases the organisms certainly diminished. The urea increased somewhat, but not to the extent that was observed in most of the cases.

Effects on Blood.

Owing to the comparative feebleness of the organism only the $\frac{1}{1}$ test shewed any result, but this shewed marked improvement diminishing later.

Agglutination results.

There was slight partial agglutination at dilutions of 1 in 20 and 1 in 100.

Obsonic results.

Before injection the patient's obsonic index was .74, after injection it increased to 1.3. The slides were however unsatisfactory.

Case XVII

1

Elizabeth L., aged 65, admitted 20th September, 1912.

She was admitted suffering from melancholia. She was restless, agitated and apprehensive. She believed she caused distress to her relatives and brooded over the fact considerably. She believed that the nurses at the Infirmary ill-treated her in attending to her. She was dull, resistive and obstinate.

Physically, she had considerable arterio-sclerosis, was very thin, emaciated, and her lungs showed evidence of chronic bronchitis. Weight 6 stones. Height 5' 1".

She remained very agitated and depressed and gave evidence of aural hallucinations. She developed the idea that she had no feeling in her stomach and at times stated she had no stomach and became very difficult with her food, requiring to be hand fed for a long time.

Later she required constant nasal feeding. She lost weight rapidly, became so restless and agitated that she could not be kept in bed. She has had no natural sleep recently. On March 9th she had an attack of diarrhoea with blood and mucus in the stool. She is a chronic masturbator.

Isolation of the organism.

A mucoid form of B. Coli was isolated. This form grows large heaped colonies rapidly. It fermented the usual sugars and it was a freely motile organism $2\frac{1}{4}$ in length and less than $1\frac{1}{4}$ broad. No liquifaction of gelatin occurred.

Administration and dosage of vaccine.

She was injected with 200 millions on the 19th January, and this was followed with slight pyrexia and some local reaction.

On the 23rd this was followed with an injection of 1000 millions which caused slight pyrexia and some local reaction.

On the 30th, as little improvement had occurred in her urine 1500 millions were injected. There was a slight pyrexia and fair local reaction, and this was followed by marked pus in the urine and an increase of urea and organisms. On February 16th the urine cleared distinctly below the previous level.

(See urine table.)

The opsonic power was not estimated after injection:
before it was 1.21, which is too indeterminate to be of value.

Agglutination results were negative.

Conclusions.

The Dosage of vaccine and effects on urine.

The lower doses had no real effect, and even the injection of 1500 millions had not a great result although the urine cleared somewhat. While after the 1000 a negative phase of 6 days only occurred, after the 1500 million the phase lasted 16 days. Experience had taught me by this time that increasing the dosage after a prolonged negative phase is dangerous, and as the habits of the patient and her general condition were unfavourable to improvement the case was left. The improvement then noticed was considerable, but the case has since relapsed.

The bacteriolytic table shows some improvement, with however some retrogression later.

The patient's general health is bad, partly on account of her mental condition, which is that of the most extreme agitation and restlessness. Her delusions about her food tend to accentuate her emaciated condition.

In this case a reference may be made to the difficulties imposed by the patient's mental state, which militated against tabulated results -

1. Difficulty in obtaining catheter specimens.
2. Inability to measure amount of urine passed in 24 hours.
3. Difficulty in taking blood.
4. Patient's masturbation causing likelihood of reinfection from below.

CASE XVIII.

Patient Emily A. Ch., admitted December 8th, 1911, aged 40.

She was admitted in a state of acute mania, being restless and incoherent. She was troublesome with her food.

She settled down into an agitated restless condition, being hallucinated and deluded: she believed she was to be cut up and burnt, and constantly heard voices telling her she misconducted herself with a man who worked for her brother. She was a chronic masturbator.

Physically she had marked bronchitis on admission and some pyrexia. She had some arterio sclerosis and was generally thin and emaciated. Her weight however was 7 st.13 lbs and her height 5' 1". Her urine had a sp.gr.of 1022, a deposit of and contained many motile organisms.

She continued depressed and required nasal feeding at times. She examined all food utensils carefully, looking for any deposit, etc., and obviously believed she was being poisoned. She was troublesome and resistive to attention frequently.

She continued in feeble health, although her bronchitis entirely cleared up.

Isolation of organisms.

Two types of organism were isolated -

1. A coliform organism not liquifying gelatin, actively motile, and forming acid and gas on the usual sugars.

2. A rapidly liquifying organism which did not form acid and gas and which formed a bluish growth on agar and in peptone. The patient being a masturbator, this was probably contamination and tended to a mixed growth in the urine. But at the same time the constancy of her bacilluria suggests an infection of the renal pelvis with *B. coli*.

Her grossly toxic appearance also suggests some marked absorption and a more serious condition than a mere cystitis.

No attempt has been made to treat her so far, as she is a recent case and came into this series very late.

CASE XIX.

Eliza J., aged 41, admitted 12th December, 1904.

She was admitted suffering from Systematised Delusional Insanity. She had vivid hallucinations of sight and hearing and other senses. She believed she was being tortured and that her frame was being altered. She was dangerous and hostile.

Physically she was in good health on admission, and her urine was clear - sp. gr. 1015, amount passed 51 ozs.

In March 1907 she had some diarrhoea with blood and mucus in the stool.

In November, 1911, she developed the idea that she was constantly being called a paramour, and had various pronounced sexual delusions. She is now a chronic masturbator, is in poor physical health, and is less dangerous.

This case was complicated also by the fact that her urine reduced Fehling, forming that curious canary coloured deposit which occurs in cases that have taken sulphonal and trional in long continued small ~~doses~~ doses.

An organism was isolated which was motile and fermented glucose, lactose, etc., formed acid and clot in milk, and did not liquify gelatin.

Administration of vaccine.

50 millions were first administered and little result occurred. This was followed 9 days later by 200 millions, the result being a marked rise in the urea and some increase in pus: later there was a fall in the urea and a general improvement followed. This was followed 6 days later by an injection of 1000 millions and no improvement occurred until 6 days later when a diminution of pus and organisms was noted. This

continued for about twelve days when the pus and organisms were noticed to be increasing, so another 1000 million injection was given. This time an improvement occurred in four days, but only lasted for ten days when again the condition became worse. The urine still gives a copious growth of organisms although some slight improvement is noted.

The bacteriolytic results were very dissappointing and contradictory. After injection it improved somewhat towards the organism, but a relapse soon occurred.

The opsonic slides were unsatisfactory and the agglutinating tests were negative.

Conclusions. This ~~was~~ a distinctly dissappointing case, but a degenerate paranoic of uncertain disposition and degraded habits is hardly a fair test of any form of therapy.

REVIEW OF COLON CASES.

REVIEW OF THE COLON CASES.

These are considered under the following heads -

1. General considerations.
2. Precautions to be adopted in selecting ^{CASES} and in isolating the organisms. Under this head the difficulties and advantages in dealing with Asylum cases will be considered.
3. Mode of preparation of the vaccine, and the variations and effects obtained with the different methods adopted.
4. The suitable dosage.
5. The results -
 - (a) on Urine
 - (b) on the blood
 - i. Bacteriolytic effects
 - ii. Opsonic ,,
 - iii. Agglutination ,,
 - (c) On general health, including symptoms
 - (d) On mental state.

In reviewing these cases and comparing the results with those of other observers I have come across only two articles which give the results of the experience gained in a series of cases, of the effects obtained by vaccine therapy in colon bacilluria and its allied diseases. These are articles by Prof. Billings of ⁽¹⁾ Rush College, and Dr. Wulff of Copenhagen working under Prof. Rovsing ⁽²⁾.

A number of other references have been traced which however only quote individual cases and generally speaking are indefinite

(1) American Journal of Medical Science, May 1910, p.625

(2) Presse Medicale, p. 97, 9th February, 1910.

about the dosage, the length of time which should elapse between inoculation, and the results.

After having worked for some time at these cases one is bound to be impressed with the general work of both the above-mentioned observers, although personally I have come to the conclusion that in the chronic type of case they tend to be too optimistic as to the permanence of the results obtained.

General considerations.

These fall under three heads ↓

- (1) Acute cases.
- (2) Sub-acute cases.
- (3) Chronic cases.

1. The Acute Cases.

These include ~~sinuses~~^{abscesses} due to B.Coli and acute colon infections in general. In these cases where the spontaneous cure tends to be delayed autogenous colon inoculation has undoubtedly proved of great value.

Only one acute case occurs in this series, and the results of inoculation in this case (No.III) showed relief from pain, lowering of temperature and slowing of pulse, On two occasions when doses of 50 and 200 millions were given. The area for absorption in this case in the pelvic cellular tissue was however so extensive that ultimate recovery would have been miraculous.

2. Sub-acute cases.

By this is meant the exacerbations which supervene at intervals in a number of the cases of chronic infection. In these cases one may fairly say the results of bladder washings, urotropin, etc., are usually very unsatisfactory, while vaccine therapy certainly tends to produce a great improvement though

infrequently inducing a cure, especially in cases of long standing, will reduce an acute exacerbation to the former chronic condition. In these cases it is usually obvious which organ or organs are affected, an active pyelo nephritis becoming superadded to a cystitis or low form of infection of the renal pelvis itself. When this occurs, unless some treatment is adopted, the patient tends to die. This is well illustrated in this series in cases VII and XIV, whereas in case VI, when vaccine therapy was adopted under these circumstances, an aged and moribund dement improved in health, ceased to have a nightly temperature and benefitted in having his bacilluria cured and his urine freed from pus.

In case VII The patient progressed steadily downhill and eventually died although every effort was being made with urotropin bladder washings, etc. His end occurred rather suddenly, or the patient would have been given the chance of vaccine treatment.

Case XIV, although not so acute, also illustrates this point.

3. Chronic cases.

The symptoms in these cases are usually slight, although pain on percussion over the renal region can usually be elicited. Pain in the supra pubic region is exceptional. During mild exacerbations in these cases pain becomes more marked over the renal regions, and supra pubic pain on micturition is frequently added. There is concentration of the urine and increase of pus with, in a number of cases, pyrexia. The condition closely resembles the effects produced by heavy doses of vaccine, except that with the latter the pyrexia disappears in 48 hours whereas in the former, when untreated, the condition takes a week to a fortnight to settle down and pyrexia usually lasts for a number of days. Usually these mild exacerbations settle down without ill

results. The exceptional case becomes a pronounced pyelo nephritis, with occasionally uraemic and more frequently general toxic symptoms developing, and unless treated a fatal issue is more than probable.

Mode of Infection.

This opens up a very large subject which closely resembles the kindred subject of tubercular infection. The literature on this subject is very extensive, and consideration of this hardly comes within the scope of the present work. All authorities however seem to agree that the infection may either be ascending from the bladder or by lymphatic spread from the neighbouring bowel. Posner, Lewin and others (1) have shown that a pathological intestinal wall allows the colon bacillus to pass through, and the bacteria to enter the blood. It is also shown that the virulence of the bacillus is markedly increased in diarrhoea and other intestinal diseases, and this explains why results of such varied grades of infection are obtained, extending from simple bacilluria to marked pyelo nephritis. The post mortem findings in cases XIII and XIV point to lymphatic or blood infection and confirm these views. Case VII seems a clear case of cystitis followed by ascending infection.

Predisposing causes.

In the article just quoted (1) the following causes are quoted as predisposing to colon infection of the urinary tract.

1. Chronic constipation of long standing
2. Injury to the bowel wall.
3. Diseases of the bowel such as diarrhoea and dysentery.

(1) Osler and Macrae, p.239. Vol VI.

My series shewed -

1. 9 cases suffered from chronic constipation.
2. 6 cases have had some or many attacks of diarrhoea associated with blood and mucus in the stools.
3. 2 cases gave evidence of injury to bowel wall.
4. 2 cases showed evidence of chronic cholecystitis with an occasional acute exacerbation, and suggest a similar condition existing to that occurring in some typhoid carriers.

2. Precautions adopted in selecting cases and in isolating the organisms.

Under this head the difficulties and advantages in dealing with Asylum cases are considered.

Advantages.

1. Ease in controlling the treatment.
2. Ease in collecting histories in a well regulated modern institution with exhaustive case books.
3. Ease in following up cases after treatment.

Difficulties.

1. Difficulty in properly examining cases whose mental state is excited.
2. Difficulty in obtaining catheter and blood specimens during an excited phase in the patient. This also militates against ureteral catheterisation and cystoscopic examination.
3. X-ray apparatus is not supplied to most asylums.
4. Difficulty in getting the amount of urine passed in 24 hours measured.
 - i. Owing to defective control of sphincters.
 - ii. In women a mixture of faeces and urine in using bed pan.
 - iii. Difficulty in inducing cases which are not in bed to use utensils.

All authorities concur in emphasising the importance of isolating the organisms in pure culture, preferably by ureteral catheterisation. This has been impossible in this series, but a constant recurrence of the same organism in either pure culture or greatly predominating in specimens obtained by ordinary catheterisation have been made to take the place of the more satisfactory method.

The following points need careful attention:-

(1) Recognition of cases of calculus

Wulff favourably reports on a case of treatment with vaccines in renal calculus.

(2) Recognition of tubercular cases. This certainly needs recognition as while great improvement is wrought in the patient's condition when superadded colon infection is ameliorated or cured, great damage may be caused by a heavy dose of colon vaccine given in a pronouncedly negative tubercular phase with the result that a gross exacerbation of the latter condition may be precipitated. Case VIII illustrates this point.

Billings lays great emphasis on adequate drainage of the urinary tract. This is a question which principally occurs in men, but in cases of obstruction of the urinary tract above the bladder the matter involves both sexes. He believes that good results cannot be obtained without attention to these points.

The points he looks for are -

1. Renal calculus
2. Sacculation of the renal pelvis.
3. Obstruction to the ureter either by kinking or from pressure from without.
4. Sacculation of the bladder.
5. The usual urethral causes of obstruction in men, enlarged prostate, stricture, etc.

III. Mode of Preparation of Vaccines

and variations in effect obtained with methods adopted.

In view of the work of Semple and Harrison on the varying nature of the toxicity and potency and also the lasting nature of their immunising power in typhoid vaccine according as to whether they were sterilised by heat or by an antiseptic mixture, various experiments were carried out with B.Coli.

At first the vaccines were sterilised by putting them in a paraffin oven for one hour at 60° C. and repeating this 24 hours later. The vaccines prepared in this manner proved highly toxic and produced a high temperature and much local reaction, irrespective of their immunising efficiency. Later this was modified, and the method adopted was to place the vaccine in the oven for half-an-hour, reliance being placed on adding .5 % pure carbolic. Later still the heating was abandoned and reliance placed on the ~~heating~~ antiseptic only.

A number of control injections were done on healthy cases with no bacilluria, and it was found that with -

- 1 - vaccines prepared from the same culture, half of which was sterilised by antiseptic and half by heat that while the former produced no reaction at all beyond a slight local reaction, that sterilised by heat produced a temperature of 100.6, a pulse of 106, and general malaise. Doses of 25 million were given in each instance.
- 2 - A moderately toxic vaccine sterilised by heat produced nearly the same local and general reaction in 25 million doses as in 200 to 500 million doses.
- 3.- That a vaccine prepared with antiseptic which, when injected into its own infected case, produced a remarkably severe reaction, when injected into a healthy person produced little or no result beyond slight local reaction.

Professor Billing states that the reaction of the patient gives an indication as to the specific nature of the organism injected. I would qualify this by saying that -

i. General malaise, high temperature, quickening of pulse, and marked local reaction, may be produced by overheating the vaccine, this occurring even when a small dosage is given and being irrespective of the immunising power.

ii. Increased renal pain and supra pubic pain with increased pus and organisms in the urine generally indicate that the causal organism has been injected. A kindred organism, when injected in a case of bacilluria, causes however similar symptoms.

In all cases of bacilluria the urea was observed to rise on injection with their own organism and also it must be admitted to a fair degree with kindred organisms. Cases without bacilluria injected with colon vaccines showed no such rise in their urea. The figures in four cases in which this was done proving the urea to be practically unaltered.

Billings recommends preparation of fresh vaccines for second and subsequent injections, and claims improved results with this. My experience has shewn me that the less result is usually obtained with a repetition of the same dose; but that this is much more marked in cases in which heat has been employed.

I conclude -

i. Fresh vaccines do shew an improved result. I was accidentally compelled to investigate this point owing to the supply of the first vaccine being exhausted.

ii. Preparation of fresh vaccines is more important with those which have been greatly heated. This is confirmed by Semple's work in relation to typhoid, as he found that the immunising power of heated vaccines deteriorated much more after three months than occurred with vaccines sterilised with heat after a year.

IV. Suitable Dosage of Vaccine.

In considering this question, I assume that little or no heat has been employed in sterilising the vaccine.

In my earlier cases I spent a long time in injecting minute doses which produced little or no result on the urine, although, owing to the heat employed, in sterilising the vaccines, a fair amount of local and general reaction was obtained. I must admit that in one case the injection of 2 millions followed by 5, 8 and 20 millions at short intervals did produce a slight transient result on the urine. ^{Case K} After giving what I now regard as hopelessly inadequate doses I found that 200 millions was the least dosage that produced any reasonable effect, and doses of 500 millions in most cases did good even as an initial dose.

Having slowly and with much trepidation discovered what was a reasonable and initial dose for chronic cases investigation was continued as to what constituted the upper limit of doses. In some cases trespass beyond 1000 million proved disastrous, this being marked in two cases. In other cases, however, 2000 millions were quite well tolerated, although a prolonged negative phase always resulted. Doses greater than this always resulted in a prolonged negative phase without any corresponding positive phase later.

In three cases, in which more acute symptoms were present, 200 millions was followed by relief from pain and a lowered temperature. 600 millions caused much relief after some negative phase which was not prolonged in two other instances.

1000 millions always caused some prolonged negative phase, usually with a pronounced positive phase following. In two cases, ~~while~~ 2000 millions while producing a marked negative phase with considerable increase in pain, in brief, producing

the same state of affairs found in an exacerbation of the disease, was followed by a better positive phase than after the smaller injections.

In the ^{only} acute case taken up 50 millions followed 5 days ~~it~~ later by 200 millions produced relief of pain, a lower temperature and slowing of the pulse rate.

My conclusions are -

1. In cases with marked pain and temperature 2000 millions will cause relief from pain with a fall in temperature and pulse rate, usually within 24 hours.

2. Most chronic cases tolerate 600 millions well with however a temporary exacerbation of symptoms.

3. A thousand million will in many cases, after a lengthy negative period, produce a positive phase with diminution of pus and organisms. 200

4. 2000 millions will also do this in some cases. This however does not occur in other cases, The expected positive phase not occurring.

In the only series of cases which I have traced that report favourable results are those of Wulff and Billings. Both these observers give their dosage of from 200 to 500 millions for initial doses, and stop at 1000 millions for their largest doses.

Wulff gradually worked from the small doses recommended in the English literature on the subject, and he expresses his opinion of the utter inadequacy of these. His final conclusion is that no harm results from an initial dose of 500 millions.

Billings simply states that he starts at from 200 to 400 millions and rises to 1000 millions. While working at this

subject, I have been much impressed with the fact that this is the correct view.

(1)
Various books recommend much smaller doses. Emery gives 10 to 40 millions as an initial dose (1) and states that 250 millions should not be exceeded. He however refers to the more acute cases which undoubtedly do not need such heavy doses. But with the elimination of the toxicity which occurs from heating, it seems certain that his figures may be exceeded without fear.

Basanquet and Eyre (2) quote 2 to 5 millions as the ^{INITIAL} dosage but Dr. Eyre, in casual conversation, which should not perhaps be quoted, expressed no surprise at the dosage I have given, when applied to chronic cases.

Allen (3) gives ten to 25 millions as the limit dosage, but in the only case he quotes from his own experience he mentions no good results were obtained until 500 millions were given.

Hicks (4) quotes no definite doses in reference to a case of pyeolitis of pregnancy. His results were disappointingly transient.

Gray (5) quotes a case with no definite dosage, except such as can be deduced from the weight. He gives improvement of a somewhat theoretical nature namely reduction of bacteria from 30 millions to 1 million per c.c.

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- (1) Emery, Immunity and Vaccine Therapy, p. 394.
 (2) Basanquet and Eyre, Serums, Vaccines and Toxines, p. 320.
 (3) Allen, Vaccine Therapy, 4th Ed. p. 242.
 (4) Hicks, British Medical Journal, 1909 1. 203.
 (5) Gray, Lancet, 1906, 1. 1102.

Thomas R. Brown (1) quotes three cases he himself treated with autogenous vaccines. Two of these were cases of infection with B. proteus, and one of colon infection. The dosage he quotes is from 150 to 500 millions, given "once or twice a week". His results, he states, were inconclusive.

Wright and Reidd (2) quote doses of 100 to 200 millions, in a case of persistent sinus after an operation on the gall bladder. Their results were entirely successful.

Emery quotes doses of 10 to 40 millions as an initial dose, and states that doses exceeding 250 millions should not be given. With this dosage he obtained remarkably favourable results in a case of acute cystitis. (3)

(1) Thomas R. Brown. Osler and Macrae, Vol. XI. p. 265.

(2) Wright and Reid, Brit. Med. Journ. 1906, i, 43.

(3) Immunity and specific therapy, p. 395.

Summary of Results on Urine.

These may be summarised as follows.

1. One case (VI) cured, Pus and bacilli disappearing entirely.
2. One case (IX) nearly cured of pus and organisms, with no appreciable relapse for over six months.
3. One case (~~X~~) temporarily cured of organisms, with a slight gradual steady relapse.
4. ~~Four~~ cases (IV, ~~X~~, XI, XII and XVI) shewed distinct improvement, but with considerable tendency to relapse directly the the injections ceased.
5. Three cases (XV, XVII, XIX) shewed very ^{transient or but} slight improvement. Two of these cases were chronic masturbators, and therefore it may not be altogether the fault of the method. Case XV was an infection with a protein organism, and this did not shew much improvement.
6. Case VIII, that in which the colon infection was associated with tubercle, shewed marked improvement on colon injection, followed by a sharp relapse on a heavy dose being given, and thereafter failed to show similar adequate improvement after reinjection with smaller doses. This was probably due to the second injection corresponding with a marked negative tubercular phase.

Changes in the urine during inoculation.

These were interesting as during inoculation in a pronouncedly negative phase the urine became concentrated and the pus and organisms increased in number. One point noted was the marked increase in the urea, which occurred especially after the first injection. This occurred with a case of bacilluria injected

with non-autogenous colon vaccine, but did not incur in three cases with no bacilluria which were injected with colon vaccine. When the urine cleared in a positive phase the proportion of urea still remained high.

Blood Changes.

1. Six cases (I) IV, VII and XI, XII and XVI) showed distinct bacteriolysis before injection compared with the control blood. Three cases (X, XVII and XIX) shewed far less bacteriolytic power than the control blood.
2. All the organisms shewed considerable improvement during injection, but a great tendency to relapse quickly shortly afterwards.

An exception must be made in cases XVII and XIX which shewed marked absence of bacteriolytic power, never reaching the degree shewn by a control serum, although they slightly improved during injection.

The marked loss in bacteriolytic power in case VI after the disappearance of the bacilluria was remarkable.

Opsonic power.

In ~~four~~^{three} cases the opsonic index was markedly positive prior to injection, viz., I X and XII. In ~~two~~^{one} cases (VI) it was markedly diminished; in three cases it was indeterminate. A complete series was not done owing to the difficulties with these organisms, and the results generally were variable.

Agglutination results.

These were disappointingly negative. In only two cases was anything approaching agglutination obtained, viz., in cases IV and XVI. In case IV partial results were obtained in a 1 in 20 dilution, and in case XVI partial results were obtained in dilutions of 1 in 20 and 1 in 100.

Effects on General Health.

1. Before injection.

All chronic cases, apart from those in which exacerbations occurred were people in indifferent health. Without being able to point to any particular trouble, except a dull pain over the renal region which varied greatly, not one of these cases could be described as being in robust health.

I was much struck in reading an article by Mr. Lane (1) to notice the close resemblance of a number of his cases to the condition present in the general health in my series. He described cases showing pigmentation of the skin and marked aesthesia often with evidence of incipient or marked chronic Bright's disease. Several of my cases showed distinct resemblance to one or more of these conditions.

These cases of bacilluria are usually associated, as previously noted, with chronic constipation, and I firmly believe that if the intestinal stasis was relieved in these cases the improvement under vaccine therapy might be permanent instead of temporary.

2. During inoculation in a marked negative phase renal pain was pronounced and frequency of micturition and supra pubic pain occurred in a few cases in fact the clinical picture exactly resembled the exacerbations most of these cases had shown at different times. When the urine began to clear up at the beginning of a positive phase it resembled the clearance which in some cases had followed recovery from the acute attack.

The points in favour of the vaccine were that in proper doses the positive phase produced better results than a natural attack usually did, and the general condition of the patient was not upset for so long a time.

3. After inoculation, during the positive phase the well-being of the patient was most marked. This was especially noted in case VIII whose general condition had not been so well for years.

Effects on Mental State.

A number of cases were so demented that no effect could be produced; at the same time a distinct improvement was noted in cases IV, VI, X and XV, although their dementia was very considerable. Cases XI and IX were somewhat remarkable. In case XI the dementia had so far progressed that she was no longer certified as melancholia but as a secondary dementia. The patient became actively hallucinated and very agitated, and her condition approximated to that existing on admission four years ago. In case IX the patient had never ~~fallen~~^{SUNK} into an advanced state of dementia, but had settled down into a quiet, brooding, melancholic condition. After injection he became intensely

restless and agitated. It may be argued that this stimulation of the mental condition was due to pronounced leucocytosis, but in a number of cases the leucocytosis was done during the pyrexia following injection and the leucocytosis was not found to exceed 9,500 in any case. It usually ran at about 8,000. Added to this the stimulation of the mental condition continued long after any leucocyte rise due to the reaction after injection must have passed away.

The conclusion I am inclined to draw is that a temporary lifting of the profound toxæmia which is present in these cases must have occurred.

A curious feature of the mental condition in practically all this series was that all the patients were of the melancholic type, most of them before dementia supervened were agitated and had marked persecutory delusions; a considerable number of them refused their food on account of ideas of poisoning, etc.

Another curious feature was the great proportion that had hallucinations and delusions of the sexual type. This presumably comes under the head of hallucinations founded on a physical basis.

General Summary of Results.

1. Relief of pain and lowering of temperature after injection in the acute case, No III.
2. Cure in one case of ? Pyelonephritis and Cystitis (Case VI)
3. Relief of pain, reduction of temperature to normal, and diminution of bacilluria in two chronic cases which had sub-acute exacerbations of their condition (Cases IV and XI).
4. Five cases, Nos. IV, X, XI, XII and XVI, showed such marked decrease in bacilluria that at one time in their course the bacilluria was not apparent on naked eye examination. A slow and gradual but steady relapse occurred in all these cases.
5. One case (IX) relapsed very slowly after the bacilluria had been reduced to a negligible degree.
6. In case VIII where colon and tubercular infections were associated very marked amelioration of both signs and symptoms occurred after one of the injections. The case did not prove so satisfactory on further treatment, probably owing to the tubercular phases not being carefully considered.
7. Three cases improved very slightly, and a very complete relapse occurred in a very short time. These cases were unsatisfactory on account of the general mental and physical condition of the patients.

One of these three cases was a case of proteus infection.

Comparison with some of the results of other observers.

In considering this subject distinction must be drawn between acute and sub-acute cases of short standing and chronic cases of long standing which show occasional sub-acute exacerbations. Prognosis of the former is naturally far more favourable. (1)

Billings (1) only quotes three actual cases, although he states he hopes to publish a detailed account of a series. This second paper has not been traced. The cases quoted are -

(1) A case with haematuria which was operated on in view of the possibility of tumour. A congested state of the mucosa of the pelvis was found and colon infection proved on examination. This case was cured after the administration of a vaccine.

(2) A case associated with uraemic symptoms injected with 400 millions every seventh day for about two months with absolute cure resulting.

(3) A case associated with tubercle which was injected with 500 millions and was colon free after the third injection. Tubercular injections were carried on synchronously. Results

Results with chronic cases are not quoted.

Wulff (2) quotes two sub-acute cases fully -

i. the case of a girl aged 12 with advanced cystitis and pyelo nephritis, treated with vaccines with complete cure in one month.

ii. the case of a man aged 47, in which bacilluria was associated with haematuria absolute cure resulting from treatment

(1) Billings, loc.cit.

(2) Wulff, loc cit.

He also quoted that four cases of pyelitis were cured.

He summarises as follows -

"Ces 7 malades ont été incontestablement guéris tous les symptômes de maladie ont disparu".

His summary of his full results is as follows -

"Sur ce nombre de 23 malades il y en a 7 qui incontestablement ont été guéris et dans 11 cas nous avons observé une amélioration sensible consecutive au traitement par le vaccin".

I quote this fully as Allen (1) in his book on vaccine therapy states (The best recorded results are those of Wulff who claims to have cured eighteen cases out of twenty-one, the other three not being much benefitted." This was followed by a reference to the Press Medicale 1910, p. 97, from which the previous quotation was taken bodily. The difference in results between ^{this statement &} seven cures in more acute cases and "appreciable improvement" in eleven chronic cases in which Wulff admits he has had no absolute cures is considerable.

In view of the temporary improvement generally observable in chronic cases, the view taken of what constitutes improvement is a most important matter. That Wulff has been persevering is obvious as he quotes one case which was "relieved" after 18 injections extending over three months. Most patients would not stand this amount of treatment in view of the reaction, temperature and malaise which is most commonly induced.

Referring to Wright's method of counting vaccines Wulff states "Il devient possible de doser le vaccin avec une précision absolue". The above quotation seems rather to suggest that Wulff is inclined to describe effects in an optimistic spirit.

Later Wulff abandoned this method of estimating the dosage in favour of estimation judged from the density of the emulsion. Wulff refers to a series of ten cases read by Schneider at the second Congress of Urology, April, 1909. He adds that the results were favourable. This reference has not been followed up. Other references found referred mainly to isolated cases and few definite results were quoted. Among these may be mentioned references to the cases of Hicks, Brown, and Gray, which have been previously referred to. In the last mentioned case the reduction of the bacilluria from thirty millions to one million per c.c. is quoted as a favourable result. This seems rather a theoretical improvement.

I may perhaps conclude by giving the lines along which I believe further treatment might be followed. -

1. Endeavours to cure the intestinal stasis either by short circuiting of the colon as recommended by Lane⁽²⁾ or by other methods.

2. Injection of live organisms into a sterile abscess cavity produced by a chemical agent as done by Lewis Bruce in a case of streptococci.

3. Injection of attenuated organisms as is being done with the *E. Typhosus* by Castellini and others (1).

In this connection it may be said that a number of the vaccines employed shewed distinct motility of the organisms when examined in a hanging drop preparation, although no colonies developed when 1 c.c. of the emulsion was cultivated.

(1) *B. M. J.* March 1913 ?

(2) *Lancet* 21-12-1912

. The local reaction following injection of these in heavy doses was never so marked as occurred with a 25 million injection of an overheated into a normal subject.

The reason I suggest the above lines of treatment as future possibilities is the inefficiency of present methods to entirely remove the disease in the bulk of cases; and the above mentioned indications might by removing the cause and greatly increasing the immunising power produce permanent cures in a large number of cases.

Advance^{ly} along the lines of improved methods of local application such as instillation of the renal pelvis with local applications by means of a ureteral catheter may probably also help in obtaining successful results.

Order The Res Vol V 1 P 264

S U M M A R Y O F R E S U L T S

(a) Typhoid

(b) Colon

C O N C L U S I O N S .

Summary of Results.

I. The case of ? Typhoid Bacilluria with chronic Periosteal abscesses which gave pure cultures of the same organism.

Results of administration of autogenous vaccine -

i. Clinical effects -

- a. The rapid drying up of the chronic ~~abscesses~~ abscesses which contained the organism in pure culture.
- b. Cessation of ? typhoid bacilluria after its continuance for nearly ten months.

ii.

Effects on Blood.

- a. Bacteriolytic power increased very greatly towards patient's own organism, and also to a stock strain on B.typhosus.
- b. Increase in opsonic power which however was positive originally.
- c. Agglutinating power greatly increased towards patient's own organism, and also towards the stock strain of B.typhosus.

The Widal reaction was totally lost $3\frac{1}{2}$ months after injection after being present towards the stock strain for ten months prior to injection.

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7. Three cases improved very slightly, and a very complete relapse occurred in a very short time. These cases were unsatisfactory on account of the general mental and physical condition of the patients.

Conclusions.

I. Colon vaccines should be sterilised as far as possible by the addition of .5 % carbolic or other antiseptic in sufficient quantities in relation to the strength of the emulsion, and not by heat. More results in the way of pyrexia and malaise ~~having~~ were induced in a healthy case by the injection of 25 millions of a vaccine prepared by heat than were induced with the injection of 2000 millions which was sterilised by carbolic into a case of bacilluria.

II. Acute and sub-acute cases of infection by B.Coli experience relief from pain together with fall of temperature and slowing of the pulse rate when moderate doses such as 200 million are given.

III. Doses of 500 to 1000 millions can usually safely be given. In some cases 2000 millions while producing a lengthy negative phase later produce a better positive phase than is obtainable with smaller doses. 200 millions should not be exceeded as an initial dose.

IV. In dealing with mixed tubercular and colon infections of the kidney, great care should be taken to follow the tubercular as well as the colon phases.

V. Pyelo nephritis and cystitis can be cured or relieved by vaccines in cases which resist other treatment.

VI. Chronic bacilluria of long standing can be improved probably only very temporarily, but the acute exacerbations of these cases can be readily brought back to a chronic and comparatively innocuous condition by this method .

VII. Increased bacteriolytic power can be induced in most cases but there is a marked tendency for this to be quickly lost, although a considerable time is taken for it to return to its former condition.

VIII. The tendency for the urinary condition to relapse is probably due to

1 - the evanescent effects of vaccine injection in colon cases.

2. The existence of the original cause, which in many cases is chronic intestinal stasis.

Finally, Emery's⁽¹⁾ well considered opinion may well be quoted:-

"Vaccine treatment cures all the symptoms and reduces the pus and bacilli present in the urine to a small fraction of its original amount, but fails to remove them entirely".

I will only say in regard to this opinion that while greater results follow larger doses than he recommends, these are but evanescent in effect and in cases of long standing the observer will usually be disappointed with the ultimate state of affairs.

(1) Immune & Specific Therapy P 394

Enclosed with thesis are

1. A set of growths of actual organisms, with slides.
2. Charts (a) Chart of Urinary changes
(b) Chart of Bacteriolytic changes
(c) Chart of characters of organisms.

In conclusion I may say every effort has been made to verify the results contained in the chart on the biological characters of the organisms met with. Discrepant results have at times been obtained, but when these occurred the organism has been re-tested many times and the ultimate results recorded. In working outside of an organised bacteriological laboratory enormous difficulty is experienced in keeping alive and uncontaminated a number of organisms for a considerable period. It is believed this has been done, but failure may have occurred. Reliance has principally been placed on the gelatin slope cultures in preserving the organisms from dying out.

In Case I, the typhoid organism, it is particularly requested that if marked discrepancy occurs between the description and the specimen sent that a chance may be given me of re-testing the cultures I have and sending up another specimen. The same applies in a minor degree to the other organisms sent.

I am much indebted to Dr. Stansfield, the Medical Superintendent of the London County Asylum, Bexley, for granting me permission to use these cases.