T H E S I S

FOR THE DEGREE OF M.D.

Presented by

Ralph Paterson Smith M.B., Ch.B. (1918), D.P.H. (1922).

1 9 2 7.

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

T Y P H O I D C A R R I E R S.

An investigation in the Pathology and Bacteriology of the typhoid carrier condition based on the study of eight cases of the disease, personally observed in Glasgow and District from 1921 to 1926 together with some observations on the therapeutic and economic aspects.

****

T H E S I S

FOR THE DEGREE OF M.D.

by

Ralph Paterson Smith M.B., Ch.B.(1918).

1 9 2 7.

****

INDEX OF CONTENTS.

INDEX OF CONTENTS CONTINUED.

ILLUSTRATIONS.

ILLUSTRATIONS CONTINUED -

Fig. 16. Magnified 1000 times opposite page 393. B.bulgaricus from milk two days old.

Fig.17 and Fig. 18. Magnified 1000 times opposite page 398.

Smears from faeces of carriers fed with B. acidophilus milk to show abundance of Gram positive aciduric bacilli.

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

This work was carried out under the supervision of Professor C.H. Browning on behalf of the Medical Research Council at the Pathological Department of the Western Infirmary, Glasgow. My thanks are due to the co-operation of all the members of the Staff particularly Professor Browning for his guidance and help. Part of the examinations were made by Dr. Guthrie from 1924 onwards, to whom my thanks are also due. The invaluable co-operation of the Medical Officers in the various Institutions enabled the investigation to be carried out from the therapeutic aspect and I beg to record my thanks to Mr. Farquhar Macrae, Dr. G. Paterson, and Dr. W. Whitelaw, The his tology was carried out under the supervision of Professor Stuart McDonald, Durham University, College of Medicine, Newcastle-upon-Tyne, to whom I am also indebted.

The Bibliography has been placed at the end of each chapter for convenience.

****

Chapter I.

Historical review of the carrier condition in enteric fever.

The importance of typhoid carriers to the community has been emphasized by numerous investigators since Koch's historic address in 1902 when he suggested the possibility of the typhoid bacillus leading a saprophytic existence in the intestinal tract. But it was not till 1904 that Drigalski recorded the first chronic carrier traced from convalescence onwards also the first female chronic carrier who gave no history of having passed through an attack, and not till 1905 that Lentz recognised the existence of persistent paratyphoid excreters. While the occurrence of typhoid infections have been greatly reduced by improved social conditions such as modern sanitation and prophylactic vaccination a considerable number of cases of typhoid fever still occur and their source in many cases may be traced to carriers, who appear to be principally responsible for maintaining the disease in the periods between epidemics. It is estimated by various observers that from 9 to 55 per cent. of present typhoid infections are caused by carriers. Meyer (1921) states that 6 per cent.of the cases of milk-borne typhoid fever from 1915 - 1918 inclusive have been traced to carriers while Garbat

estimates that 55 per cent. of all typhoid cases are due to persistent excreters. In U.S.A. typhoid fever is the ninth highest cause of mortality and the fifth among Infectious Diseases. According to Gay (1918) there occur 150,000 cases yearly from which he estimates some 7,500 carriers are produced. From 0.1 to 0.8 per cent. of the general population would thus seem to be carriers. Prigge (1910) reported 0.29 per cent. out of 10,841 healthy individuals; Müller (1917) 0.8 per cent. of 20,019 persons in 1916 and Nichols (1922) states that not less than 0.1 per cent. of 30,000 food-handlers in the U.S. Army during the Great War were found to be chronic typhoid excreters. From a summary of over 1700 convalescents from typhoid reported by various observers between 1905 and 1910, Gay (1918) concludes that continued typhoid excretion largely ~~thr~~ through the faeces may be anticipated in from 4 to 5 per cent. of all recovered cases. McCarthy and Simmons (1924) found 4 chronic typhoid and one paratyphoid B carriers among 84 convalescents all of whom had received triple vaccine, previous to their infections.

In a survey of the findings of various investigators approximately 4 or 5 per cent. of all acute cases become chronic intestinal excreters. The following table gives the results of some of the more recent investigators.

Kayser (1906) - 5.0 per cent.
Park (1908) - 5.0 per cent.
Kircher (1908) - 5.0 per cent.
Frosch (1908) - 2.47 per cent.
Hässch (1909) - 4.62 per cent.
Fornet (1910) - 0.90 per cent.
Mayer (1910) - 4.0 per cent.
Vincent and Murilet (1917) - 1.0 per cent.
Garbat (1922) - 2.4 - 4.2 per cent.
Gay (1922) - 4 to 5 per cent.
McCarthy and Simmons (1924) - 6.0 per cent.

These figures alone are sufficient justification for the present investigation.

Classification of typhoid carriers.

An arbitary classification has been made into temporary, chronic, and paradoxical excreters; those who continue to excrete the specific organisms after the acute attack has subsided for periods less than three months being regarded as temporary; for periods over three months onwards as chronic and those who excrete the organisms but have never suffered from the disease as paradoxical. More recently Garbat (1922) and some of the American investigators have differentiated carriers into two further groups, namely Intestinal and Biliary. In the case of the former they claim the organisms to be of purely intestinal origin, and not derived from an infected gall-bladder, and in the latter to be derived from the gall-bladder or bile ducts in the Liver. The latter group is sub-divided into Gall-bladder (bile) carriers and Liver or duct (Hepatic) carriers.

The gall-bladder carrier is the variety most

commonly met with and many workers (Nichols etc.) including the author doubt whether true intestinal varieties ever occur at all unless of the temporary type. The three cases of the author's series submitted to operation (cholecystectomy and cholecyst-gastrostomy) were apparently of the gall-bladder variety. Garbat states that in intestinal carriers typhoid colonies are found in great numbers on every plate of media or even in pure culture, whereas in "Bile carriers" the culture media plates show the presence of typhoid colonies in very small numbers (1 to 75 B. coli) but that the final diagnosis of the latter type is possible only by Duodenal culture and not by faeces culture.

It would appear to the author that any intestinal infection in chronic carriers is secondary to the biliary infection. Cases which fail to be cured by Cholecystectomy are probably due to infection of the bile ducts with the liver.

BIBLIOGRAPHY.

Drigalski (1904): Centralbl.f.Bakt. Abt. orig., XXXV, p. 776.

Fornet (1910): Münch. Med. Woch., 1910, 57, p. 218.

Frosch (1908): Klin. Jahrb., 1908, XIX, p. 537.

Garbat (1922): Monos. Rockefeller Inst. No 16., May 10th 1922.

Gay (1918): Typhoid Fever, New York, 1918, p. 128.

Hitsch (1909): Centralbl.f. Bakt., I, abt. Ref., 1909, XLIII, p. 161.

Kayser (1906): Arb. k. Gandhtsamte, 1906, XXIV, p. 176; ibid, 1907, XXV, p. 223.

Kircher (1908): Klin. Jahrb., 1908, XIX, p. 471.

Koch (1902): Die Bekämpfung des Typhus, Nov. 1902.

Lentz (1905): Klin. Jahrb., XIV, p. 475.

McCarthy and Simmons (1924): Journ. Lab. Clin. Med., 1924, IX, p. 513.

Mayer (1910): Centralbl.f. Bakt., 1910, 53, p. 234.

Meyer (1921): Journ. Infect. Dis., 1921, 28, p. 381.

Müller (1917): Münch.Med.Woch., 1917, 64, p.1.

Nichols (1922): Carriers in Infectious Diseases, Balt., 1922, p. 45.

Park (1908): J.A.M.A., 1908, 51, p. 981.

Prigge (1910): Klin. Jahrb. 1909, XXII, p. 245.

Vincent and Murilet (1917): Typhoid and Paratyphoid Fever, London 1917, p. 66.

Chapter II.

Histories of the Enteric Carriers concerned in the present investigation.

In all, eight cases were investigated. Of these five proved to be chronic intestinal B. typhosus excreters, two B. paratyphosus B, and one a mixed excreter of B. typhosus and B. paratyphosus B. (See Table I). (The asterisk opposite name indicates insane carrier).

Case I. Mrs. M. Griffen (Mrs.G.), a woman aged 76 years, contracted typhoid fever 25 years previously, and was discovered to be a carrier following an investigation into the source of the infection in her three grandchildren. She was in good health and robust for her years, but suffered from chronic constipation, and, not-withstanding, her stools were swarming with B. typhosus. The blood gave a positive Widal reaction for B. typhosus. This patient came under observation on 16.11.21.

Case II. Kate Ogg (K.O.), a woman aged 27 years, suffered from enteric fever in August 1914 for which she was treated in the Isolation Hospital, Dufftown, and was discharged in October 1914. In 1915 she became kitchenmaid at a farm near Keith. Six months after one of the

TABLE I.

TABLE TO ILLUSTRATE HISTORIES AND CONDITION FOUND IN THE ENTERIC CARRIERS CONCERNED IN THE FOLLOWING INVESTIGATION.

Case	Name.	Age in yrs.	Sex	Type of Excreter	Excretion of organisms in		Widal Reaction			Years since attack of Enteric Fever.	No. of persons infected.	Relatives	Deaths
					Faeces	Urine	T	A	B				
I	Mrs. Griffen (Mrs. G.).	76	F	B. typhosus.	+	+(occasionally)	+			25	3	3	-
II	Kate Ogg(K.O)	27	F	B. typhosus.	+	+(occasionally)	+			9(Aug1914	2	1	-
III	Mary Duncan (M.D.).	39	F.	B. paratyphs. B.	+	+(occasionally)			+	4¾ (Dec.1918)	3	0	-
IV.	Helen Turnbull (H.T.).	52	F.	B. typh.& B. paratyph. B.	+	+(very rarely)	+		+	10 (Oct.1913)	8	1	1
V.	James Murray (J.M.).	75	M*	B. typhosus.	+	+(once only).	+			No evidence that disease contracted in last 19 yrs.	23 (18 sane 5 insane)	0	-
VI.	Margaret Moyes (M.M.)	40	F*	B. typhosus	+	-	+			unknown	?	?	?
VII	Bertha Evans, (B.E.).	60	F*	B. paratyph. B.	+	-			+	"	11 (6 sane 5 insane)	0	0
VIII	Mrs. Riddett (Mrs. R.).	68	F*	B. typhosus.	+	+	+			"	?	?	?

men servants developed typhoid fever. In consequence the faeces of everybody about the farm were examined and B. typhosus was isolated from the stools of Kate Ogg. She left the farm and kept house for her two brothers one of whom in 1919 developed a severe attack of typhoid fever. She never suffered from any symptoms referable to the gall-bladder and a few months before coming under the present observation on 27.10.21. typhoid bacilli were again found in her faeces.

Case III. Mary Duncan (M.D.), a woman aged 39 years, was treated in the Fever Hospital, Dumbarton for "typhoid fever" from December 1918 till February 1919, In May 1920 she became a servant at a farm (Maryfield) near Buckie, and in the July of the same year two farm servants sickened and left the farm, one said to be suffering from rheumatic fever and the other from influenza. In December 1920, a lad who had just gone to the farm took ill and was subsequently found to be suffering from enteric fever. He was treated at the Isolation Hospital, Elgin, and his blood gave a positive Widal reaction for B. paratyphosus B. Mary Duncan was now suspected to be the source of the infection, and her blood on examination gave a positive agglutination for B. paratyphosus B. This organism was also found in her faeces at an examination made in October 1921. She gave no history of cholecystitis and had remained well since her acute attack. She came under observation

in the present investigation on 27.10.21.

Case IV. Helen Turnbull, (H.T.), a woman, aged 52 years, had enteric fever, when acting as housekeeper to her brother at Langknowe in Northumberland in October 1913. She was treated at home. Her brother, shortly afterwards developed a similar infection and died. The infection was derived from cases at a neighbouring farm.

On leaving Langknowe in February 1914, she had three changes of residence:- (1). About October 1914 she went to Heatherhope to a family living in an isolated cottage, consisting of the husband, a shepherd, named Telfer, and his wife and the assistant shepherd, named Inglis. Here "H.T." stayed a fortnight doing work for Mrs. Telfer who was under medical treatment (no symptoms of enteric fever) and on Nov. 17th 1914 Inglis sickened with enteric fever and was notified on the 30th of that month. Telfer had received some of the clothing from Langknowe from his relative who had died but this appears to have been disinfected and was only used when Inglis was away and was never in the lad's room.

A boy from Hownam (McClements) who had one meal in the house while Inglis was ill also took typhoid fever, and was the only case in Hownam. The cottage at Heatherhope was new, built at the cost of the Burgh of Kelso to replace one removed when the waterworks were established,

and the drainage and water-supply were both above suspicion.

(2). At the end of March 1915, "H.T." went to a family at Belford to act as housekeeper and cook. Early in May 1915 the householder William Davidson, aged 57 years, took typhoid fever and a fortnight later a man in a neighbouring cottage, Kerr, also became infected. During the lambing season Kerr had some of his meals at Davidson's house. The water-supply of the house showed no signs of pollution and was of good quality on chemical analysis. In June 1915, an examination of the blood, urine and faeces of Helen Turnbull made at the Royal College of Physicians' Laboratory, Edinburgh, gave an entirely negative result. The house was disinfected at the termination of Davidson's illness as well as all the clothes etc. The hired lad left at the end of May, his place being taken by Frank Anderson, who did not come to live in the house or have meals there until after the disinfection in the end of June. He also was notified as having enteric fever on September 24th 1915, and removed to hospital. During August and September "H.T." milked two cows, and supplied the farm house with milk and butter. The cook there became ill and on removal to the Cottage Hospital, Coldstream, was diagnosed to be suffering from typhoid fever. In October 1915 her faeces were again sent to the Royal College of Physicians' Laboratory, Edinburgh, and found to contain typhoid bacilli.

In all, 6 examinations were made in 1915 and the specific organisms found on 2 occasions.

(3). In May 1916 she moved to a cottage at Yetholm and in

September 1916 two cases of typhoid fever occurred in the family next door. The infection was thought to have been fly-borne from the pail-privy.

No further cases appear to have been caused by this carrier in spite of various changes of residence. Owing to the amount of feeling caused in Yetholm she was admitted to the Hospital for Infectious Diseases, Kelso, in Oct. 1916 (where she remained till 30th March 1917) and was given employment in the garden. In March 30th 1917 she went to a Children's Home at Greenock and remained until 11th Oct. 1920, and shortly before she left her faeces again gave a positive result.

She never suffered from any symptoms referable to the gall-bladder and came under observation for the present investigation on 14.4.22.

The first four cases were kept under observation in Belvidere Fever Hospital, Glasgow.

Case V. James Murray (J.M.), aged 75 years, was admitted to the Lunatic Wards of Linlithgow Poorhouse on 21st June 1904. He had become chargeable on 5th December 1887, being found wandering in Abercorn Parish in a demented state. Prior to admission to Linlithgow Poorhouse he had been treated in Morningside and at Larbert. On admission he was demented, utterly irrelevant in his disjointed remarks, having no initiative and requiring treatment like a child. He has enjoyed good health during his sojourn there except that he had a double hydrocoele which required occasional tapping. In consequence of out-breaks of

enteric fever in the establishment on 21.4.14 his serum along with other twenty samples of blood from all the male lunatic inmates was sent to the Royal College of Physician's Laboratory, Edinburgh, for examination and also the the Usher Institute of the University. As a result of the tests made, three cases, of whom one was known to have had typhoid fever, were regarded as possible carriers, and samples of urine and faeces were obtained from each on April 27th 1914 for detailed bacteriological investigation. In Murray's faeces B. typhosus was discovered in large numbers. His blood gave a very high agglutinative reaction, namely, a complete reaction in 1 in 30, and in 1 in 60, and a partial reaction in 1 in 120, 1 in 240, 1 in 480, and 1 in 960. No history of Enteric Fever was obtainable but, as he was an old soldier, he may have suffered from the disease while in the Army. He was thus discovered to be a carrier 10 years after his admission to the institution. From the time of his admission till January 1909 - a period of fully four and a half years - no known case of enteric fever arose in the institution. An outbreak which was confined to the poor house and comprised fifteen sane and one insane persons occurred early in 1909. Three male lunatic inmates were found ill with the infection in April 1909, June 1910, and March 1913 respectively. These persons were thought to have been infected by soiled clothing being handled in the laundry as the milk and water-supply were above suspicion. Murray's period of residence in the institution therefore covered

all the foregoing outbreaks of enteric fever. Subsequent to the discovery of him as a carrier the following cases arose:- (1). A male sane inmate, who may have been infected through contact with enteric infection in a privy pail, was found to have contracted enteric fever in March 1916. (2). A male lunatic was found to have contracted enteric fever in March 1918. (3). A male sane inmate took enteric fever in April 1921. (4). In May 1921, the male lunatic attendant who had held this position for seven and a half years took enteric fever.

There is no evidence that Murray contracted the disease after admission to the poor house, and it is highly probable that he had suffered from typhoid fever during his period of military service in India.

While under observation for the present investigation he was detained in the lunatic wards of Merryflatts' Poorhouse, Glasgow. The first specimens of blood, urine and faeces were examined on 16.5.22.

Case VI.

Margaret Moyes, (M.M.), aged 40 years, was admitted to Hawkhead Mental Hospital, Crookston, Cardonald, Glasgow in October 1905 at the age of 23 years suffering from dementia praecox. The patient, herself, to the knowledge of the Medical Staff, has never suffered from typhoid fever, but in the course of a routine examination of the laundry patients by Widal reactions to find the cause of a typhoid outbreak in the asylum, her serum was found

to agglutinate B. typhosus and also to a slight degree B. paratyphosus B. on 7.8.22. by Dr. William Whitelaw, Director of Western Asylum Laboratory. The only illness she suffered from was impetigo and conjunctivitis in August 1920, hence she was probably a carrier before admission. For the last 6 years or so she has been dirty in her habits though quite physically sound.

In the Asylum from June 1916 till September 1923 there were 63 cases of typhoid fever of whom 55 were females with 18 deaths (15 females and 3 males). The death rate constituted 28.3 per cent of all the cases. In a search for the cause of the outbreak Dr. Whitelaw found 11 patients on the female side to be excreters of B. typhosus and to give a positive agglutinative reaction for that organism in their sera, among whom were Margaret Moyes and Mrs Riddett (Case VIII). Owing to the number of carriers discovered it is impossible to state how many cases of infection Moyes and Riddett gave rise to.

Record of Examinations on Margaret Moyes by Dr. Whitelaw.

On 18.8.22 both faeces and urine gave positive cultures of B. typhosus. Negative examinations were made on 23.8.22, 30.8.22 and 6.9.22. B. typhosus was again isolated from the faeces in the next two examinations on 18.9.22. and 24.9.22. Specimens from this patient were first sent for examination in the present investigation on 19.10.22.

Case VII. Bertha Evans, a woman aged 60 years, was admitted to Kirklands Lunatic Asylum, Bothwell on 27th May 1910 from Dalziel

Parish suffering from delusions of a persecutory and expansive nature, definitely systematized, and first noticed during the menopause. Her mental condition is one of paranoia. There was no history of enteric infection prior to admission (She is an intelligent woman with a good memory and would be able to state if she had been treated for such an illness). There was no record of any illness of a pyrexial nature of any duration, suggesting enteric group infection during her residence in the institution. She had, however, for many years suffered at intervals of a few weeks from "bilious attacks", requiring to stay in bed and complaining of vomiting, giddiness, headache, and anorexia. These were relieved by the taking of calomel and magnesium sulphate. Cases of obscure illness began to occur in the hospital in August 1922. Serological examinations and clinical observation by the staff of the Medical Officer of the County of Lanark failed to reveal the nature of the illness. About the middle of October 1922 the illness was diagnosed as paratyphoid B. fever as a result of positive agglutination tests and isolation of B. paratyphosus B. from the faeces of patients by Dr. Whitelaw, Director of the Western Asylum's Laboratory. In the search for a carrier bloods of a group of patients and staff who had "influenza"

in the Spring of 1922 were examined; bloods of ex-service men and of a group of patients and staff who had suffered from enteric fever 14 years before (i.e. prior to Mrs. Evans' admission) and lastly the bloods of the Kitchen Staff were examined. A miscellaneous group was then dealt with and the serum of Mrs. Evans (who was included on account of her bilious attacks) agglutinated B. paratyphosus B. in a dilution of 1 in 40 (13 standard agglutinin units per c.c. of blood) and subsequently that organism was isolated from her stools in both the Western Asylum's Laboratory and the County Laboratory.

On isolation of this patient no further cases of the disease occurred. During the epidemic of B. paratyphosus B. which commenced in the middle of August 1922 and lasted till about the middle of Nov. 1922 caused by this carrier 11 persons - the Clinical Assistant, 2 nurses, the cook and kitchen maid, 5 patients and the Doctor's infant daughter - were infected. None of the suffers were dangerously ill and no deaths occurred. The average length of the fever was 9 or 10 days.

Specimens from Mrs. Evans were first examined in the present investigation on 17.12.22.

Case VIII. Mrs. Riddett, aged 68 years a female dement, was admitted on 18th September 1895 to Hawkhead

Lunatic Asylum, Glasgow, and has had no attack of typhoid fever since admission or before as far as is known. She was found to be a carrier on 12th May 1923 during the investigation for the cause of the repeated outbreaks of enteric fever in the institution by Dr. Whitelaw. Her blood gave a positive Widal reaction for B. typhosus and the organisms were isolated from her faeces. She is exceedingly dirty in her habits. The outbreak is dealt with under the history of Case VI. She came first under observation in the present investigation on 31.5.23.

Cases IV and V of this series are described by Dr. Dittmar in a Report to the Scottish Board of Health in 1922; Section (III) outbreaks traced to a working housekeeper refers to Turnbull and section (I) Enteric Fever in a Scottish Poorhouse refers to Murray.

Commentary on the histories of the carriers and on infections derived from them and some general considerations of the carrier state.

Age and Sex Prevalence.

It may be said that the majority of carriers to whom outbreaks of enteric fever have been traced are women, usually middle-aged. From the statistics of

Frosch (1908), Vincent and Murilet (1917), and Gay (1918), chronic carriers occur maximally between 40 - 45 years of age and are in the proportion of 5 females to one male. Of the series investigated by the author 7 out of 8 were women and the onset of the condition occurred mainly in the third and fourth decades of life. Frosch stated that 10 - 25 per cent. of all women infected with typhoid fever became carriers.

TABLE II.

Table to illustrate age of onset of infection in the carriers and duration of the carrier state.

Case.	Name.	Sex.	Age of onset of attack in years.	Number of years a carrier.	Remarks.
1.	Mrs.G.	F.	51	25	
2.	K.O.	F.	20	11	
3.	M.D.	F.	35	$6\frac{3}{4}$	
4.	H.T.	F.	42	12?	
5.	J.M.	M.	40?	35?	Lunatic carrier.
6.	M.M.	F.	20?	20?	Lunatic carrier.
7.	B.E.	F.	45?	15?	Lunatic carrier.
8.	Mrs.R.	F.	35?	13?	Lunatic carrier.

The reason given as the cause of this is that women's dress predisposes to biliary stagnation and hence lesions of the mucous membrane.

Children seldom become chronic carriers. The site of multiplication of the enteric bacilli in chronic carriers is the gall-bladder in which the organisms may persist for many years, often giving rise to gall-stones. The fact that women appear to be more liable to gall-stones than men consitutes a serious factor in relation to the problem of the typhoid carrier, as women are more concerned in the preparation of food.

Mode of spread of infections.

The majority of infections caused by the carriers investigated as shown by their histories have been derived from the contamination of food-stuffs or milk (Mrs.G., K.O., M.D., and H.T.). In some the infection was spread by the soiling of linen in laundrying and by direct infection while being nursed (J.M., M.M., B.E., and Mrs.R.). In the case of H.T. fly-borne infection from a privy may possibly have caused a few cases.

The literature on the spread of infection from carriers is too extensive to give in detail. Milk infections are described by Frost (1917) and Osborn and Beckler (1920) while ice-cream from infection of the milk employed in the making of it is given by Cumming (1917). Infection of the water-supply by carriers is much rarer and is usually from contamination by infected faeces.

Normal state of health of carriers.

An additional danger lies in the fact that carriers usually appear to be in perfect health or may only suffer from slight, and to them, unimportant pains in the region of the gall-bladder, it being well known that in only a proportion of patients suffering from gall-stones do severe symptoms arise. None of the present series had symptoms referable to the gall-bladder nor did any of them suffer from continuous ill health. One (M.M.) suffered from recurrent bilious attacks. In addition to lesions in the biliary tract carriers may suffer from repeated attacks of intestinal catarrh with diarrhoea (Mayer 1910). Cases have been reported of re-infection of carriers by themselves with the occurrence of a generalised typhoid infection (Kasper, Kamm, Jones and Grimme quoted Arnd 1923). Mayer (1910) describes three similar cases. In one Mrs.B. the acute attack took place in 1903 and an attack of "biliary typhoid" took place in 1907 during the puerperium. The child was born with typhoid fever. The other two cases were girls whose acute attacks occurred in 1903 and 1904 and who developed second attacks in 1905 and 1906 respectively.

Carriers, it should be noted do not arise merely from typical clinical cases, nor do they depend on the severity of the original illness, for in many cases

there has been no recognised attack.

Duration of the carrier state.

The expression "once a carrier always a carrier" (Osborn and Beckler 1920) seems to hold good as cases have been recorded as long as 40 years after the original typhoid attack (Dean 1908). Of the carriers in the present investigation the acute typhoid infection took place 6,11,12, and 25 years before (M.D., K.O., H.T., and Mrs. G. respectively), while in the insane patients 13, 15, 20 and 35 years, if any acute attack was experienced at all, seem to be an approximate estimate of the duration of the condition. The figures are however only problematical as regards the length of time of the carrier state as no reliable histories were obtained from these cases. It is only since 1902 that individual cases have been kept under continuous observation so that any figures beyond that date must, of necessity, be problematical. In three of the cases (K.O., M.D., and H.T.,) there appears to be no doubt that the carrier state resulted immediately after convalescence, as they gave rise to clinical cases shortly afterwards.

Infections derived from carriers.

That many of the cases of typhoid fever occurring at the present time are sporadic and derived from

carriers is borne out by examination of the histories of the same carriers of this series. The epidemics which arise in asylums and similar institutions appear to be derived from a similar source (vide J.M., M.M., B.E., and Mrs. R.). The fact that these carriers are insane and probably none too cleanly in their habits intensifies the risk of epidemics.

Examination of Table I on page 8 shews that the six carriers (excluding M.M. and Mrs. R. who along with other carriers gave rise to a large number of cases) infected in all 50 persons (with enteric fever). This works out at an average of 8 persons to each carrier.

These figures agree with the findings of other investigators e.g.

TABLE III.

AUTHORS.	NO. OF CARRIERS.	NUMBER OF PERSONS INFECTED.
Chesley, Burns, Wade and Greene (1917).	30	213.
Osborn and Beckler (1920)	51	493.
Meyer (1921)	14	249.
Lutz (1921)	1	45.

Quite long periods may elapse between the various outbreaks derived from the same carrier. Osborn and

Beckler (1920) state that one carrier (a dairy-man) caused no known cases from 1909 to 1919 when 29 cases of typhoid fever developed on his milk-route. Similarly 3 other chronic excreters showed the same peculiarity.

The intermittent nature of the carrier danger is manifest in several of the carriers of the present series. Thus Mrs. G. set up cases for the first time 24 years after the acute infection and K.O. gave rise to no known cases from 1915 to 1919.

The intermittency of infections derived from carriers indicate the grave menace they constitute during their life-time, and the necessity for their strict supervision.

****

B I B L I O G R A P H Y.

Arnd (1923): Schweiz. Med. Woch., 1923, 53, p. 423.

Chesley, Burns, Wade and Greene (1917): J.A.M.A. 1917, 68, p. 1882.

Cumming (1917): J.A.M.A. 1917, 68, p. 1163.

Dean (1908): B.M.J. March 7th 1908.

Frosch (1908): Klin. Jahrb., 1908, XIX, p. 537.

Frost (1917): J.A.M.A., 1917, 68, p. 609.

Gay (1918): Typhoid Fever, New York 1918, p. 128.

Lutz (1921): Centralbl.f. Bakt., 1921, 86, p. 550.

Mayer (1910): Centralbl.f. Bakt., 1910, 53, p. 234.

Meyer (1921): J.Infect. Dis., 1921, 28, p.381.

Osborn and Beckler (1920): J. Infect. Dis., 1920, 27, p. 145.

Vincent and Murilet (1917): Typhoid and Paratyphoid Fever, London 1907, p. 66.

****

CHAPTER III.

Comparison of methods of isolation and identification of Typhoid and Paratyphoid bacilli from the stools of Enteric Carriers.

TABLE OF CONTENTS.

1. A historical survey of the various methods employed in the isolation of typhoid and paratyphoid organisms from the faeces is given.
2. Comparison of various methods with particular reference to brilliant-green enrichment.
3. A description of methods of collection of specimens of faeces and bile for the identification of enteric carriers, and method for preservation of faeces for delayed examinations.
4. Results of a series of examinations of faeces in 8 enteric carriers with comparison between diredt plating and brilliant-green peptone water enrichment. B. typhosus carriers:- Out of 340 examinations by both methods, positive results by direct plate were 305, by brilliant green 145.

B. paratyphosus B excreters:- 242 examinations; positive - 171 by direct plate, positive 226 by brilliant green.

5 Suggested explanation of failure of brilliant-green method in case of typhoid excreters, from interaction of B. coli isolated from carriers on B. typhosus.

6. Optimum concentration of brilliant green for isolation of B. typhosus proved to be 1:500,000; for B. paratyphosus B, 1:285,700; necessity for series of dilutions of dye antiseptic is emphasized.

7 Enrichment by preliminary inoculation of litmus milk proved superior to the brilliant-green method but inferior to direct plating on to MacConkey in the case of carriers of B. typhosus.

8 Detailed description of Milk enrichment method with reference to time of exposure and temperature. A short exposure, 2-3 hours at room temperature, gave the best results.

9 Effect of nature of stool on positive results; value of glycerine-saline as a preservative for faeces in delayed examination; suggested employment of 24 hours action with a view to increasing positive findings, since by this means 40 positive results would have been missed.

COMPARISON OF METHODS OF ISOLATION AND IDENTIFICATION OF TYPHOID AND PARATYPHOID BACILLI FROM THE STOOLS OF ENTERIC CARRIERS.

The vast number and variety of methods and media which have been recommended in the recovery of the typhoid group of organisms from the faeces indicates the limited value of the majority of them.

There have been two general types of media devised for this purpose, namely Solid and Fluid.

Solid Media are of different types; I. Media on which there is a sharp differentiation between lactose-fermenting and non-lactose-fermenting colonies shown by a colour change in an indicator added along with the sugar to the agar, but little or no inhibition of the growth of organisms accompanying the typhoid bacillus except certain cocci. Among these differential media several are worthy of note; MacConkey's (1901 and 1908) bile-salt-lactose-agar, to which neutral red is added as an indicator (Grunbaum and Hume, 1902). At the present time this medium is mostly employed in this country. Drigalski and Conradi's (1902) lactose-litmus-nutrose-crystal-violet-agar; Endo's (1903) lactose-fuchsin-sodium sulphite-agar; numerous modifications of the latter medium have been advocated in recent years by Kendal (1911-12) and Robinson and Rettger (1916). Endo-agar has many supporters

especially in America. The most recent development of this class of medium was introduced by Holt-Harris and Teague (1916) in their eosin-methylene-blue-agar, containing 0.5 per cent. of both lactose and saccharose.

II. Media which inhibit the growth of many strains of B. coli and of other faecal bacteria to a much greater extent than they inhibit B. typhosus due to the action of a differential antiseptic. Loeffler (1903-1906) first pointed out the difference of susceptibility of B. typhosus and B. coli to ~~the dye~~ malachite green, whereas of these two organisms B. coli is the more resistent to most antiseptics, in the case of malachite green the order of susceptibility is reversed. Conradi (1908) then demonstrated a similar property in the case of brilliant green. Various media were devised to take advantage of the selective action of these dyes but without much success, as is shown by Conradi's brilliant green-picric acid-agar which was modified by Fawcus (1909) who added bile salt and lactose. The differential action of the dyes does not seem to have been generally admitted. The question was again taken up by Browning, Gilmour and Mackie (1913), and Torrey (1913) independently and they proved conclusively that brilliant green had a greater selective action against B. coli as compared with B. typhosus and B. paratyphosus B than malachite green. Confirmation of their work

was also given by Tidy and Dunn (1916) and Teague and Clurman (1916). Krumwiede and Pratt (1914) on the other hand, found that none of the "green" dyes are more selective than the others.

Krumwiede, Pratt, and McWilliams (1916) employed agar containing 1 per cent. lactose and 0.1 per cent. glucose and brilliant green in dilutions of 1:200,000, 1:330,000, and 1:500,000, using Andrade's acid fushin indicator. Krumwiede, Kohn, Kuttner and Schumm (1918) describe the preparation of this medium, with minor modifications, for practical use and stress the necessity of preliminary standardisation to obtain the optimum value of the antiseptic dye.

Teague and Clurman (1916) devised an agar medium which contains 3/50 per cent. eosin and 1/300 per cent. brilliant green with 1 per cent. of lactose and saccharose.

Fluid enriching media which allow the typhoid bacilli to multiphy more rapidly than the accompanying faecal flora.

Carbolic acid was first used but was found to inhibit the typhoid bacilli as much as the other faecal bacteria. Caffein broth (Roth 1904) and malachite green broth (Peabody and Pratt 1908) were next employed. Jackson and Melia (1909) recommended lactose bile as an

enrichment broth and Robinson (1916) found that in conjunction with Endo-agar in his experience it constituted the most efficient and expedient method of isolation. His results are based on only six specimens of faeces from persons giving a positive Widal reaction and are too few to be of value. Lactose bile never came into general use. Tonney, Caldwell and Griffen (1916) in the examination of a large number of typhoid stools had better results by direct plating on to Endo agar. No specimen which was negative on the direct plate gave a positive result by the use of bile, while 38 out of 40 were lost through passage in bile. Winslow and Dolloff (1922) found that the toxicity of brilliant green to B. coli and B. lactis aerogenes is greatly diminished in bile. Torrey (1913) recommended a glucose broth enrichment medium containing brilliant green which he claimed to have a marked selective propensity for the paratyphoid-enteritidis group of organisms. About the same time Browning, Mackie and Gilmour (1913) introduced a similar preliminary enrichment peptone water medium containing brilliant green for the isolation of B. typhosus. Browning's (1918) medium consists of 2 per cent peptone and 0.5 per cent. sodium chloride in distilled water, steamed for three-quarters of an hour and filtered through ordinary filter paper and made faintly

alkaline to litmus. The medium is distributed in amounts of 10 c.cms. in test tubes, then sterilised by steaming or in the autoclave. A stock 1 per cent. solution of brilliant green (sulphate, zinc free) in distilled water is prepared. Immediately before use is added a 1 in 10,000 dilution freshly made up by adding 0.1 c.cm. of the stock solution to 9.9 c.cms. of distilled water and then this dilution is added to the peptone water in the following amounts:- 0.1, 0.2, 0.35, 0.5, 0.7 c.cm. (corresponding to dilutions of 1:1,000,000, 1:500,000, 1:285,700, 1;200,000, 1:143,000). The addition of telluric acid to a second series of brilliant green tubes with a view to inhibiting the growth of B. lactis aerogenes and other inosite-fermenting dye-resisting types is recommended (Browning, Mackie, and Smith 1914). The telluric acid is added to each tube to give a dilution of 1:25,000 (0.4 c.cm. of a 1 in 1,000 solution). When a large number of examinations has to be carried out Browning recommends the use of a single concentration of 1 in 200,000 or 1 in 250,000 brilliant green in 10 c.cms. of peptone water. According to the experience of Stokes and Clarke(1916), Leitch (1916) and Tidy and Dunn (1916) this is on the average the optimum concentration for the isolation of the specific organisms. In the author's own experience this is the case (see p. 54).

Cole and Onslow (1916) suggest that their medium prepared from casein digested with trypsin is likely to prove especially suitable for eliciting the differential antiseptic effect of brilliant green. Meyer and Stickel (1918) found that peptic casein digests are better for the growth of typhoid organisms in this respect and that it applies also to the solid brilliant green-agar medium of Krumwiede, Pratt and McWilliams and the eosin-brilliant green-agar medium of Teague and Clurman.

In regard to the amount of faeces to be employed to each tube, Browning advocates one platinum loopful; in the case of very fluid faeces a large loop-up to 1/6th of an inch in diameter-being employed; solid faeces are emulsified by rubbing up with several volumes of sterile water. The cultures are then incubated for twenty to twenty-four hours at 37°C. and subcultures made on MacConkey's or Endo's medium. Three successive strokes, without re-charging the needle, are made from each tube-in this way one 4-in plate accomodates the subcultures made from three peptone-water tubes; the plates are then incubated at 37°C. for from eighteen to twenty-four hours, as in the case of direct plates.

In 1916 Teague and Clurman brought forward a further fluid enrichment dextrose bromoform-congo-red-

brilliant green-gelatin medium which they claim to be superior to Browning's when used in conjunction with their brilliant green-eosin-agar plate procedure.

Other Methods.

Bierast (1914) found that in mixtures of B. coli and B. typhosus submitted to the action of benzine or petroleum ether for 5 hours, B. coli is more rapidly destroyed. In view of his results in artificial mixtures he recommended its use in the preliminary isolation of B. typhosus from the faeces, and obtained two positive results when other methods proved negative. His method was to add one finger's breadth of petroleum ether to a faecal suspension in broth, shake at intervals for ½ - 1 hour and leave for 16 hours in a cool place, decant supernatant fluid and plate sediment on to an Endo agar plate. Hall (1915) found the fraction of the petroleum with a boiling point 40°C. gave the best results (Pentane C_5H_{12}). He modified Bierast's technique by adding to a heavy suspension of faeces in broth a half of its volume of pentane, shaking for ½ hour, allowed mixture to stand for 1 - 1½ hours at room temperature and plated sediment. In 21 positive specimens of faeces he got 7 positive by pentane method alone to 1 positive by direct plate. The other 13 were positive

by both methods. Jaffé (1915) and Heyn (1917) also had favourable results but the number of examinations are too few to prove what they claim the Bierast's method to be. Ickert (1917) modified Bierast's technique by emulsifying the faeces (size of haricot bean) in 3 or 4 c.cms. of bile before adding the petroleum ether. From 26 stools of typhoid convalescents he found 8 positive by Bierast's method to 15 by bile and ether. Nedrigailoff (1917) suggested the use of 2 or 3 c.cms. of Benzine to 7 or 8 c.cms. of a mixture of faeces in broth (a teaspoonful of faeces to each 2 c.cms of broth), vigorous shaking (5 - 10 minutes) repeated in 20 minutes, and tube set aside, protected from light, for 8 - 10 hours at room temperature. The supernatant fluid is decanted and the sediment inoculated on to Endo agar. On the other hand Schuscha (1916) found the results by the petroleum ether method to be less satisfactory than by direct plating. In addition the ether is highly inflammable and the method dangerous, since two laboratory workers developed typhoid fever. Krumwiede and Kohn (1918) agree with Schuscha but state that with short periods of exposure to petroleum ether (10 minutes shaking and leaving for 1 hour) the method may be successful where

direct plating alone with Endo agar fails, though the results are distinctly inferior to their brilliant-green agar medium.

Among other procedures for the isolation of typhoid and paratyphoid bacilli from enteric stools, Dreyer proposed the use of the actinic rays from an electric arc between water-cooled silver electrodes, since experiments with certain laboratory strains of B. typhosus and B. coli yielded promising results. In a later communication he states that from practical experience in cases of enteric the results are in no way as good as those obtained by direct plating and are distinctly inferior to the results of Browning's brilliant-green method. Tidy and Dunn likewise by the use of the arc lamp were unable to obtain any specific action. It appeared to kill B. coli rather less than it did B. typhosus.

Wordley (1921) employed Dudgeon's method in preference to Browning's brilliant green fluid enrichment method. The faeces are dried to a powder and spread over convenient culture media, either MacConkey or litmus lactose agar (the latter medium, he states, is better). In 64 stools of typhoid patients, 42 were negative by both brilliant green fluid enrichment and the dry method, 19 positive by the latter

to 10 by the former.

Collection of specimens of faeces for examination.

As is generally recognised, cultures should be made from the faeces as soon as possible after evacuation, preferable within several hours, since the isolation of typhoid bacilli becomes increasingly difficult when faeces are allowed to stand for some time. Freshly taken rectal swabs are preferred by some workers but as a general rule small corked glass tubes with a small metal scoop inserted into the cork, are employed. When a delay must occur before the faeces are examined Teague and Clurman's (1916) method of preserving typhoid stools for delayed examination by means of emulsifying one part of faeces in two parts of a 30 per cent. dilution of glycerine in 0.6 per cent. NaCl solution, should be employed. Where the faeces are of normal consistency - for example in suspected carriers - it is advisable to administer purgatives (calomel or elaterin) as advocated by Tonney, Caldwell and Griffen (1916) and confirmed by Stokes and Clarke(1916), and then examine the resulting fluid evacuations: it is generally agreed that the chances of successful isolation of the specific organisms are considerably increased thereby. Tonney, Caldwell and Griffen recommend 0.1 - 0.2 grains of

elaterin to be given the previous evening. . The first portion of the resultant copious movement should be discarded and the remaining liquid or fluid portion retained. There is a marked increase in the ratio of B. typhosus to B. coli in the faeces, especially of carriers. The technique suggested by these investigators is to use wide mouthed screw-topped bottles (sputum) filled two-thirds full with the glycerine saline solution and stool is added until the bottle is nearly full. If the stools are fluid it is necessary only to shake to mix; if solid emulsify as far as possible witha glass rod. The glycerine saline solution inhibits the growth of both typhoid and coliform bacilli to some extent but apparently the coli-typhoid ratio is maintained more or less in the same state as when faeces were first passed. If anything the coliform organisms appear to be more inhibited than the typhoid. B. typhosus can be isolated from the faeces for more than six days after evacuation when the same faeces emulsified in saline alone have become negative. Benians (1918) has confirmed this observation of Teague and Clurman and has further extended the observation to cases of Paratyphoid A and B infections. He investigated twelve carriers, two of B. typhosus,

one of B. paratyphosus A and nine of B. paratyphosus B, all of which yielded colonies of the specific organism on direct MacConkey plates. Control experiments were made in each case with faeces emulsions in 30 per cent. glycerine and 0.6 per cent. saline solution and in saline alone. In one set of experiments the emulsions were allowed to stand at room temperature (16° C) and in another test they were kept at 37° C throughout. In the first experimental series, except for the first two days, the specific organisms were in all but two cases isolated from the glycerine-saline tubes throughout but their numbers fell rapidly till the sixth day, but none of the saline tubes were positive. The organisms in these two cases were scanty from the first. In the second set where a high temperature (37° C) was maintained throughout, the number of non-lactose fermenting bacilli (B. paratyphosus B) was maintained in the glycerine - the number of coliform organisms falling somewhat - whereas in the plain saline emulsions the lactose fermenters increased enormously and the paratyphoid bacilli fell rapidly in numbers, disappearing entirely by the fourth day. Osborn and Beckler (1920) have further confirmed the observations of Teague and Clurman and found plates made from specimens after standing for 24 hours occas-

ionally give positive results when negative following immediate examination after emulsification, because there is less overgrowth of B. typhosus with other organisms.

Scheer (1918) on the basis of experimental centrifugation of artificial mixtures of B. coli and B. typhosus for twenty minutes and then allowing to stand for an hour and inoculation of plates from the supernatant fluid, recommends a similar procedure for the isolation of B. typhosus from stools. He found B. typhosus regularly almost in pure culture in the supernatant fluid while B. coli is only with difficulty isolated. The number of tests on the stools of typhoid patients is scanty.

Duodenal Cultures for the isolation of B. typhosus from the bile of typhoid carriers.

Since it has been recognised that the gall-bladder constitutes the main seat of multiplication of enteric organisms in carriers, cultures of bile secured from the duodenum by the passage of an Einhorn tube, have been recommended by numerous observers, who claim that the specific organisms are overgrown by the other intestinal organisms, so that negative faeces examinations occur when positive cultures can be obtained from the duodenum. In support of this, Garbat

(1922) quotes Von Drigalski and Jurgens who at post-mortem examinations showed that by cultural methods in the intestinal tract from duodenum down to rectum the number of typhoid bacilli decrease. At a necropsy on 19/4/23 on an acute case of typhoid fever the author, however, got a negative result in the duodenum, while positive cultures of B. typhosus were obtained from the ileum and gall-bladder (see page 83, section on Altered Biological Reactivity). The intermittent excretion of typhoid bacilli in the bile Garbat regards as only apparent and not real. Only on a few occasions were duodenal bile cultures negative intermittently. Schievelbein (1919) studied the bacterial content in bile secured from the duodenum in 71 cases of typhoid and paratyphoid fever, and in chronic and convalescent bacillus carriers. Of 5 typhoid carriers 3 became negative at the end of a month as shown by 10 consecutive daily examinations of both stools and bile. In one case the bile was positive for B. typhosus in 100 per cent. of examinations and in the stools in only 50 per cent. In two paratyphosus A carriers both faeces and bile were constantly positive. In 36 chronic B. paratyphosus B carriers who regularly gave positive cultures in faeces examinations, the bile examinations gave positive results in 30 or 84 per cent. He con-

cluded that while a large proportion of carriers have a chronic gall-bladder infection, this is not true of all carriers. Stepp (1918) found that by means of a preliminary duodenal injection of Witte's peptone before withdrawing the material for culture a greater number of positive results could be obtained. Apparently the peptone solution causes contraction of the gall-bladder.

Henes (1920) urges the necessity for culture of the duodenal contents in all cases of typhoid fever in convalescence. He advocates periodic cultural examinations from the duodenal tube allowed to remain in situ all day, claiming thereby to get more accurate and dependable results. Three such negative examinations at weekly intervals constitute his criterion of cure both of the acute and carrier conditions. Nichols, Simmons and Stimmel (1919), Garbat (1922) and Simmons and McCarthy (1924), have all employed this method in the detection of carriers and have had positive duodenal cultures when negative results have been obtained in the faeces.

AUTHORS OWN OBSERVATIONS.

Technique. The technique employed throughout in the examination of the stools of eight enteric carriers, is that described by Browning (1918). Specimens of faeces

three to six hours after evacuation, were received bi-weekly on Mondays and Thursdays, when obtainable, in small scooped sterile bottles or occasionally on sterile swabs. Observations as to the nature of the stools were noted and the reaction tested by litmus paper (method described by Goeffon see p. 210). The faeces were emulsified in 30 per cent. glycerine-saline in the proportion of 1:2 and sometimes according to the hardness of the specimen and abundance of resultant coliform growth in proportion of 1:4 or even higher. The faeces were then allowed to stand at room temperature for 1 hour before the examination proper was begun, in the hope that the more motile typhoid bacilli would rise to the surface. A platinum loopful of uniform size was taken from the surface and stroked directly on to a MacConkey agar plate in the manner described in Medical Research Council series report No. 21 p.37, and the plate incubated at 37°C. for 18 - 24 hours. A series of 5 brilliant-green peptone water tubes were inoculated with one or two loopfuls of the faeces emulsion according to the consistency of the specimen. The dilutions of brilliant green were 1:1,000,000; 1;500,000; 1:285,700; 1:200,000; 1:143,000; i.e. 0.1, 0.2, 0.35, 0.5 and 0.7 c.cms of 1:10,00 stock solution of brilliant green in distilled water to

10 c.cms of peptone water in each tube. Another series with telluric-acid as well was occasionally tried (see p.32). After incubation for 18 - 24 hours one or two loopfuls from each tube were plated on to a Mac-Conkey agar plate. The five tubes were plated onto the same plate and three or four strokes were usually made from each without recharging the needle. The plate was then incubated overnight. When the direct MacConkey plate showed no likely non-lactose fermenting colonies a fresh plate was stroked from the glycerine-saline faecal emulsion which had stood at room temperature for 20-24 hours. Agar slopes were then inoculated from likely colonies from the various MacConkey agar plates. After incubation these were emulsified in 0.85 per cent. saline, any likely or doubtful colonies being tested for motility and inoculated into fluid media containing the following series of sugars - lactose, glucose, mannite, dulcite, maltose and saccharose. Litmus milk, gelatin and peptone water tubes were also inoculated, and a test for indol formation made. The saline-organism mixture was next killed at 56° C. in the water bath ($\frac{1}{2}$ to $\frac{3}{4}$ hours exposure) and agglutinated with antityphoid or anti-paratyphoid B rabbit serum at 56° C. for two hours by the Macroscopic Method. The stock antisera were made by

inoculating rabbits intravenously in increasing doses with killed saline suspensions of laboratory cultures of B. typhosus (R.L.L.) and B. paratyphosus A (Primrose) until high titre agglutinating sera were obtained.

The next table (IV) gives a detailed description of the number of examinations performed on each patient and on carriers (K.O., H.T., and M.D.) who had the operations of cholecystgastrostomy and cholecystectomy performed for the cure of their condition are subdivided to show the effect of these operations upon the excretion of the specific organisms.

TABLE IV.

Results of Examination of Faeces of Enteric Carriers.

The following table shows the findings in the whole series.

Patient.	Organism Isolated.	Number of Examinations.	Results		Date of 1st. Examination.	Date of last Examination	Remarks.
			Pos.	Neg.			
Mrs. G.	B.typhosus	36	36	0	16/11/21	13/3/22	
J.M.	B.typhosus	104	79	25	16/5/22	7/6/23	
M.M.	B.typhosus	50	22	28	19/10/22	21/5/23	
Mrs.R.	B.typhosus	3	1	2	31/5/23	7/6/23	
K.O.	B.typhosus	155	132	23	27/10/21	4/5/23+	+ To operation.
		8	7	1	4/5/23	31/5/23*	* Last positive.
		162	0	162	31/5/23	19/11/25	
H.T.	B.typhosus & B. paratyphosus B	108	56	52	14/4/22	4/5/23 +	+ Up to operation.
		15	5	10	4/5/23	28/6/23 *	* Last date on which specific organisms were isolated.
		91	0	91	28/6/23	12/2/25	Subsequent examinations.
M.D.	B. paratyphosus B.	178	168	10	27/10/21	17/7/23	Up to 1st. operation on 17.7.23
		85	67	18	17/7/23	19/7/24	From 1st. to 2nd operation on 19.7.24.
		48	6	42	19/7/24.	12/3/25.	After 2nd. operation.
		58	1 (24.7.25)	57	21/3/23	16/5/26	In last 9months (30 neg. examinations since last positive on 24.7.25.
B.E.	B.paratyph. B.	19	1	18	7/12/22	10/5/23	

Note. In the case of H.T. the faeces were positive for B. paratyphosus B on four occasions before and once after operation.

The examinations on the faeces of K.O. and M.D. from 1.8.24 onwards were performed by Dr. Guthrie.

Table V deals with the comparison between direct plating on to MacConkey agar and the brilliant green peptone fluid enrichment method.

TABLE V.

Comparison between direct plating on to MacConkey agar and the brilliant-green fluid enrichment methods.

	I	II	III	IV	V	VI.	
Patient.	Total No of Exams.	No. of Exams. with positive results by either method.	Positive Direct Plate.	Positive Brilliant-green.	Positive direct plate when brilliant-green negative.	Positive Brilliant-green when direct plate negative.	Type of Carrier.
Mrs. G.	36	36	34	34	2	2	B. typhosus.
J.M.	104	78	72	46	18	6	"
M.M.	50	21	16	12	8	6	"
Mrs. R.	3	1	1	0	1	0	"
K.O.	165	137	133	43	70	2	"
H.T.	123	57*	49*	8*	40*	6*	Mixed B. typhosus & B. para. B.
M.D.	310	241	171	225	7	57	B. paratyph. B.
B.E.	17	1	0	1	0	1	"

* In the case of H.T. on 5 occasions B. paratyphosus B was isolated from the faeces; by the brilliant green method three times (twice alone) and by Direct plate once. On the other occasion preliminary benzine treatment of the faeces gave the positive result when the other methods were negative.

Analysis of the results according to the type of carrier, especially when of the intermittent excreting variety, reveals a distinct failure of the brilliant green method as compared with direct plating in B. typhosus carriers e.g.(J.M., M.M., K.O. and H.T.). Thus out of 340 examinations on all the excreters of B. typhosus, 305 positive results were obtained by the direct method to 143 by brilliant green. When the typhoid excreter passed the specific organisms abundantly in the faeces the positive findings were on a par (e.g. Mrs. G.). On the other hand in the case of B. paratyphosus B carriers the reverse holds good (e.g. M.D. and B.E.). Out of 242 examinations 171 were positive by the direct and 226 by brilliant green. Again this does not reveal the ease with which the specific organisms were isolated. Often when comparatively scanty by direct plate they were in pure culture in one or other of the brilliant green dilutions.

These findings disagree with the results of Browning and his co-workers with reference to B. typhosus but

support his contention as regards B. paratyphosus B Cultures of B. typhosus from these carriers were tested for undue susceptibility to brilliant green but this was not found to be the case. A number of other observers have expressed a favourable opinion of the method (Dreyer, Stokes and Clarke, Leitch, Tidy and Dunn). The 622 individuals Leitch examined included the two known typhoid carriers. Typhoid bacilli were picked out on all of five occasions in which the faeces of these carriers were examined by the brilliant green method and on four occasions by the direct plate. No less than five paratyphoid B carriers were detected by brilliant green which were altogether missed by the direct method, whilst on the other hand, two carriers were found by the latter method and missed by the former. It is to be noted, however, that the original observations on the isolation of B. typhosus from faeces by means of brilliant green were made not on carriers, but on recent cases of the disease.

<u>The influence of B. coli on the growth of B. typhosus with special reference to enrichment by brilliant green in typhoid carriers.</u>

The brilliant green method of enrichment may fail,

as has been shown previously, even when B. typhosus is sufficiently abundant to be found on direct plates; one reason for such failure has been shown to be the presence of types of B. coli which are resistant to brilliant green, but which may be suppressed by the action of telluric acid. A further cause forfailure has been elucidated in the course of this work on the four chronic typhoid carriers of intestinal type (J.M., M.M., K.O., and H.T.). In these cases it was found, on repeated examinations carried out over periods of many months, that even by the use of a series of concentrations of brilliant green, enrichment of the typhoid bacilli, as a rule, was not obtained; the addition of telluric acid to the medium on several occasions failed to improve the results. Similarly, variation in the amount of the inoculum and of the period of incubation of the brilliant green cultures (two hours, as suggestd by Krumwiede and his co-workers, and twenty hours) were without notable effect. The reaction of the peptone water was invariably tested before use, and the dye solution freshly prepared. The strains of B. typhosus inseveral of the cases (J.M., M.M., K.O., and H.T.) were tested after isolation, and were not unusually susceptible to brilliant green; and cultures of the prevailing types of B. coli (of common variety)

isolated from these patients' faeces were not abnormally resistant to the dye. Accordingly, mixtures were made of approximately similar saline suspensions of 24 hours' growth of a stock culture of B. typhosus (R.L.L.) with the various cultures of B. coli from the carriers and with a stock culture of B. coli (Baby) respectively, in the proportion of one of B. typhosus to four of B. coli. The mixtures were then used to inoculate a series of tubes of fluid medium containing brilliant green in concentrations ranging from 1:1,000,000 to 1:143,000 which were incubated and subcultured thereafter as usual. The result was that B. typhosus could not be recovered from any of the growths in the mixtures containing B. coli derived from the carriers, although it was found in practically pure culture from the mixture with the stock B. coli in the presence of suitable amounts of the dye. The experiment was then carried out in a different form; thus, varying volumes of B. coli suspension were added to one volume of B. typhosus culture, and after the mixtures had stood overnight at room-temperature each was used to inoculate peptone water containing brilliant green 1:286,000; the result was that B. typhosus was recovered from the mixture containing 800 volumes of the stock B. coli culture to 1 volume of B. typhosus, whereas in the case of the carrier's B. coli, B. typhosus

failed to be recovered from a mixture in which the relative proportions were 50 to 1. Thus it is apparent that the strains of B. coli present in these cases possessed in marked degree the property of inhibiting the growth of B. typhosus. Direct plating of mixtures of B. typhosus with the stock B. coli showed that the latter also had an inhibitory effect on B. typhosus; this was demonstrated in the following way. From a mixture of B. typhosus (1 volume of a saline suspension of a young agar culture) with B. coli (4 volumes of a suspension of similar opacity to that of the typhoid culture) a series of decimal dilutions was prepared - in a control series the typhoid suspension, without B. coli, was diluted similarly with saline. Then successive stroke cultures on plates of MacConkey's medium were made with a constant loop. On incubation at 37°C. it was found that the proportion of typhoid colonies which developed in the series containing B. coli was much smaller than in the controls without B. coli. The result, with the higher dilutions, where widely isolated colonies developed, proved that B. typhosus was inhibited and not merely masked by the abundant growth of colonies of B. coli (such colonies as developed were, with few exceptions so minute and delicate as to resemble a colony of pneumococcus);

also from the dense parts of the growth B. typhosus could not be isolated by means of brilliant green. The phenomenon appears to hold good no matter what medium is used (MacConkey, Endo, and Eosin-methylene blue agar being all tested). An attempt was made to distinguish between the inhibitary powers of the different strains of B. coli by growing fluid cultures in neutral peptone water (2 per. cent.), for 24 hours at 37° C., then filtering through Maassen porcelain candles and inoculating the sterile filtrates with B. typhosus. Agar slope cultures were inoculated by means of a platinum loop of a young culture of B. typhosus and the filtrate poured over the surface, and also tubes of filtrate and saline control tubes. No gross difference of the resulting growths in filtrates from stock B. coli and from carrier's B. coli could be detected; both showed well-marked proliferation of B. typhosus.

Conclusion: It is possible that exposure to brilliant green kills off the typhoid bacilli which have already been attenuated in some way by the action of carrier's B. coli and thus a negative result is more frequently obtained by the brilliant green method than by direct plating, where no additional antiseptic

action occurs. B. paratyphosus B does not appear to be so susceptible to the action of brilliant green or to be affected to the same extent by the carrier B coli, hence enrichment can more readily occur.

Optimum concentration of brilliant green for the isolation of B. typhosus and B. paratyphosus B from the stools of carriers.

A record was kept throughout the whole series of examinations by the brilliant green peptone water enrichment method of the number of non-lactose fermenting colonies which appeared on the plate from the various dilutions of the dye, also as to the state of turbidity or discolouration of the tubes. It may be generally stated that where discolouration is present after incubation, this results from the abundant growth of coliform bacilli and from these tubes the specific organisms are unlikely to be obtained. This agrees with Browning and Thornton's findings. The value of a series of varying doses of the dye lies in the fact that when the lower ones show discolouration the next higher doses are then most likely to yield positive results. Occasionally when all tubes appeared clear after 24 hours and failed to yield growths on subculture, a positive result was obtained after the fluid cultures had been incubated for a further period.

Browning recommends the use of a single concentration of brilliant green (1:200,000 or 1:250,000) when for any reason the full procedure cannot be adopted, e.g. owing to the necessity for dealing with large numbers of specimens. According to the experience of Stokes and Clarke, Smith, Leitch, and Tidy and Dunn this is, on the average, the optimum concentration for the isolation of the specific organisms.

In the experience of the author as illustrated by the subsequent table (VI) the optimum concentration would appear to lie between 1:500,000 and 1:200,000 for any type of carrier; 1:285,700 would appear to give the highest number of positive results. This holds good especially for B. paratyphosus B while for B typhosus 1:500,000 appears to be the optimum concentration. The majority of positive results for the latter are got in the first four dilutions while for B. paratyphosus B the last four gave the greatest number, but as the result with B.E.(paratyphoid carrier) shows there is no invariable rule. The necessity for a series of dilutions of the green dye antiseptic is accordingly emphasized.

TABLE VI.

TABLE TO ILLUSTRATE OPTIMUM CONCENTRATION OF BRILLIANT GREEN FOR THE ISOLATION OF B. TYPHOSUS AND B. PARATYPHOSUS B. FROM THE FAECES.

Case.	Number of Exams.	Positive results in the various dilutions of the dye					Type of Carrier.
		1:1,000,000	1:500,000	1:285,700	1:200,000	1:143,000	
Mrs.G.	23	8	21	16	12	4	B. typhosus.
J.M.	46	26	26	19	12	10	B. typhosus.
M.M.	12	9	7	4	3	2	B. typhosus.
K.O.	30	9	24	20	13	10	B. typhosus.
H.T.	8	4 B.typhosus 0 B. para.B.	0 B.typh. 2.B.para B	1B.typh. 2 B.para.B	0 B.typh. 1 B.para.B.	1 B.typh 1Bpara B	B. typhosus & B. paratyphosus B.
M.D.	200	107	154	180	165	141	B. paratyphosus B.
B.E.	1	1	0	0	0	0	B. paratyphosus B
Total	320	164	234	242	206	169	

SUBDIVISION OF TOTAL POSITIVE RESULTS ACCORDING TO NATURE OF ORGANISM.

Nature of organism.	Concentration of dye.				
	1:1,000,000	1:500,000	1:285,700	1:200,000	1:143,000.
B. typhosus.	56	78	60	40	27
B. paratyphosus B.	108	156	182	166	142

TABLE VII.

TABLE TO ILLUSTRATE COMPARISON BETWEEN RESULTS BY EXAMINATION OF THE SAME SPECIMENS OF FAECES WITH MILK, DIRECT PLATE AND BRILLIANT-GREEN METHODS.

Case.	Total Exams.	Direct Plate		Brilliant-green		Milk.		Positive by Milk when others negative.	Positive by either Direct Plate or Brilliant green.	No. of exams. in which all three methods are negative.
		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.			
J.M.[1]	57	36	21	19	38	26	31	1	40	16
M.M.[1]	13	6	7	3	10	2	11	1	6	6
K.O.[1]	56	49	7	7	49	26	30	2	50	4
H.T.[1,2]	55	19 (1 para B)	36	4 (3 para B)	51	14 (1 para B)	41	4	21	30
M.D.[2]	55	44	11	48	7	40	15	0	55	0
Total	236	154	82	81	115	108	128	8	172	56

1 = Typhoid carrier. 2 = Paratyphoid B. carrier.

Enrichment by preliminary inoculation of Litmus milk and comparison of results with direct plating and brilliant green enrichment.

For a period, as a routine measure, a tube containing 10 c.cms. of litmus milk was inoculated with a loopful of the glycerine-saline faeces emulsion and incubated at 37° C. or left at room temperature for varying periods, and then subcultured on to a MacConkey agar plate. (See Table VII, p. 59).

From examination of the tables it is thus seen that milk enrichment on occasion may give a positive finding when the other methods are negative. Generally speaking, however, milk enrichment is distinctly inferior to the direct plate method, but in most cases equals if not surpasses, especially in the case of B. typhosus carriers, the brilliant green fluid enrichment method. As will be shown later the time of incubation at 37° C., when 18 -24 hours, adversely affects the positive findings from the milk tubes. Earlier on in the examinations most of the milk tubes were incubated for this period so that with less incubation or simply leaving the milk at room temperature for varying periods a higher proportion of positive results might have been obtained.

TABLE VIII.

TABLE TO ILLUSTRATE MILK EXAMINATIONS WITH REFERENCE TO TIME AND TEMPERATURE FACTORS.

Case.	Time of exposure in milk before plating.								Remarks
	2 - 3 hours.				18 - 24 hours.				
	20°C.		37°C.		20°C.		37°C.		
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	
J.M.	22	15	3	11	4	16	0	22	
M.M.	2	11	-	-	0	8	-	-	
K.O.	22	15	2	10	7	16	0	17	
H.T.	10	25	3*	9	1	21	0	19	* 1 positive for B.paratyph B.
M.D.	30	6	11	1	4	16	0	19	
Total	86	72	19	31	16	77	0	77	

The addition of brilliant green to the litmus milk was next tried, in a final dilution of 1:285,700, in the case of the faeces of the B. typhosus carrier J.M. While the milk was positive the number of colonies present on direct plate and from the first three dilutions of brilliant green peptone water tubes was much greater. As the number of experiments done in this connection was few, no conclusion can be come to from the findings.

Analysis of Milk Enrichment Results with reference to time of exposure before plating and the temperature factor. (See Table VIII, p. 59).

The largest number of positive results (54 per cent.) with milk enrichment are obtained by inoculating the litmus milk tube and allowing it to stand at room temperature (20° C.) for 2 or 3 hours before plating. Incubation at 37° C. for the same period gives relatively few positives (38 per cent.) while after 18-24 hours the specific organisms have been completely overgrown by coliform organisms. The number of positive findings diminishes with the length of exposure in milk as after 18 - 24 hours at room temperature (20° C.) the percentage falls from 54 to 17.

This is shown by a comparison of results made on the same day with the same specimen of faeces, but from the different carriers.

Further table to show analysis of milk examinations made on the same day with the same specimens of faeces.

Case.	Positive 2-3 hrs at 20° C. when negative 18-24 hrs. at 20° C.	Positive 2-3 hrs. & 18-24 hrs. at 20° C.	Positive 2-3 hrs. at 37° C. and negative 18-24 hrs. at 37° C.
J.M.	5	3	3
M.M.	2	-	-
K.O.	8	4	1
H.T.	3	-	2
M.D.	12	2	8.
Total	30	9	14.

On three occasions the milk tubes were positive for the specific organisms after 2-3 hours exposure at 20° C. and 37° C. and after 18 - 24 hours at 20° C. but negative after the same exposure at 37° C. Likewise on four occasions positive results were obtained

after 18 - 24 hours exposure at 20°C. when negative after 2 - 3 hours.

Effect of nature of stool on positive results.

As is generally recognised the chances of successful isolation of typhoid organisms from the faeces are considerably increased when the stools are soft or of fluid consistency. Purgatives given with a view to hastening the passage of the contents of the small intestine and examination of the resultant fluid stool have been shown to increase the number of positive findings (Stokes and Clarke, Tonney, Caldwell and Griffen). The latter workers got positive results in carriers by the use of elaterin as described on p. 37 . They give a table illustrating the effect of a soft stool upon positive results.

Elaterin stools	35.
Soft Stools	18.
Hard Stools	4.

The author agrees with their findings as on many occasions positive results were obtained by the use of calomel and elaterin.

TABLE IX.

Table to illustrate effect of nature of stool on positive result.

Case.	Nature of Stool.					
	Fluid or semi-fluid.		Soft formed.		Hard.	
	Positive.	Negative.	Positive.	Negative.	Positive.	Negative.
J.M.	24	2	45	6	10	17
M.M.	8	4	12	9	2	15
K.O.	77	6	50	10	3	2
H.T.	38	23	22	26	1	13
M.D.	95	3	122	16	4	4.
Total	242	38	251	67	20	51.

It must be noted, however, that many of the negative results occurred not as isolated observations but during periods which often extended over some weeks, up to four, especially so in cases of K.O. and H.T. During these periods the stool was usually soft or fluid and even the use of a purgative at times failed to give a positive result. The numbers of colonies of the specific organisms present on the Mac-Conkey plates was almost invariably considerably higher when the stool was fluid or of soft consistency. When

hard, the organisms were scanty or absent. Calomel and elaterin caused a distinct lowering of the coli-typhoid ratio, and on various occasions caused the reappearance of typhoid colonies after a negative phase. Table IX illustrates that when the stool is hard the chances of a positive result are greatly diminished while when fluid or semi- fluid the greatest number of positives are obtained.

The isolation of typhoid and paratyphoid bacilli from faeces treated with glycerine - saline.

The technique followed was as described by Teague and Clurman. The value of the method is illustrated in ~~the subject~~ Table X which shows that when the specimen of faeces in glycerine-saline has been allowed to stand for 18-24 hours at room temperature before inoculating the media, positive results may be obtained even when the result is negative after only 1 hour's contact. As a routine the author stroked a second direct MacConkey plate, from the faeces-glycerine-saline emulsion, after 18-24 hours when the first plate showed no non-lactose-fermenting colonies or only doubtful colonies. In the majority of instances when the plates from both examinations were positive, the numbers of non-lactose fermenting colonies tended to be increased after the longer

exposure to glycerine, with a slight corresponding inhibition of the numbers of coliform colonies present. This is well illustrated in the case of J.M.

Number of Non-lactose fermenting colonies on MacConkey agar.

Date.	After 1 hour's exposure to glycerine-saline.	After 18-24 hours exposure to glycerine saline.
30.10.22.	2.	51.
13.11.22.	1.	25.
23. 4.23.	2.	14.
7. 5.23.	0.	6.

TABLE X.

Table to illustrate value of Glycerine-saline in delayed examination of faeces.

Case.	Positive findings on MacConkey agar Direct plates.			Type of Carrier,
	Exposure to glycerine - saline both for 1 hour & 18-24 hours.	Exposure to glycerine-saline for 1 hour alone when 18-24 hrs. negative.	Exposure to glyc. saline for 18-24 hrs. when 1 hr. negative.	
J.M.	6.	0.	9.	B. typhosus.
M.M.	3.	1.	4.	"
K.O.	10.	9.	8.	"
H.T.	7.	6.	10.	Mixed B.typhosus & B.paratyphosus.
M.D.	8.	3.	9.	B.paratyphosus B.
Total	34.	19.	40.	

Consequently 40 positive results would have been missed had the second plate after exposure of faeces to glycerine-saline for 18-24 hours not been made. Thus as a routine procedure it would appear well worth carrying out this measure in all cases.

An experimental comparison between the use of ordinary saline and 30 per cent. glycerine-saline with coli-typhoid culture mixtures in the proportion of 4 volumes of B. coli to 1 of B.typhosus, was made. Immediate direct plating or by brilliant green showed no difference in the respective growths, but after allowing the mixtures to stand for 24 hours at room temperature no typhoid colonies developed on direct plate from the saline while numerous colonies grew from the glycerine-saline mixture. 30 per cent. glycerine gave better results than 10, 40, or 75 per cent.

Effect of centrifugalisation of the faeces emulsion.

Centrifuging the faeces glycerine-saline emulsion did not show any great advantage over simply allowing the mixture to settle for 1 or 18-24 hours before plating. Variable results were obtained. Thus sometimes more non-lactose fermenting colonies were obtained with a diminution in the number of coliform but on the other occasions the converse was true. In

all, about a dozen tests of this description were made.

When the mixture was shaken up or when plates were made immediately after emulsification invariably a greater number of coliform colonies were present while often no non-lactose fermenting colonies could be detected even though they were quite numerous on plates made from the emulsion after allowing settling to take placefor 1 to 24 hours.

Comparison between MacConkey agar, Endo agar and Eosin-methylene-blue agar of Teague and Clurman.

These media were made according to the description given previously, and comparative tests were made in conjunction with the experiments on the interaction of B. coli on B. typhosus. In all a matter of six tests were made, and as far as these experiments go MacConkey agar seems to be the best differential medium of the three. Difficulty was experienced in differentiating the non-lactose fermenting colonies on Endo on account of diffusion of the red from the coliform colonies.

Preliminary treatment of Faeces of Carriers with Benzene.

The technique employed was similar to that

described by Nedrigailoff (1917), only peptone water was used instead of broth for emulsifying the faeces in; 1 part of faeces about the size of a "pea" was added to 4 c.cms of peptone water with 3 c.cms. of benzene, and shaken vigorously by hand for about 10 minutes, repeated in twenty minutes and the tube put aside for 6 to 18 hours. The supernatant fluid was discarded and the sediment plated on to MacConkey agar. Eight tests were made with the carrier faeces. Only one positive result was obtained, while the whole eight were positive by direct plate or brilliant green enrichment.

Table to illustrate Benzine Treatment of Faeces in isolation of the specific organism from Enteric Carriers.

Case.	Benzine.		Direct Plate or Brilliant-green.		Type of Carrier.
	Positive.	Negative.	Positive.	Negative.	
J.M.	0	2	2	0	B. typhosus.
K.O.	0	2	2	0	"
H.T.	1(B.para. B)	1	2	0	Mixed B. typhosus and B. paratyphosus B.
M.D.	0	2	2	0	B. paratyphosus B.
Total.	1	7	8	0	

The sole occasion in which a positive result was obtained by the benzine method was in the case of H.T. on 24.5.23 when a B. paratyphosus B colony developed from the benzine tube while B. typhosus was isolated via brilliant green on the same day. The method gave very poor results and the shaking of the tubes constitutes a grave danger of infection. In addition the inflammable nature of the benzine necessitates great care.

Comparison of Methods, with Review of Literature.

All solid media, even when so constituted that cocci are inhibited and the common varieties of B. coli distinguished by a colour reaction due to the presence of a fermentable sugar and an indicator as in Drigalski and Conradi's, Endo's and MacConkey's media, leave much to be desired as none of these media interferes notably with the growth of coliform bacilli, hence where the typhoid or paratyphoid organisms are small in number a very large surface must be spread in order that discrete colonies of the pathogenic bacillus may be obtained. Glen-Liston and Goré (1919) recommend a method of the latter variety, based on the observation of 140 carrier stools examined. Definite quantities of a series of definite dilutions of an emulsion of faeces to secure a sufficient number of isolated colonies are distributed on dry agar

slopes. They state there was never less than 1 typhoid or allied organism to 20 of the common intestinal bacteria and hold it unnecessary to examine more than 50 isolated colonies. Where a large number of examinations are required this method would appear to be almost impracticable. Most investigators are agreed that none of the differential media is superior to the rest, but that the advantage lies wholly in the familiarity of the worker with one particular medium. Comparisons have been made between the media in common use (MacConkey, Drigalski-Conradi and Endo) as a result of a series of tests by Drayer, Walker and Gibson (1915) and they concluded that the Drigalski Conradi medium is least efficient and Endo decidedly best. Tidy and Dunn (1916) confirmed these observations but did not find such great differences between the other two. One advantage which the MacConkey medium has is that it indicates with striking clearness the non-lactose fermenting colonies, which enables the picking out of pure colonies far better than the Endo medium. The reason for this is that the coliform organisms colour the plates around them and obscure the non-lactose fermenters. This defect of the latter medium is particularly marked when a soft agar substratum and faintly alkaline reaction to litmus is chosen. Numerous modifications have been employed to remedy this

(Kendall 1911-1912, Robinson and Rettger 1916). Various indicators have been tried, such as neutral red, Congo red, water blue and China Blue. Schmitz (1915) described a congo red agar while Bronfenbrenner, Schlesinger and Soletsky employed a China Blue- rosolic acid-peptone fluid medium. The latter claim that the bactericidal action of their C.R. mixture is due to the rosolic acid and that the inhibition is directed against Gram-positive bacteria. In the eosin-methylene-blue-agar (Holt-Harris and Teague) after 18 hours incubation typhoid colonies are colourless and coliform colonies have black centres and do not colour the surrounding medium. The authors of the medium claim that B. coli colonies are differentiated earlier on this plate than on Endo and that a greater percentage of colourless colonies turn out to be typhoid while it inhibits certain organisms which Endo does not. Only few coli develop on the medium. Meyer and Stickel (1918) prefer the brilliant green eosin agar medium of Teague and Clurman in preference to Endo, Holt-Harris and Teague's medium, and Krumwiede's brilliant green agar even with the use of peptone digest agar.

Before any final conclusion as to the merits of the various solid differential media with or without the addition of a selective antiseptic, a larger

series of controlled tests would have to be performed. Each author of any particular medium advocates the virtues of his own and emphasizes the disadvantages of the others, often without making adequate and equal controls.

Owing to the fact that typhoid bacilli are often few in number in comparison with other intestinal organisms an enrichment medium seems advisable before plating. The advantage of a fluid enriching medium for the typhoid bacillus would lie in the fact that a larger amount of faecal material would be subjected to examination than upon solid media. If the proportion of typhoid to other organisms falls below a certain figure (1:300 Ficker and Hofmann 1904) it becomes impossible to isolate typhoid bacilli on solid media. Dreyer, Walker and Gibson (1915) indicated that the typhoid bacillus fails to grow on selective media when the proportion of typhoid to the colon bacillus in mixtures, was lower than 1:15. If a larger amount of such faeces than the plate medium permits is inoculated ina fluid enrichment medium that allows the rapid development of B. typhosus while it inhibits the growth of most other organisms present after 24 hours incubation the percentage of typhoid will have greatly increased and thus they can be readily isolated subsequently on solid media.

Since the introduction by Browning of his peptone water brilliant green enrichment medium, numerous workers have decried it while others have been equally emphatic about its value. Browning, in his Applied Bacteriology, discusses fully the various objections levelled at the medium and points out how often the criticism has been made on scanty findings and on experiments which have not been properly controlled.

Particular reference is made to the work of Krumwiede and his co-workers, and the other American investigators who have introduced the recent new enrichment fluid and solid media. As all this work has been already covered by Browning the author proposes only to add a few of the more recent criticisms. Houston in a report (10th) to the Metropolitan Water Board on the isolation of the typhoid bacillus from a sewage, reports a complete failure by brilliant-green fluid enrichment which he ascribes due to the presence of preponderating numbers of highly resistant coliform and other microbes. These grow well in doses of inhibitory agents that are either fatal to the typhoid bacillus or markedly inimical. Meyer and Stickel (1918) found this brilliant green fluid method unreliable even with peptic or tryptic digests and less efficacious than direct plate on eosin-

brilliant-green-agar. The gelatin-congo-red-brilliant green-bromoform enrichment method of Teague and Clurman gave promising results with 2 human stools, but they state the disadvantages of all enrichment fluids is that a few more positive findings are only obtained at increased time, cost and labour. They are convinced that those apparently favourable results noted with fluid enrichment media are counterbalanced by equally dependable and quicker results obtained by the use of peptic digest eosin-brilliant-green-agar. The comparative study of Meyer and Stickel was, however, mainly directed between Krumwiede's, Teague and Clurman's and Holt-Harris and Teague's media. In 1919 McLeod reported his observations on Browning's method on the work of a mobile Laboratory on acute typhoid and paratyphoid infections, during the previous four years. At first only one tube of Brilliant green was used, a 1:250,000 dilution, but in the last three years a second dilution, 1:500,000 was employed in addition and a comparison with a literally direct method, i.e. the plate was inoculated with a small portion of faeces and spread immediately. The first series relates chiefly to paratyphoid infections and his results show that out of 16 results 15 were positive by the brilliant green method and only 7 by the direct method. By means of brilliant green a B.

paratyphosus A carrier was detected when nothing but B. coli and a few coarse non-lactose fermenting colonies were present in directplate. In the second series described by McLeod the original claim made by Browning, Gilmour and Mackie, that their method the isolation of typhoid and paratyphoid bacilli are both facilitated, is supported. Excluding two paratyphoid infections, there were 14 specimens derived from 11 different cases, 13 of stool and one of urine, in which a positive result was obtained. In 13 of these the result was positive by the brilliant green method whereas in 6 only by the direct method.

Mention has already been made to Wordley's (1921) article (page 36). In addition he made a comparison by mixtures of B. typhosus with normal stools seeding tubes containing 5 c.cms. of peptone water (0.1 and 0.2 c.cms. of 1:10,000 brilliant green in each) from the mixtures, incubated for 24 hours at 37° C. and plated on to MacConkey agar plates. Of 100 specimens examined 27 were positive by the "Dry" method and 5 by brilliant green.

As far as the results of the present writer are concerned failure with brilliant-green enrichment occurred in relation to B. typhosus carriers but marked enrichment in the case of B. paratyphosus ?B excreters.

As Browning recommends, a combination of direct plating on a differential medium such as MacConkey or Endo agar and the use of a brilliant green fluid enrichment medium gives the greatest chance of successful isolation of the specific organisms.

*****

BIBLIOGRAPHY.

Benians (1918): Lancet, 1918.

Bierast (1914): Centralbl. f. Bakt., I, orig., 74, p. 348.

Bronfenbrenner, Schlesinger and Soletsky (1920): Journ. of Bact., 5, p. 79.

Browning (1918): Text-book on Applied Bacteriology, pp. 94-140.

Browning, Gilmour and Mackie (1913): J. of Path. and Bact., XVIII, p. 146, and Journ. of Hyg., XIII, p. 335.

Browning, Mackie and Smith (1914): J. of Path. and Bact., XIX, p. 127.

Browning and Thornton (1915): B.M.J. August 14th., 1915 and May 13th 1916.

Cole and Onslow (1916): Lancet, July 1st, 1916.

Conradi (1908): Münch Med. Woch., p. 1523; Centralbl. f. Bakt., Ref., 1908, 42, p. 47.

Dreyer (1915): Proc. Roy. Soc. Med., Vol. IX, No. 2, p. 15.

Dreyer, Walker and Gibson (1915): Lancet, 1915, I, p. 643.

Drigalski and Conradi (1902): Zeitsch. f. Hyg., vol XXXIX, p. 283.

Endo (1903): Centralbl. f. Bakt., Abt. I, orig., XXXV, p. 109.

Fawcus (1909): J. Roy. Arm. Med. Corps, XII, P. 147.

Ficker and Hofmann (1904): Arch. f. Hyg., 49, p. 229.

Garbat (1922): Monographs Rockefeller Instit. for Med. Res., No. 16, May 10th 1922.

Glen-Liston and Gore (1919): Indian Journ. Med. Res. 1919., Spec. Ind. Sc. Congress., pp. 107-123.

Grunbaum and Hume (1902): B.M.J., vol. I, p. 1473.

Hall (1915): Wien. Klin. Woch., 16, p. 418.

Henes (1920): J.A.M.A., 75, p. 1771.

Heyn (1917): Centralbl. f. Bakt., I, orig., LCCIX, pp. 185-191.

Holt-Harris and Teague (1916): J. Infect. Diseases, 18, p. 596.

Ickert (1917): Berl. Klin. Woch., pp. 458-560.

Jackson and Melia (1909): Journ. Infect. Dis., VI, p. 194.

Jaffe (1915): Berlin Klin. Woch., No. 52.

Kendall (1911-12): J. Med. Research, 25, p. 95.

Krumwiede and Pratt (1914): Journ. Exper. Med., XIX, 20, p. 501.

Krumwiede, Pratt and McWilliams (1916): Journ. Infect Dis., XVIII, pp. i - 13.

Krumwiede, Kohn, Kuttner and Schumm (1918): Journ. Infect. Dis., XXIII, pp. 275 - 289.

Krumwiede and Kohn (1918): Journ. of Bact., 3, p. 361.

Leitch (1916): B.M.J., Sept. 2nd. 1916.

Loeffler (1903): Deutsch. Med. Woch., 1903, Vereins-Beilage, p. 286.

Loeffler (1906): Deutsch. Med. Woch, XXXII, p. 289.

MacConkey (1901): Thomson-Yates Laboratory Rep. vol. III, pt. ii, p. 151.

MacConkey (1908): Journ. of Hyg., vol. III, p. 322; ibid., 1909, IX, p. 86.

McLeod (1919): Journ. of Hyg., XVIII, p. 260.

Meyer and Stickel (1918): Journ. Infect. Dis., 28. pp. 48-67.

Nedrigailoff (1917): Russky Vrach, Petrograd, 1917, XVI, pp. 73-96; ref. J.A.M.A., 68, p. 1789.

Nichols, Simmons and Stimmel (1919): J.A.M.A., 73, p. 680.

Osborn and Beckler (1920): J. Infect. Dis., 27, p. 145.

Peabody and Pratt (1908): Boston Med. and Surg. Journ., CLVIII, p. 213.

Robinson (1915): Journ. Med. Research, 32, p. 399.

Robinson and Rettger (1916): J. Med. Research, 1916, 34, p. 363.

Roth (1904); Arch. f. Hyg., XLIX, p. 199.

Scheer (1918): Mediz. Klin. 1918, Aug. 4th., pp. 758-761.

Schievelbein (1919): Centralbl. f. Bakt., Jena, 1 Abt., orig., 83, pp. 97-101.

Schmitz (1915): Deutsch. med. Woch., 41, p. 427.

Schuscha (1916): Centralbl. f. Bakt., I, orig., 78, p. 226-231.

Simmons and McCarthy (1924): Journ. of Lab. and Clin Med., IX, No. 8, pp. 513-518.

Smith (1923): Journ. Path. and Bact., XXVI, pp. 122-123.

Stepp (1918): Munch. Med. Woch., 1918, No. 22.

Stokes and Clarke (1916): Journ. Roy. Arm. Med. Corps, XXVI, pp. 461-470.

Teague and Clurman (1916): Journ. Infect. Dis., 18, p. 647; ibid, p. 653.

Teague and Clurman (1916): Journ. Med. Research, 35, p. 107.

Tidy and Dunn (1916): Journ. Roy. Arm. Corps, XXVII, p. 482.

Tonney, Caldwell and Griffen (1916): Journ. Infect. Dis., 18, pp. 239-246.

Torrey (1913): Journ. Infect. Dis., 1916, 13, p. 263.

Winslow and Doloff (1922): Journ. Infect. Dis., 31, pp. 301-304.

Wordley (1921): Journ. of Hyg., XX, p. 360.

****

CHAPTER IV.

Altered biological reactivity of chronic enteric carriers.

Among the methods described for the discovery of carriers are the following:-

A. Antibody reactions of the serum.

1. Agglutinating action, i.e. the Widal Reaction.
2. Complement fixation.
3. The autolysate precipitin reaction.
4. Opsonic action.

B. Cutaneous Hypersensitiveness.

C. Alteration of the Blood Picture.

D. Blood Culture.

Widal Reaction. In dealing with large institutions such as asylums, where enteric fever has been endemic, a fair indication may be got of the presence of carriers by performing an extensive series of Widal tests, commencing with subjects who are ascertained to have suffered from typhoid fever in previous

years, or who may happen to have symptoms of gall-stone trouble.

The criterion of what constitutes a positive Widal varies greatly. As the hanging drop method of performing the test has now been generally discarded it is not necessary to discuss the results obtained with it. Browning (1918) gives the following as the criteria of a positive reaction in the case of persons not vaccinated with the organism, where marked agglutination of saline suspensions of average cultures by the sedimentation method, occurs.

Organism.	Serum dilution.
B. typhosus.....	1 : 100.
B. paratyphosus A.....	1 : 50 (or even lower, 1:20
B. paratyphosus B.....	1 : 200.

In the case of paratyphoid A infection the development of agglutinins is often especially poor, and Dreyer (1909) has concluded that agglutination with serum 1:10 is for all practical purposes diagnostic.

Co-agglutination may also practically invalidate the result. In some cases of the author's series the agglutinating power of the sera fluctuated markedly so that in several carriers of B. typhosus increased agglutinating power for B. paratyphosus A or B was

observed from time to time; similarly the serum of the B. paratyphosus B carrier (M.D.) showed at times a marked power of agglutinating B. typhosus. Thus, on occasion, the Widal test might give no help as to the causal organism. These findings are discussed in detail later. Leiss (1918) states that in more than half of the uncomplicated acute typhoid cases, whether they have received prophylactic inoculation or not, there is a co-agglutination for B. paratyphosus A. An interesting case which corroborates Leiss's statement occurred in the Western Infirmary, Glasgow, on 17.4.23, of a sailor aged 30 years who suffered from an acute attack of typhoid fever followed by perforation of one of the intestinal ulcers, with generalised peritonitis and death. During his sojourn in hospital the Widal reaction was repeatedly tested with his serum by Dreyer's procedure, and on each occasion there were 100 standard agglutinin units per cc. for B. typhosus, 600 for B. paratyphosus A and 0 for B. paratyphosus B. At the post-mortem examination on 19.4.23 the perforation was found near to the ileo-caecal valve. All the Peyer's Patches showed extensive ulceration and there was a marked focal necrosis in the liver in addition to a generalised peritonitis. B. typhosus was isolated from the pus from the peritoneal cavity and from the ileum,

from the liver, and from the gall-bladder and spleen in pure culture, but not from the duodenum at the biliary papilla. Numerous non-lactose fermenting colonies from these sites were tested against Standard B. paratyphosus A anti-serum (Series 45, No 1200, received from the Standard Laboratories, Oxford) but none gave any agglutination in dilutions of 1:50, 1:100, 1:200, 1:400, 1:800.

This anti-serum was tested against Standard Anti-paratyphoid A emulsion and a stock B. paratyphosus A (Ward) emulsion and in both cases agglutination occurred up to a dilution of 1:400.

A similar case of paratyphoid B. fever in a boy is also illustrative of the variation in agglutinins present in the serum (Dunlop unpublished).

The patient took ill on 3.6.24. On examination of his serum on 23.6.24. the Widal reaction gave a strong positive result for "T.,A., and B.". Standard agglutination for B. typhosus occurred in a serum dilution of 1:20,000, for B. paratyphosus A 1:1000 and for B. paratyphosus B 1:10,000. 3,125 standard agglutinin units per c.c. were present for "T"; 232 for "A" and 2,439 for "B". The test was repeated with the same sample of serum on two

subsequent occasions and the readings were identical. Examination of the faeces on 30.6.24. revealed abundant non-lactose fermenters both on direct plate and after brilliant-green enrichment. Eight colonies were tested for their fermentativereactions and proved to be of the paratyphoid group. Two were further tested with high agglutinating sera and agglutinated up to titre with B. paratyphosus B. anti-serum. They were not agglutinated by antityphoid and anti-paratyphosus A sera.

On 8.7.24. the Widal reaction was still positive for all three organisms as shown in the table:-

Organisms tested.	Standard Agglutination with a Serum Dilution of	Number of Standard Agglutinin Units per c.c.
B. typhosus.	1:10,000.	1,562.
B. paratyphosus A.	1:500 up to 1:1,000.	116 to 232.
B. paratyphosus B.	1:20,000 to 1:50,000.	4875 to 12,195.

The faeces on the same day showed abundant B. paratyphosus B. colonies to be present, but in spite of testing 25 colonies, none proved to be either B. typhosus or B. paratyphosus A.

A sister of the same patient, who had nursed him,

also developed enteric fever later on (18.6.24) but on 27.6.24 her serum showed no agglutination with B. typhosus or B. paratyphosus A. It contained at this time 122 standard agglutinin units per c.c. for B. paratyphosus B, standard agglutination occurring with a serum dilution of 1:500. Large numbers of B. paratyphosus B colonies were present in the faeces.

In each instance the Widal reactions were tested for by Dreyer's method and the same-batches of Standard Cultures were used in all the tests. The patients' Sera were also tested against B. Gaertner and B. Aertrycke suspensions but with negative results.

Do all carriers give a positive Widal reaction?

Cler and Ferazzi (1905) recorded 6 cases in which typhoid bacilli were found in the faeces as a result of food infection, without any disturbance of health. The Widal reaction was negative in all cases, but the bacilli disappeared in several weeks.

Busse (1908) isolated typhoid bacilli in pure culture from the blood of four contacts with typhoid patients but found the serum in all without agglutinating power.

Kamm (1909) and Gaehtgens (1909) both consider the Widal test of little diagnostic value in the

detection of bacillus carriers. It is stated by Kayser (1909) that about 75 per cent. of carriers give a well-marked Widal reaction, but Ledingham and Arkwright (1912) think this percentage rather high, if agglutination at 1 in 100 be taken as the criterion of a positive result. They employed the hanging drop method, however. Five out of nine of their carriers gave a positive result, while the other four gave an incomplete or absolutely negative result, Again, a patient's serum may acquire the power of agglutinating the typhoid bacillus during an infection which is in no way related to it as shown by Wilson (1909) in cases of typhus fever. On the other hand, positive Widal reactions are got in inmates of institutions in whose excreta the bacilli have never been demonstrable. In a recent report to the Scottish Board of Health by Dr. Dittmar (1924) out of 28 persons who gave a positive Widal reaction, 11 were found to be carriers, but in none of the remainder could the specific organisms be demonstrated on repeated examinations. Kamm (1909) during examination of 136 blood-samples in the search for carriers in an asylum, found 8 cases with positive Widal reactions. of these, 4 gave the reaction with a serum dilution of 1 in 50, 1 with 1 in 100, and 3 with 1 in 200. Four of these cases had had enteric fever about 8 or

12 years previously, while the other four gave no record of infection. In none of the cases could the bacilli be isolated from the faeces, in spite of frequent purgation , or from the urine. Chesley, Burns, Greene and Wade (1917) who examined 32 typhoid carriers found the Widal reaction positive in 26, partially positive in 4 and absent in 2.

Hilgermann (1917) definitely states that there do not exist carriers of B. typhosus who gave a negative Widal reaction and emphasizes its value in their discovery. This may be due to the fact that he considers as a positive result agglutination with a serum dilution of 1 in 25 after 8 hours at 37°C. Persons who give a positive serum reaction, but in whose excreta the organisms cannot be discovered are regarded by him as "hidden carriers" and are hot dangerous from the point of view of dissemination of the virus.

Simmons and McCarthy (1924), on the other hand, found that typhoid agglutinins were present in the sera of their four typhoid carriers, the titres being 1 in 640, 1 in 1280, 1 in 320 and 1 in 640 respectively. The serum of a B. paratyphoid B carrier, however, agglutinated B. paratyphosus B only in a dilution of 1 in 40. From their findings in the examination of 84 convalescents, they con-

cluded that these tests were of no diagnostic value, since agglutinins were demonstrable in the sera of individuals who were not carriers but who had passed through an attack of enteric fever six months previously.

In the majority of carriers a positive reaction is obtained but this is liable to considerable fluctuations from time to time and may even be absent. The results obtained in the author's series, as will be seen later, confirm these findings.

The effect of previous anti-typhoid vaccination on the specificity of the Widal reaction both in carriers and healthy individuals is of importance. As is well known, agglutinins develope in the serum of inoculated persons and persist there for a variable period. In a survey of 1,000 inoculated persons Baker (1917) found that in a third the agglutinin titre fell below 1 in 25 within the first six months, but in the majority a titre of 1 in 50 persisted up to a year. Where a titre of 1 in 250 was obtained it was invariably found that the individual had suffered from typhoid fever at some previous date and only rarely was this titre due to the effects of inoculation. As a criterion for diagnosis of typhoid fever in inoculated persons he

maintains that the titre must be over 1 in 250 though this may be got, of course, in recently vaccinated individuals. Where a titre of 1 in 125 is present a year after inoculation he holds that in the large proportion of cases it is due to a previous attack of typhoid fever. Brösamlen (1919) whose criterion of a positive reaction, by the microscopic method, is agglutination with a serum dilution of 1 in 100, found in healthy individuals a positive reaction in 100 per cent. of cases examined within 2 or 3 weeks after anti-typhoid vaccination. After 1 to 3 months 95 per cent. still gave a positive result, and thereafter the number slowly decreased. At the end of a year 56 per cent. still remained positive, and after 2 years 41 per cent. agglutinated in a dilution of 1 in 100. He found that the agglutinin titre did not become affected by bacterial or toxic stimuli other than by infection with B, typhosus or related organisms. Likewise Shera (1922) found a positive serum reaction for B. typhosus in 6 inoculated ex-soldiers persisting for 4 or 5 years. In all of these men the serum agglutinated in a dilution of 1 in 50 for B. typhosus and in 4 up to 1 in 250. The paratyphoid agglutinins were very slight in all. Similar results were found

by Pijper (1922) and by Dunlop (unpublished). The latter studied a case of a healthy man, prophylactically vaccinated a number of times in the Army with "T" alone and with mixed "T.A.B." vaccine, who never suffered from typhoid fever, but whose serum 3½ years after the last vaccination agglutinated B. typhosus in a dilution never less than 1 in 125 for any suspension used. By Dreyer's technique there were 30 and 12 standard agglutinin units present per c.c. for B. typhosus and B. paratyphosus A respectively. Where there has been antityphoid vaccination within 6 to 18 months previously Browning suggests on the basis of Martin and Upjohn's (1916) studies, that agglutination with a dilution of more than 1:1,000 strongly suggests true infection with B. typhosus.

Thus it will be seen that care must be exercised in determining the influence of previous antityphoid vaccination on carriers.

Can the agglutinins due to vaccination be distinguished from those due to a present or previous infection?

The studies of Mackie and Wiltshire (1917) on the agglutinins found in the blood of persons suffering from one of the paratyphoid infections who had been inoculated at some time previously

against B. typhosus alone, are worth recording. In practically every case there was a definitely elevated degree of agglutination with B. typhosus and this was frequently equal to that obtained with the infecting paratyphoid bacillus. Absorption tests showed that these results were due to the presence of two specific agglutinins. In two cases there were agglutinins present for both B. paratyphosus A and B in addition to B. typhosus. Absorption tests showed the presence of three specific agglutinins.

Thus it would appear impossible to distinguish these in the case of carriers.

Diagnosis of Mixed Infections by agglutinin absorption and other methods.

The absorption or saturation test of Castellani (1902) might also be employed in carriers to prove which is the primary specific agglutinin present and which are the heterologous or co-agglutinins in the patient's serum.

From his experimental work on animals immunised against a certain micro-organism, Castellani found that two or more agglutinins might be present in the serum and that when saturated with the first micro-organism all agglutinins were removed, but when saturated with the others its agglutinating power upon the first was reduced little, or not at all.

Likewise, the serum of an animal immunised against two micro-organisms, A and B, loses its agglutinability, when saturated with A, only for A. Saturated with A and B it loses its agglutinating power for both. These facts may be applied in the diagnosis of mixed infections, and to the differentiation between between closely allied germs. Suppose, for instance, the serum from a typhoid case agglutinates the laboratory cultures of B. typhosus and of a variety of B. coli, saturate the serum with typhoid bacilli; if the serum loses its agglutinating power for the typhoid bacillus only, it is a case of mixed infection with both the typhoid and colon bacillus; if the serum loses its agglutinating power for both B. typhosus and B. coli, it is a pure typhoid infection, the coliform organism having been agglutinated by the group-agglutinins produced by the typhoid bacillus.

Castellani's work is confirmed by Conradi(1904), Harvey (1909 and 1915), Gratton and Wood (1911), Gratton and Harvey (1911) and Taylor (1918). These last four observers all emphasize its value in determining mixed infections of typhoid with paratyphoid A and B bacilli.

The suggestion made by Dreyer and Inman (1917) on the necessity of carrying out successive observat-

ions at four day intervals so as to yield agglutination curves for the diagnosis of typhoid and paratyphoid fevers in inoculated persons might possibly be of value in the diagnosis of carriers. They point out how misleading it is to draw conclusions from a single observation of the agglutinin content of the serum, since in some instances the titre for the infecting organism may be found less than that of inoculation agglutinins and may, indeed, be lower throughout the whole infection. The value of continued examinations of the agglutinins content of the sera of carriers, illustrating the fluctuation in the homologous and co-agglutinins is discussed fully under the author's own observations.

Aoki and Konno (1921), by the use of typhoid immune serum obtained from hyper-immune animals and showing strong group-agglutination, demonstrated that paratyphoid B. bacilli can be divided into two sub-groups, one which easily, and the other which with difficulty, group-agglutinated. They found that the titre of the group-agglutination of B. paratyphosus B in typhoid immune sera depended on the strain of that organism employed. Typhoid sera which easily agglutinate B. paratyphosus B in as high a dilution as B. typhosus, i.e. 1 in 20,000, exhibited

a much lower titre, e.g. 1 in 50 or 1 in 100, in the case of a difficulty group-agglutinated strain of B. paratyphosus B. By the application of a difficulty group-agglutinated strain of paratyphoid B. bacilli and a serum showing equally high titre with both B. paratyphosus B and typhoid bacilli one may determine whether an individual is infected with typhoid bacilli alone or is suffering from a mixed infection with B. paratyphosus B.

Such a strain might be employed with advantage for the serum of carriers which showed a marked group-agglutination for B. paratyphosus B and B. typhosus in determining which was the offending organism, or whether a mixed infection is present.

A novel means of distinguishing the primary agglutinin from heterologous agglutinins present in a patient's serum is advanced by Fishberg (1923), where the end titre of the serum for typhoid or paratpyhoid B is so nearly the same that it is impossible to say in the absence of a positive culture, which is the infecting organism. Consideration of the agglutination type, he states, may be of great aid in such a dilemna. If early inspection (after 10 - 15 minutes at 37°C) of the tubes containing typhoid bacilli shows a labilotropic

(coarse) agglutination, the infecting organism is the typhoid bacillus since the normal agglutinins are stabilotropic (fine) and the typhoid-paratyphoid group have in common only some stable receptors. But he emphasizes that absence of coarse agglutination with typhoid bacilli does not absolutely exclude a typhoid infection for there are rare typhoid sera, containing almost exclusively stabilotropic (fine) agglutinins, though in his series of 31 cases none were definitely of this kind, but all showed some stabilotropic antibodies. Similarly in paratyphoid infections the coarse agglutination is found in the tubes containing the paratyphoid bacilli, the agglutination of B. typhosus here being fine and flaky. Fishberg states that in his experience Castellani's absorption experiment often fails to attain its goal.

Thus from the literature it will be seen that the value of the Widal test in chronic enteric carriers, especially where these have received previous anti-typhoid vaccination, is slight, as the agglutinins developed by the inoculation of the specific organisms may persist for years. Repeated examinations of the excreta with isolation of the specific organisms would need to be carried out before any actual diagnosis could be made in these subjects. The chief value in the test lies in its acting as an indicator of the possible

presence of the carrier condition. Repeated tests would require to be made on subjects which give a negative result before finally deciding that they are not carriers.

Author's Observations.

The technique employed in all the Widal tests is the macroscopic method as described in the report to the Medical Research Council on "The Laboratory Diagnosis of acute intestinal infections, including the principles and practice of the Agglutination test (1920 p.128) i.e. the Oxford Standard Method (Dreyer)." Where Standard Agglutination occurred between the various dilutions an average was made by comparing the standard control tube and the tubes above and below standard agglutination, for the purpose of working out the number of standard agglutination units present per c.c. of patient's blood. 10 standard agglutination units may be taken as an ordinary standard of what constitutes a positive Widal reaction.

Variation in the agglutinating power of the sera from Chronic Enteric Carriers.

The agglutinating power of the sera from the different carriers fluctuated markedly and in several carriers of B. typhosus (M.M. & K.O.) increased agglutinating power for B. paratyphosus A

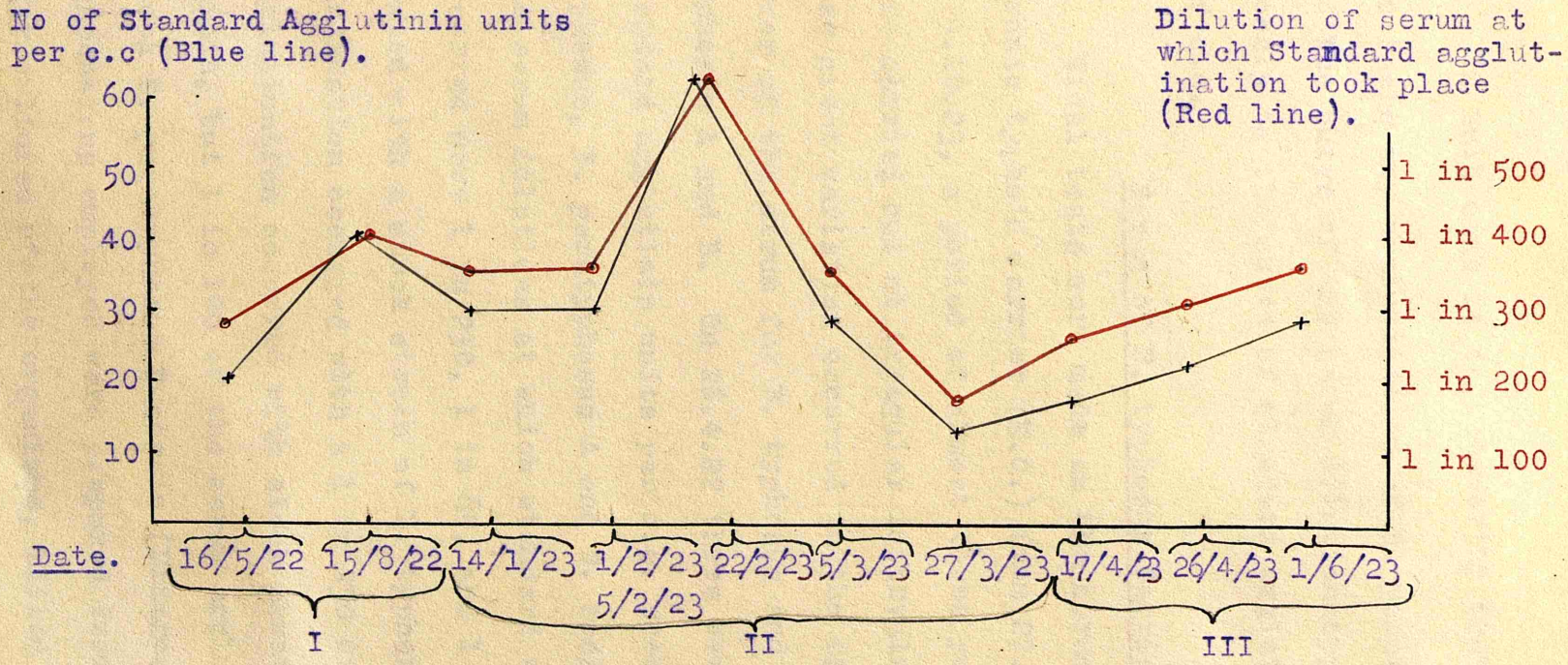
or B has been observed from time to time; similarly the sera of the carriers of B. paratyphosus B (M.D. and B.E.) have shown at times a marked power of agglutinating B. typhosus. In the case of H.T. there was a suspicion that there might be a mixed infection for B. typhosus and B. paratyphosus B, since her serum on repeated tests showed an abnormally high agglutinating power for B. paratyphosus B as well for B. typhosus, and B. paratyphosus B was isolated from the faeces on five occasions, but when the patient's gall-bladder was excised, only B. typhosus was recovered from its contents. This case was found by McKendrick to give a positive cutaneous hypersensitiveness test to B. typhosus and a slight positive to B. paratyphosus B.

Serum of B. typhosus excreter (J.M.)

The serum of the carrier J.M. was examined on eleven occasions at irregular intervals from 16.5.22. to 1.6.23. and the number of Standard agglutinin units per c.c. fluctuated from 12 to 59, while the serum dilution at which standard agglutination occurred, fluctuated from 1 in 100 to 1 in 500.(Chart I.)

CHART I.

CHART TO ILLUSTRATE FLUCTUATION OF AGGLUTINATING POWER OF SERUM OF CARRIER J.M.FOR B. TYPHOSUS.



At period 1 the standardised emulsion of B. typhosus had the factor 7.3.

" " 11 " " " " " " " " 8.5.

" " 111 " " " " " " " " 10.0

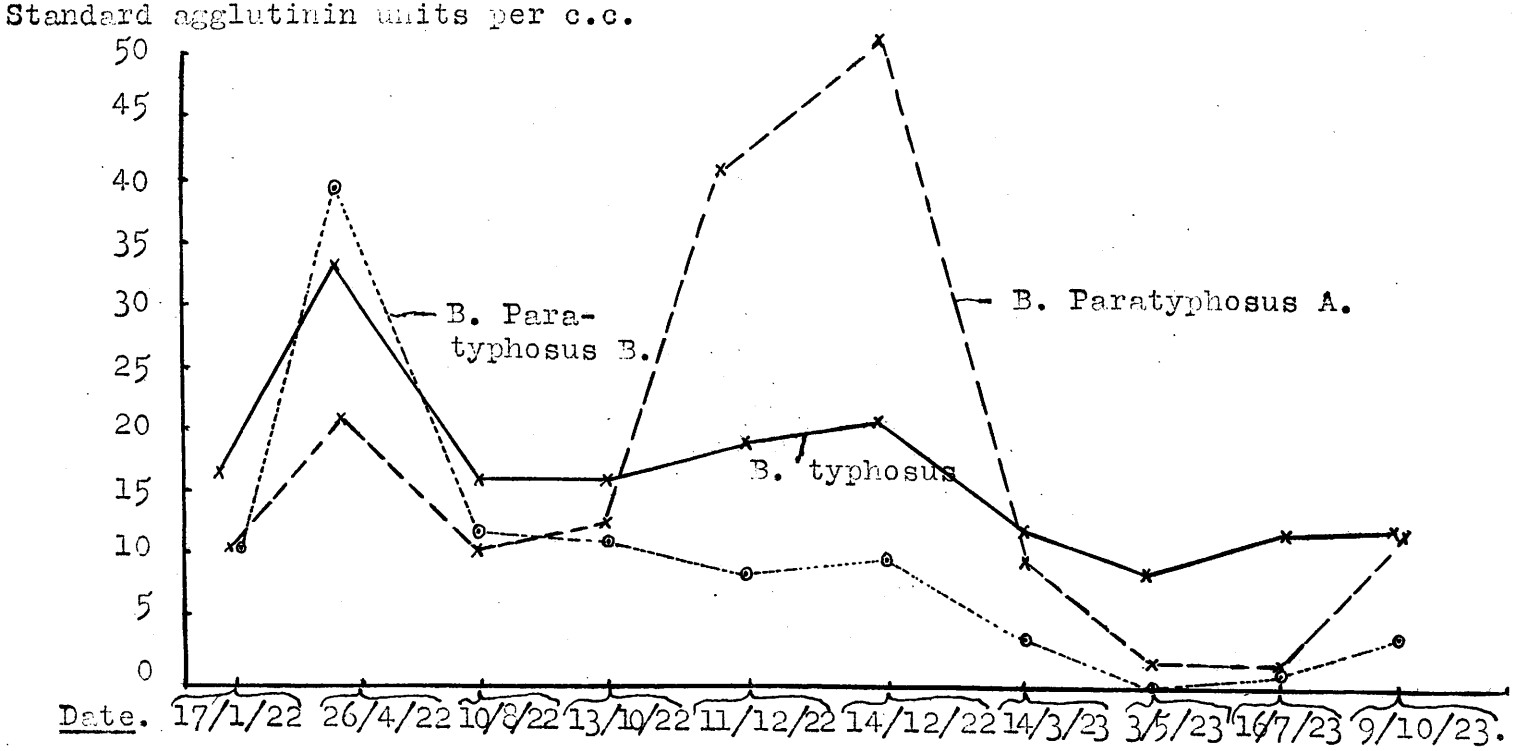
In this case it is noteworthy that at no time was there any agglutination of B. paratyphosus A or B. The curve of the serum dilution approximates very closely to the curve of standard agglutinin units per c.c.

Serum of B. typhosus Carrier (K.O.)

Widal tests were made on the serum of another chronic typhoid carrier (K.O.) from 27.10.21 to 9.10.23, a period of almost three years. These were carried out at irregular intervals. In this case great variation occurred in the agglutinating power of the serum for B. typhosus and B. paratyphosus A and B. On 26.4.22 there were 31,20 and 40 standard agglutinin units per c.c. present for B. typhosus, B. paratyphosus A and B. respectively. The serum dilutions at which standard agglutination occurred were 1 in 250, 1 in 80, and 1 in 125. Repeated with a stock strain of B. typhosus (R.L.L.) agglutination occurred with a 1 in 250 dilution, no agglutination occurred with stock paratyphosus A strain, but 1 in 100 of the serum agglutinated the stock B. paratyphosus B strain (Primrose). The suspensions employed were prepared from 24 hour agar cultures of the organisms, killed by heating in the water bath at 56° C. for one hour. They

CHART II.

CHART TO ILLUSTRATE VARIATION OF THE AGGLUTINATING POWER OF THE SERUM OF B. TYPHOSUS CARRIER K.O., FOR B. TYPHOSUS AND B. PARATYPHOSUS A AND B.



Note:- B. typhosus - continuous line.
B. paratyphosus A - broken line.
B. paratyphosus B - dotted line.

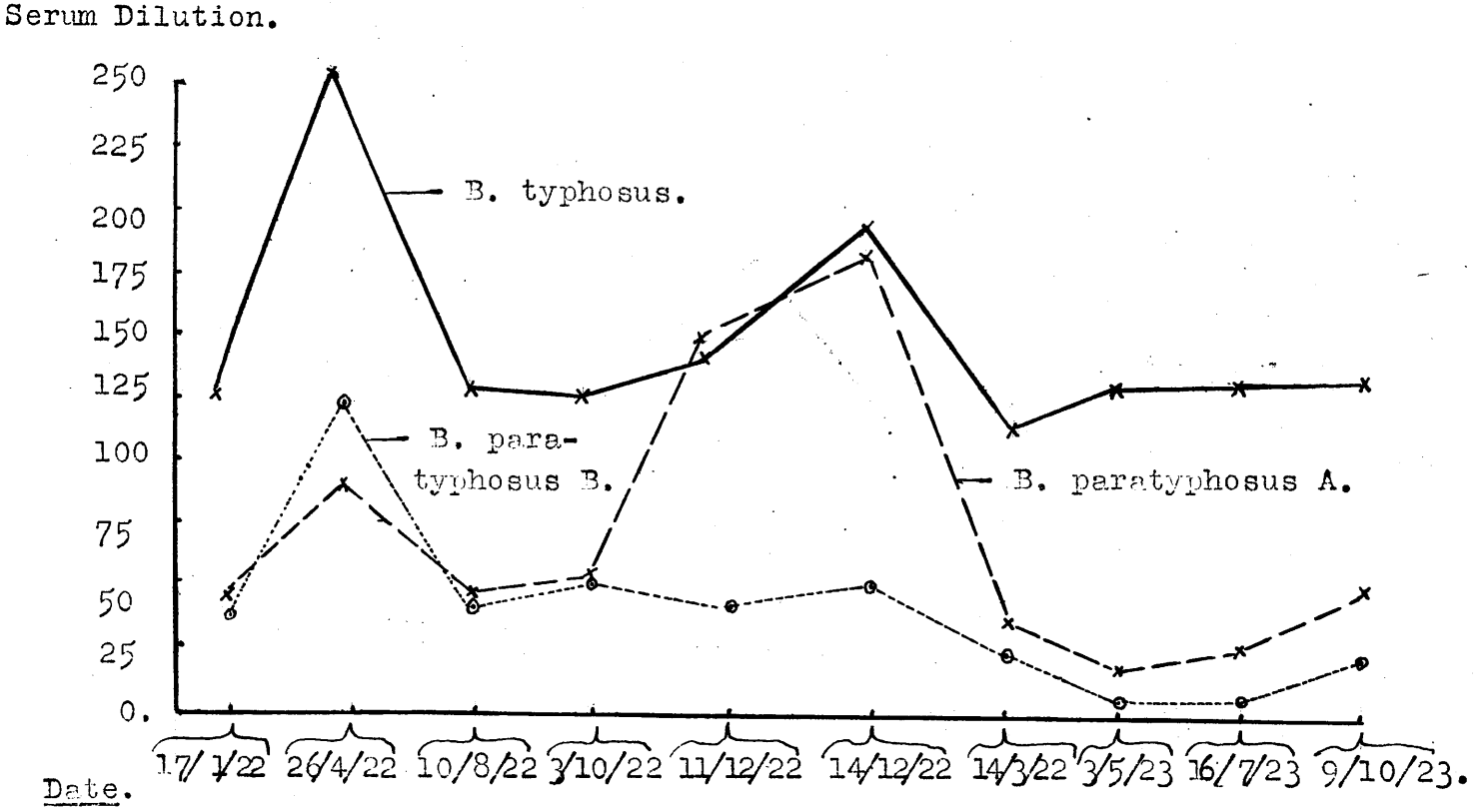
TABLE XI.

TABLE TO SHOW FACTORS WHICH THE STANDARDISED EMULSIONS OF B. TYPHOSUS, B. PARATYPHOSUS A AND B. HAD ON VARIOUS DATES USED IN PREVIOUS CHART.

Date.	B. typhosus.	B. para-typhosus A.	B. paratyphosus B.
17/1/22	7.3	3.9	322.
26/4/22.	7.3	3.9	3.2
10/8/22	7.8	3.9	3.2
13/10/22	7.8	3.7	4.6
11/12/22	8.5	3.7	4.6
14/12/22	8.5	3.7	4.6
14/3/23	8.5	3.7	5.7
3/5/23	10.0	3.7	5.7
16/7/23	10.0	4.0	5.7
9/10/23.	10.0	4.7	3.6

CHART III.

CHART TO SHOW, IN SAME CASE K.O., DILUTION OF SERUM AT WHICH STANDARD AGGLUTINATION OCCURRED.



Note:- The rulings are the same as for the previous chart.

contained approximately 100,000,000 organisms per c.c. The technique was otherwise similar to the drop method of Dreyer. Similarly on 11.12.22, repeated on 14.12.22 with the same result but with a different specimen of serum, 18 standard units per c.c. were present for B. typhosus, 41 for B. paratyphosus A and 8 for B. paratyphosus B. Here the corresponding serum dilutions at which standard agglutination occurred were 1 in 150, 1 in 150 and 1 in 40. (see Charts II and III, and Table XI).

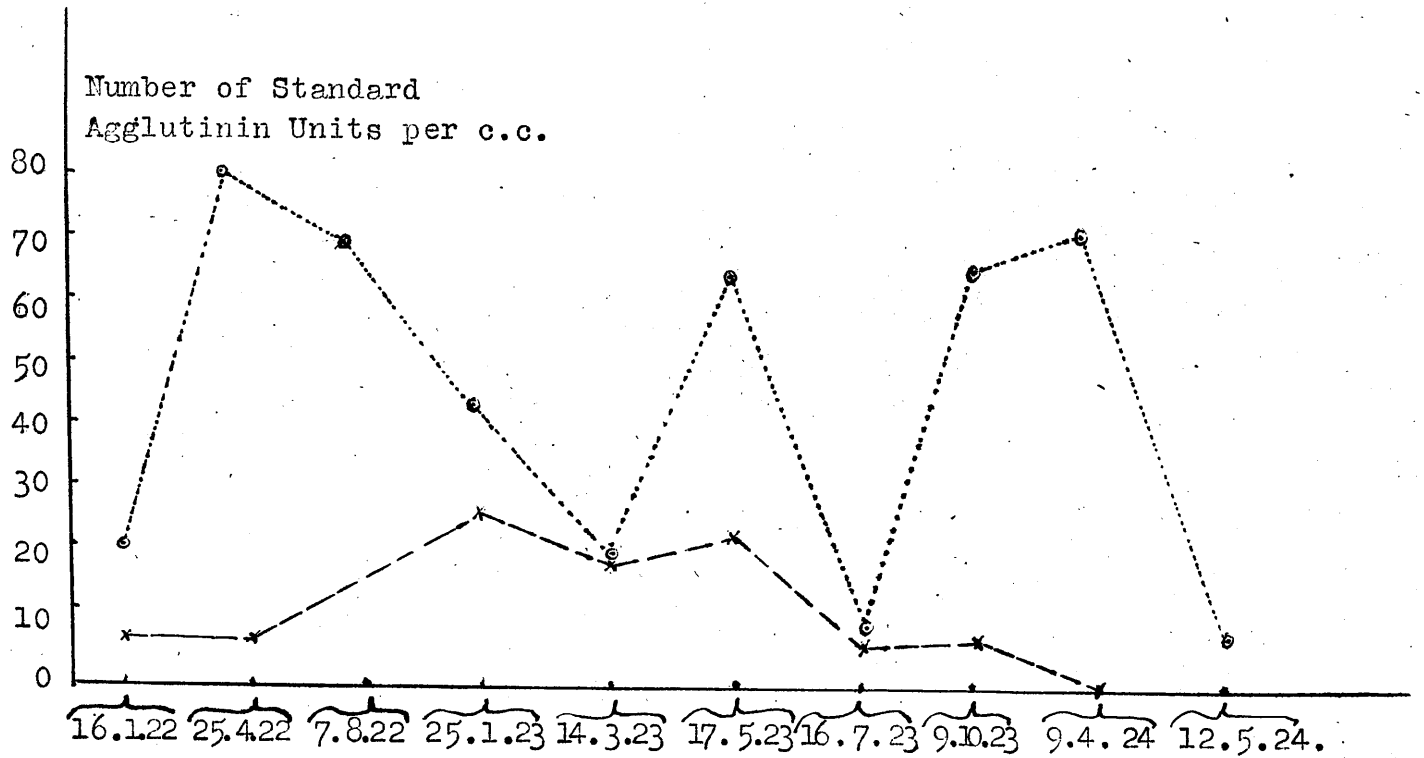
Serum of B. paratyphosus B. Carrier (M.D.).

In the case of the paratyphoid B. bacillus carrier (M.D.) practically no co-agglutination of the serum for B. typhosus and only a little for B. paratyphosus A occurred except on 27.1.23, 14.3.23 and 17.5.23, when a marked increase took place. This is well illustrated in the following chart (IV) which also shows the marked fluctuations of the agglutinins in the serum for the infecting organisms

The dilution in which the serum showed standard agglutination for B. paratyphosus B varied from 1 in 40 to 1 in 320; for B. paratyphosus A from 1 in 25 to 1 in 200; at no time did agglutination for B. typhosus occur over a dilution of 1 in 25. The titre of the serum for B. paratyphosus B. was

CHART IV.

CHART TO ILLUSTRATE THE CO-AGGLUTININS FOR B. PARATYPHOSUS A AND FLUCTUATIONS OF THE HOMOLOGOUS AGGLUTININS IN THE SERUM OF THE B. PARATYPHOSUS B. CARRIER (M.D.).



The rulings here are the same as in previous charts.

consistently higher than for B. paratyphosus A. The factors of the Standard emulsions used correspond with those shown in table for carrier K.O.(see TableXI)

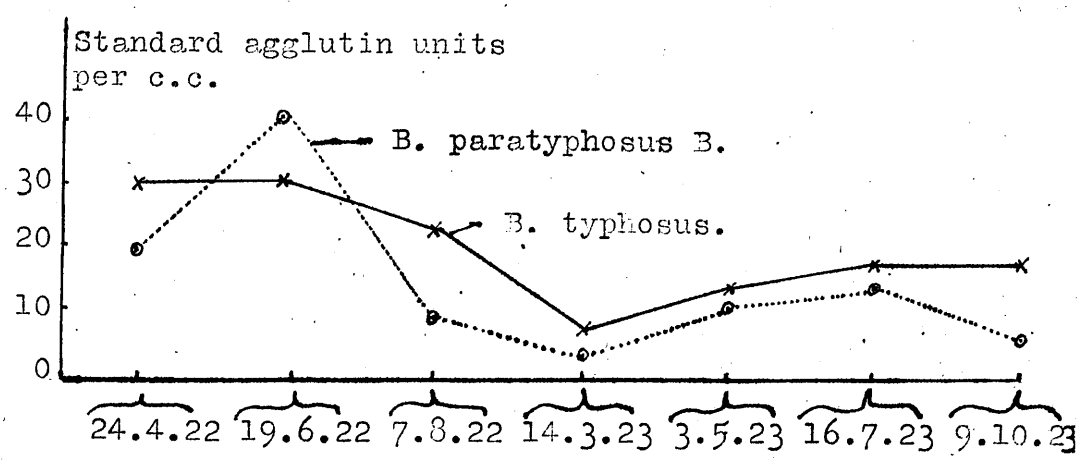
Serum of "mixed carrier" (H.T.).

H.T., the typhoid carrier from whose stools B. paratyphosus B. were occasionally isolated showed a slightly positive Widal reaction for the latter organism, and definite agglutination of B.typhosus as well. On two occasions only, 3.5.22 and 16.7.23, were any agglutinins of B. paratyphosus A observed, when 13 and 10 standard agglutinin units per c.c. were present. Seven observations were made during a period of approximately 1½ years. The standard agglutinins for B. typhosus varied from 31 to 9 and for B. paratyphosus B. from 4 to 39. On 19.6.22 there were 39 standard units present for B. paratyphosus B and 32 for B. typhosus, though on this occasion the dilution of the serum at which standard agglutination occurred was 1 in 125 for the former and 1 in 250 for the latter. The serum dilutions which pro--

duced standard agglutination varied from 1 in 75 to 1 in 250 for B. typhosus and from 1 in 25 to 1 in 125 for B. paratyphosus B.

CHART V.

Chart to illustrate variations in agglutinating power of serum of H.T. for B. typhosus and B. paratyphosus B.



The markings are as before.

On 24.4.22 the patient's serum was tested against a suspension of an agar culture of a stock B. typhosus (R.L.L.) and a stock B. paratyphosus B (Primrose) and it gave agglutination in a dilution of 1 in 200 and 1 in 30 respectively. When tested by Dreyer's method 31 standard units per c.c. were present for B. typhosus and 20 per c.c. for B. paratyphosus B. Standard agglutination occurred in dilutions of 1 in 250 for B. typhosus and 1 in 65 for B. paratyphosus B.

As regards the remainder of the series of carriers

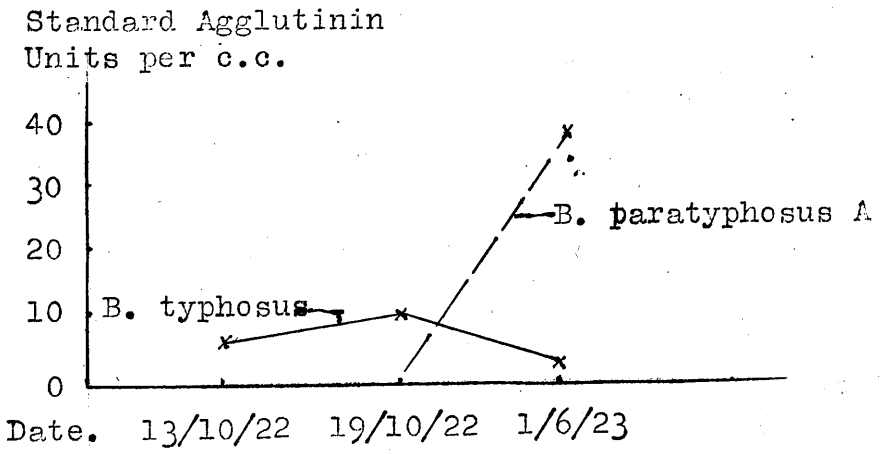
The agglutinating power of the serum of B. typhosus carrier Mrs. G. was 1 in 100 for B. typhosus, B. typhosus excreter Mrs. R. 1 in 85 or 8.5 standard agglutinin units per c.c. In neither were any co-agglutinins present. They were only tested on one occasion.

Serum of B. typhosus Excreter (M.M.).

In the case of the B. typhosus carrier M.M. it is interesting to record that on 13.10.22 and 19.10.22 no co-agglutinins were present in the serum while on these occasions there were 9 and 10 standard agglutinin units per c.c. for B. typhosus. When the serum was examined on 1.6.23, 7½ months later, 5 standard agglutinin units for B. typhosus and 34 for B. paratyphosus A were now present, standard agglutination of the serum occurring in dilutions of 1 in 50 and 1 in 125 in spite of the fact that she was indubitably a pure typhoid carrier, B. typhosus alone being isolated frequently from the faeces.

CHART VI.

Chart to illustrate variations in agglutinating power of serum of B. typhosus carrier M.M. for B. typhosus and B. paratyphosus A.



Serum of B. paratyphosus B excreter B.E.

A somewhat similar experience was met with in a paratyphoid B. bacillus carrier, B.E. On 14.12.22 29 standard units per c.c. for B. typhosus, none for B. paratyphosus A and 364 for B. paratyphosus B were recorded. Five and a half months later (30.4.23) the figuresthen were, 10, 54 and 9 respectively. As this result was so peculiar the test was repeated with the same specimen of serum with approximately the same result. Standard agglutination occurred with serum dilutions of 1 in 100 for B. typhosus, 1 in 200 for B. paratyphosus A and 1 in 50 for B. paratyphosus B.

It is noteworthy that none of the carriers examined had received previous anti-typhoid vaccination,

though, as is described later, during the time some of them were under observation vaccines were given both subcutaneously and per os. in certain cases.

SUMMARY.

Thus in all the 8 carriers tested the Widal reaction was positive for the type of organism excreted if a serum dilution of 1 in 100 for B. typhosus and 1 in 200 for B. paratyphosus B be taken as the criterion, but extreme variations occurred throughout the time they were under observation, especially with regard to the presence of co-agglutinins. Increased agglutinating power for B. paratyphosus A and B has been observed from time to time in carriers of B. typhosus (M.M. and K.O.); similarly the sera of the carriers of B. paratyphosus B (M.D. and B.E.) have shown at times a marked power of agglutinating B. typhosus. On these occasions possible mistakes in diagnosing the type of carrier could be made, if the agglutinating power of the serum were alone relied upon. The Widal reaction should, therefore, only serve as a guide as to the nature of the offending organism and the final test must necessarily be the isolation from the faeces or urine, of the organism. Periodic rises in the agglutinin content of the sera of K.O and M.D. took place, but this may

have been influenced to a certain degree by the effect of the treatment these patients were undergoing for the cure of their conditions, though this is doubtful in the case of the latter (M.D.).

Influence of treatment on the Widal reaction in Chronic Carriers.

Chemotherapy. The administration of dried Ox-bile corresponded with a fall in the agglutinating power of the serum of the typhoid carrier K.O. for B. typhosus, B. paratyphosus A and B. The ox-bile was given from 22.5.22 to 14.8.22; from the examination of the chart on p.101 it will be seen that the number of standard units per c.c. of patient's serum was:-

Date.	T.	A.	B.
26.4.22.	31	20	40
10.8.22.	16	10	11
13.10.22.	16	13	11.

This result of giving dried bile was not confirmed in the case of M.D, and only to a slight degree with H.T. No inference can, thus be drawn regarding the administration of ox-bile having any influence on the agglutinating power of the serum in carriers, nor could any relationship be established as regards any other drugs tested.

Effect of giving by mouth a stock typhoid vaccine on the Widal Reaction of the chronic typhoid carrier J.M.

This is discussed fully under the section on Vaccine Treatment, see p.243. Shortly after administration a definite rise in the agglutinin content of the patient's serum occurred; after the admin - istration had ceased a fall to the original level (29 standard agglutinin units per c.c.) soon occurred.

Effect of Pastilles Antityphique Bilieés, according to Beredka's formula, by Mouth on the Widal Reaction of typhoid carrier (K.O.).

Also fully discussed on p.240 -1 .

Only a very slight increase in the agglutinins in the patient's serum for B. typhosus and a marked rise for B. paratyphosus A took place.

Pastilles furnished by Villette, 3 Rue Maublanc, Paris XV.

Effect of "Residual" Vaccine of a stock B. typhosus (R.L.L.) on the Widal reaction of the B. typhosus carrier J.M.

Before inoculation the serum of this case contained 29 standard agglutinin units per c.c. for B. typhosus; a week after treatment it contained 12 units; then during the course of the next month the content gradually rose to 25 units (Ref. pp. 244).

A description of the preparation of the "Residual" vaccine is given on p.243 - 4.

During their experiments on the treatment of

patients suffering from Rheumatoid Arthritis by protein shock, Campbell (1924) and Dunlop (1924) inoculated a patient J.G. with some of the same residual vaccine above described and in consequence there was a marked generalised reaction, characterised by diarrhoea and a sharp rise in temperature. This case is described in detail by Dunlop. (1924).

J.G., female aged 27, was admitted to the Western Infirmary, Glasgow, on February 22nd. 1923. Her illness began in June 1920, with generalised pains, involvement of various joints and pyrexia. The patient was confined to bed for ten months, after which gradual improvement set in. Prior to admission stiffness and pain in the joints had been occasioning considerable disability. There was no history suggestive of enteric fever. Physical examination, on admission, was negative except for the local condition of the affected joints. Details of agglutinin content of serum and of treatment with residual vaccine are as follows:-

Date.	Agglutinins.	Intravenous Dose.
March 1st. 1923.	Nil.	150 millions.
" 5th	Nil.	250 "
" 13th	Nil.	400 "
" 23rd.	1/8.8	400 "

April 3rd.	58.8	Nil.
" 17th.	Nil.	100 millions.
" 24th	29.4	Nil.

The agglutinins are expressed in standard units per c.c. and the titres were confirmed by repeat tests.

The Widal reaction previous to inoculation was absolutely negative but immediately afterwards rose to 59 standard agglutinin units per c.c. for B. typhosus, then gradually fell until 29 units per c.c. were present a month later.

Another similar case, J.Mck., is described by Dunlop; female aged 17 who was admitted to the Western Infirmary, Glasgow on September 20th. 1921, and who had suffered from infective arthritis in December 1919, with pyrexia which lasted several weeks. The Widal reaction at this time was reported to be positive, although clinically the patient's doctor did not think the case to be one of enteric fever. Improvement took place but between March and June 1920 there was a recurrence of acute arthritis and the pyrexia. Similar recurrences took place in October 1920 and in February and April 1921.

On admission physical examination was negative, apart from the local condition of the affected joints which showed periarticular thickening and limitation of movement. The temperature was normal but became

slowly irregular towards the end of the first week in hospital. Details of the course of intravenous doses of residual vaccine administered and of the rise in the agglutinin titre of the serum were as follows:-

Date.	Agglutinins.	Intravenous Dose.
September 29th 1921.	3.6	125.
October 6th	3.6	200
" 11th	355.0	250
" 19th	714.0	250
" 25th	205.0	250
November 3rd	--	150
" 23rd.	35.0	Nil.

Each of the first three injections was followed by protein shock but the resulting temperature, instead of returning to normal within 24 hours, remained of remittent or irregularly intermittent type untilthe next dose of vaccine. The spleen became palpable and the affected joints tender. After the fourth dose the temperature curve returned to normal at the end of 48 hours, while those following the last two doses were of the usual protein shock types. The vaccine was being given to other patients in the ward at the same time and none of them showed any departure from normal in the resulting reactions nor

did any of them develop a rise in agglutinins. Moreover, each ampoule of vaccine contained sufficient for two doses, and the first injection administered to this patient was taken from one, the remainder of which was used for a second patient whose subsequent reaction was of the usual type. Bacteriological examination of specimens of faeces from the case, was carried out on three occasions, but no colonies of B.typhosus were obtained either by direct plating on MacConkey's Medium or by the brilliant green enrichment method.

The findings in the case J.G. are very similar to those detailed except that (a) the agglutinin titre did not reach such a high level, (b) the irregular pyrexia did not set in until after the third dose of vaccine (the first to evoke a satisfactory protein shock in this patient), but it persisted for practically two months; and (c) splenic enlargement was not found.

According to Campbell and Dunlop, in the examination of twelve patients, a rise in the agglutinin content of the serum is the usual sequel, but in two a distinct fall in the agglutinin content occurred with the ordinary commercial "T.V." vaccine. The reverse was the case after inoculation with "residual" vaccine. In ten patients and many rabbits no protein

shock and no antibody increase occurred in the serum in spite of large doses both subcutaneously and intravenously, but in the two cases above described the agglutinin content of the serum rose sharply following protein shock. This result, taken in conjunction with the evidence submitted, and which points to the absence of agglutinogenic power on the part of the "residual vaccine injected, would suggest that non-specific therapy may possibly be followed by the appearance of highly specific antibodies in the blood of a patient so treated. From the history of one of the cases (J.McK.) there were grounds for suspecting that there may have been a previous typhoid infection, but in the other such evidence is lacking. It was thought that this patient (J.G.) might in consequence be a typhoid carrier but the author was unable, in 17 bi-weekly examinations of the urine and faeces extending over a period of a month, to isolate any typhoid bacilli.

Effect of feeding B. lactis aerogenes in milk cultures and subcutaneous injection of a vaccine of the same organism, on the agglutinating power of the serum of paratyphoid B. carrier M.D., and typhoid carrier, J.M.

Full details regarding the administration and dosage of the milk and vaccine are given on pp. 224 - 6 & 405-7. Here it suffices to note that no marked variation

in the agglutinating power of the serum occurred either during or after administration in the case of J.M. In this case no vaccine was given. With the serum of M.D., however, who received subcutaneous injections of killed cultures, as well as living organisms by mouth, there occurred a definite rise in the agglutinin content.

Table to show effect of feeding of B. lactis aerogenes milk culture by mouth and vaccination by the same organism, to M.D.

Date.	Dosage of Milk feeding.	Dosage of subcutaneous injection of vaccine.	Content of serum in Standard agglutinin units per c.c. T.	A.	B.
14.3.23.	-	-	1.	16.	16.
12.4.23.)	4 ounces tid.	5 millions increase to 1,000 millions in 10 injections.			
2.5.23.)	throughout all				
17.5.23.)	this period.		0.	50.	56.
16.7.23.)	-	-	0.	5.	7.

Thus in this case a marked rise of agglutinins for B. paratyphosus A and B occurred from 16 to 50 and 56 standard units per c.c. respectively. Only a slight agglutination of B. lactis aerogenes was got with a 1:50 dilution of the patient's serum a fortnight after the vaccine was discontinued. There occurred a transformation of the intestinal flora

to one in which B. lactis aerogenes predominated. Only a few of these organisms were found for the next two or three weeks after stoppage of administration by mouth.

Effect of Surgical treatment on the Widal reaction.

K.O., the typhoid carrier, was operated on 4.5.23 when cholecystgastrostomy was performed. B. typhosus continued to be excreted in the faeces till 31.5.23 and since that date the faeces have consistently remained typhoid free- although weekly examinations have been carried out for two years subsequent to operation.

The serum on 3.5.23 just before operation showed 9 standard agglutinin units per c.c. for B. typhosus, and 3 for B. paratyphosus A. On two subsequent examinations made on 16.7.23 and 9.10.23 after operation the agglutinin content of the patient's serum was 12 units for B. typhosus and 3 for B. paratyphosus A. but in the latter examination a slight rise to 11 units per c.c. for B. paratyphosus A occurred. A cutaneous hypersensitiveness test on 2.11.23 carried out by R. Cruickshank proved negative.

A somewhat similar result was obtained in the case of the supposed mixed typhoid and paratyphoid carrier, H.T., who had cholecystectomy performed

on 4.5.23. She continued to excrete typhoid bacilli in the faeces till 28.6.23, but has subsequently been also typhoid free until her death on 28.2.25. The Widal reaction in her case was as follows:-

	Standard Units per c.c.		
	T.	A.	B.
Before operation (3.5.23)	15.	13.	12.
2½ months after operation (16.7.23)	20.	10.	16.
5 months after operation (9.10.23)	20.	1.	7.

Likewise in this case a "Skin test" was performed on 2.11.23 with a similar negative result for B. typhosus and B. paratyphosus A and B.

Accordingly in both cases there has been no material effect on the agglutinin content of the serum during 5 months following operation, although an apparent cure has occurred. If anything, there has been a slight rise in the agglutinating power. The skin test in these cases 5 and 4 months respectively after the last positive faeces result, proved negative, whereas earlier on it had been found by McKendrick to be positive when they were actively excreting the specific bacteria.

As regards the third carrier, M.D., who had cholecystgastrostomy performed on 17.7.23 but who still continued to excrete B. paratyphosus B. in the

faeces, the findings are of no value in this connection, although it might be noted that two months after operation a marked rise from 7 standard agglutinin units per c.c. to 55 was noted. In spite of the fact that the patient was still actively excreting B. paratyphosus B. in the faeces, Cruickshank on 2.11.23 had a negative result with the cutaneous hypersensitiveness skin reaction.

The Complement Fixation test has been suggested by Schone (1908) as a means of diaghosing the typhoid carrier condition. He found complement diviating substances in the blood of three typhoid carriers and claims that this reaction can be obtained at least as constantly as agglutination. His three carriers gave the following only partly satisfactory results:-

	History.	Agglutn. 1:50.	Complement Deviation.
Case I.	Typhoid fever 10 years previously.	-	+
Case II.	In contact with typhoid patient two years previously.	-	-
Case III.	Typhoid fever 12 weeks before.	+	+ +

Garbat (1922) claims that 85 per cent. of carriers give a positive test though not all carriers, even permanent ones do not necessarily continue to give the fixation test. A persisting fixation test, he

thinks, is dependent directly upon the number of bacteria which have invaded the body and the length of time they have remained there. In Nichols, Simmons and Stimmel's (1919) case the blood remained positive even after a cure by cholecystectomy had been obtained.

Henderson Smith (quoted by Ledingham and Arkwright) demonstrated specific immune bodies in the sera of two carriers, both of whom gave negative Widal tests.

Pijper (1922) regards positive complement fixation as satisfactomy since it is frequently positive when the Widal test is negative and inoculated persons usually possess no specific immune bodies. Howell (1916) disagrees with this result as she constantly found specific antibodies present in human subjects vaccinated with typhoid vaccine, and showed that they reach their highest concentration in one or two months, after which they gradually diminish.

As the agglutination titre does not rise pari passu with the increase of the immune content of the serum, it may be of value to perform a complement fixation test in addition to the preliminary Widal reaction. The complement fixation test using guinea-pig serum as the source of complement is open to fallacies (unpublished - work of Dr. E.M. Dunlop) which invalidate results of the test, and render them

inconsistent. On one occasion the author got positive results with three carriers (K.O.,H.T., and M.D.) following the technique described by Browning (1918). On another occasion a complete failure was met with in the same three cases using guinea-pig serum as complement.

It is worth noting here that in 1910 Dean described a method for the detection of B. typhosus on plates from the faeces by complement fixation. He found that it was possible to detect the presence of typhoid antigen on mixed plates by complement fixation experiments with extracts of washed off growth and a specific antityphoid serum, and obtained 81 out of 85 positive results from actual or suspected typhoid carriers.

Autolysate Precipitin Reaction. A new method for detecting typhoid infection which depends on the presence of autolysed typhoid bacilli in the excretions, along with specific antityphoid serum from the rabbit, is reported by Laird, Gonover and Butts (1923). A small quantity of the patient's stool is emulsified in normal saline and allowed to stand until the insoluble part settles. The supernatant fluid is decanted, centrifugalised and decanted again. After the addition of diatomaceous earth and filtration,

it is ready for the test. Urine free from albumen is similarly prepared. A precipitin rack is then set up with three tubes for each patient; to the first and second tubes 1 c.c. of typhoid serum is added; 1 c.c. of the filtrate of suspected faeces or urine is added to the first tube and a like amount of normal faeces or urine to the second. In the third tube 2 c.c. of the specimen undergoing examination are placed. The rack is then allowed to stand one hour at room temperature, inthe ice-box overnight and at room temperature for about four hours. Positive specimens show a precipitin or turbidity in the front row, while the controls in the second and third rows are clear. The writers have employed the test in 152 specimens of stools and urine from typhoid patients as well as with the autolysate of other pathogenic intestinal organisms, and have found that it is clearly positive in all case of typhoid fever up to the 56th day and in all carriers examined, including one of eighteen years'standing.

Opsonins. The value of opsonin determinations in the discovery of typhoid carriers was first indicated in 1908 by Ledingham, who examined the serum of two typhoid carriers with regard to their opsonin index, using inactivated and complemented serum, and found the index very high both to the carrier's own

strain and that of the other carrier. Gaehtgens (1909) published the results of his study of the opsonic index in 12 typhoid and 2 chronic paratyphoid carriers. He found that typhoid convalescents who did not become carriers exhibited an opsonic index above normal only for a short period, three or four months at the outside, while typhoid carriers had a persistently high index, irrespective of the lapse of time since recovery from typhoid fever or even in the absence of any history of typhoid fever. As all but one of these carriers had an index above 1.4, the average being 2.8 (Gaehtgens used unheated serum and his counts are therefore not as high as Ledingham's), and as 25 per cent. had failed to agglutinate in dilutions higher than 1:50, he considers the opsonic index of more value in the detection of carriers than the agglutination test. His two paratyphoid carriers, on the other hand, gave only normal indices, a result which he thinks may be due to the fact that the laboratory strain used had lost its virulence, but he believes that this may prove to be a distinction between typhoid and paratyphoid carriers. Hamilton (1910) ~~who~~, in an examination of 24 cases of chronic cholecystitis found 7 enteric carriers; 3 with B. paratyphosus B; 2 with B. paratyphosus A; 1 with B. typhosus and one mixed carrier of B. typhosus

and B. paratyphosus B. The serum of 5 (71%) agglutinated their own bacilli or a stock strain or both in a dilution of 1:50 or higher. No non-carrier agglutinated any strain in a dilution as high as 1:50, while the case of mixed infection agglutinated B. typhosus in a dilution of 1:80 and B. paratyphosus B 1:100. All seven had an abnormal opsonic index to any strain. In cases with acute symptoms the index fluctuated, falling below normal at times and again rising very high, while in cases free from acute symptoms the index was persistently high, never falling to normal. In the case of mixed infection agglutination and an abnormal opsonic index for both organisms were found. She concludes that the opsonic index is a very valuable aid in the discovery of bacillus carriers, as no decidedly abnormal index was found in any of the non-carriers examined. As in the case of agglutination a group action for opsonins may exist since Schottmüller and Much (1908) found the opsonic index of typhoid patients high for paratyphoid bacilli as well. Clark and Simmonds (1909) similarly found the index might be even higher for the paratyphoid group than for B. typhosus.

As the author has not made any observations on the opsonin content of the series of carriers investigated by him he is unable to pass any definite

opinion on the relative value of the opsonic index in the discovery of typhoid carriers. However, it provides an additional method which may be worth investigation.

B. Cutaneous Hypersensitiveness. The Cutaneous Hypersensitiveness test for the diagnosis of typhoid fever was introduced by Zupnik (1908). Gay and Force (1914) by the use of a typhoid antigen, to which they gave the name "Typhoidin", applied to a skin abrasion found a definite hypersensitiveness in cases which gave a history of typhoid fever dating back as far as 40 years and also in recently inoculated individuals, and they concluded that a positive result indicated protection against typhoid fever. Thompson (1921) was the first to comment upon the possible value of the method for the detection of carriers and suggested the use of separate inoculations with B, typhosus and B. paratyphosus A and B. McKendrick (1922) investigated 5 of the present series of chronic carriers principally with a view to determining whether, as suggested by Thompson, the skin reaction could be applied advantageously for the detection of enteric carriers. His conclusions were that the test appears to be highly specific for patients suffering from enteric fever and for chronic carriers and that a positive skin reaction in persons

apparently in good health is suggestive that they are enteric carriers. It is noteworthy that the test apparently becomes negative during convalescence, but should a relapse occur it becomes positive once more.

The results claimed by McKendrick have not been uniformly confirmed by other investigators, as Dr. W. Whitelaw (unpublished) obtained a negative result in a paratyphoid B. carrier (B.E.) and a variable result on repeated tests in a carrier of B. typhosus (M.M.) both investigated in the present series. Dr. G. Patterson also had variable results in the case of the carriers (K.O., M.D., H.T.) at a later date, who had been earlier investigated by McKendrick. On 2.11.23 Dr. R. Cruickshank performed the skin test on the carriers (K.O., H.T., and M.D.) with a negative result in all of them; in the first two the faeces had been negative for B. typhosus for 5 or 4 months respectively following Cholecystgastrostomy and Cholecystectomy; in the latter the paratyphoid B. organisms were still actively being excreted in the faeces. All these cases had been found positive by McKendrick at earlier periods in the course of the investigations. The author personally confirmed the positive result in the case of a carrier of B. typhosus (J.M.), mentioned by McKendrick. A

negative result was obtained in the case of a male, aged 48 years (M.M.) who had a perichondritis with abscess over the anterior end of the 4 th rib from which B. typhosus was isolated in pure culture. This patient suffered from his original attack of typhoid fever 6 or 7 months previously. This case is described in detail under another section (see p.359). The technique of the test and the literature on the subject are described by McKendrick. Before any final opinion can be given regarding its value in the detection of chronic carriers further confirmatory work is necessary. The main difficulty is the determination of what constitutes a positive result as, in spite of the saline control, the area of induration in some cases and maroon colour present on the fourth day in known carriers, is so slight that one hesitates to give a definite opinion whether the case is positive or not. A further difficulty is to obtain standardised suspensions of uniform toxicity for use in the test. There is no relationship between the skin test and the Widal reaction in carriers. Cases which had received antityphoid vaccination within 5 years or in which there was a history of enteric fever, were all found by McKendrick to reach negatively. 19 cases of the former and 2 cases with histories of typhoid fever five and nine years before, were tested in all.

C. Alteration of the Blood Picture. Another method described by Wodtke (1920), and recommended by him for the detection of typhoid carrier is the presence of a leucopenia associated with a marked relative lymphocytosis and sometimes eosinophilia, the diminution being in the neutrophile polymorphonuclear leucocytes. McCarthy and Simmons (1924), however, found that there was no leucopenia in any of five chronic carriers observed by them, while it was present in five individuals who were proved not to be carriers.

That an eosinophilia is developed in the blood after antityphoid vaccination was shown by Labor (1916) and Draga (1916). This apparently reaches its maximum in three months and has disappeared by the end of the six months.

To verify the work of Wodtke, blood examinations were made on three carriers (M.D.,K.O., and H.T.).

No obvious leucopenia was discovered in any of them but in one, M.D., a relative lymphocytosis was present; in the other two the differential count was quite normal. In each case 300 leucocytes were counted. There was no eosinophilia present in any.

Differential Blood Counts on Chronic Enteric Carriers.

Case.	Percentage of			
	Neutrophile Polymorphs.	Lymphocytes	Eosinophiles	Large Mononuclears and Trans.
M.D.	48.3	49	1.33	1.3
K.O.	64	36.6	2.5	2.9
H.T.	72	24	1.5	2.5

As the examination was not made until 4.10.23 and K.O. and H.T. had ceased to excrete the specific organisms, for 4 and 3 months respectively, following operations of cholecystgastrostomy and cholecystectomy on 4.5.23 for a cure of the condition and as they are still typhoid free, no definite conclusion can be drawn from their cases. However, in the case of M.D., the relative lymphocytosis would agree partially with Wodtke's work.

Dr. G. Watson, Belvidere Fever Hospital, under whose charge these patients were, for the period before their operations informs me that the blood picture was always normal, there being no leucopenia, relative lymphocytosis or eosinophilia present when he examined the blood.

D. Blood Cultures. Enteric organisms have been isolated from the blood of carriers as long as 14 years after the acute attack (Ebeling 1914). Sieliger and Ludke (quoted Arnd 1923) have also recorded carriers of B. typhosus in the blood who suffered from no ill effects.

Blood Cultures were made from the three cases (K.O., H.T., M.D.) at the same time as the blood counts were made, but with negative result.

As far as can be seen the Widal reaction still remains the safest and quickest method of detecting possible enteric carriers. This view is accepted by the majority of investigators of the problem, such as Good (1923) and others, but carriers must not be diagnosed by it alone.

****

BIBLIOGRAPHY.

Aoki and Konno (1921): Centralbl. f. Bakt., I. abt., orig., Jena, 1921, 86, pp. 330 - 336.

Baker (1917): J.R.N. Med. Service, Lond., 1917, 3, pp. 19 - 29.

Browning (1918): Applied Bacteriology, London, 1918, p. 34.

Brösamlen (1919): Deutsch. Arch. f. Klin. Med., Leipzig, 1919, 129, p. 208.

Busse (1909): Münch. med. Woch., 1908, 55, p. 1113.

Campbell (1924): B.M.J. Dec. 13th. 1924, p. 1107.

Castellani (1902): Ztschr. f. Hyg. u. Infectionskr., 1902, XL, p. 1.

Chesley, Burns, Greene and Wade (1917): A.A.M.A., 1917, 68, p. 1882.

Clark and Simmonds (1908): Munch. med. Woch., 1908, 55 p. 496.

Cler and Ferazzi (1905): Centralbl. f. Bakt. ref. 1905.

Conradi (1904): Deutsch. med. Woch., p. 1165.

Dean (1910): B.M.J. 1910, p. 1516.

Dittmar (1924): Report to Scottish Board of Health, 1924.

Draga (1916): Wien. Klin. Woch., Vienna, 1916, XXIX, p. 1559. Ref. J.A.M.A. 1917, 68, p. 409.

Dreyer and Inman (1917): Lancet, London., i, 1917, 192 pp. 365 - 369.

Dreyer (1909): Journ. Path. and Bact., 1909, XIII, p. 331.

Dunlop (1924): B.M.J. 13th Dec. 1924, p. 1108.

Fishberg (1923): J. Lab. and Clin. Med., 1923, VIII, p. 806.

Gaehtgens (1909): Deut. med. Wchnschr., 1909, 35, p. 1337.

Garbat (1922): Monos. Rockefeller Inst. for Med. Research., No. 16, May 10th 1922.

Gay and Force (1914): Arch. Int. Med., 1914, XIII, p. 471.

Good (1923): Schweiz. med. Woch., Basel, 1923, 53, p. 421.

Gratton and Harvey (1911): J.R.A.M.C. 1911, XVIII, p. 131.

Gratton and Wood (1911): J. R.A.M.C. 1911, XVIII, p. 143.

Hamilton (1910): J. Infect. Diseases, 1910, 7, p. 393.

Harvey (1909 & 1915): Abstracts from Ann. Reports of Div. San. Officers for India, 1909, p. 105, J. R.A.M.C. 1915, XXV, p. 193.

Hilgermann (1917): Deutsche. med. Woch., Berl. and Leipsig, 1917, No. 49, p. 1525.

Howell (1916): J. Infect. Diseases, 1916, 19, p. 83.

Kamm (1909): Münch. med. Woch. 1909, p. 1011.

Kayser (1909): Münch. med. Woch, 1909, pp. 1066.-1130.

Labor (1916): Wien. Klin. Woch, Vienna, 1916, XXIX, p. 1393, ref. J.A.M.A., 1917, 68, p. 233.

Laird, Conover and Butts (1923): Am. Journ. Med. Sc., 1923, 1, p. 241.

Ledingham (1908): B.M.J., 1908, 2, p. 1173.

Ledingham and Arkwright (1912): The Carrier Problem in Infectious Diseases, London 1913.

Leiss (1918): Münch. med. Woch., 1918, No. 38.

McKendrick (1923): J. Path. and Bact., 1923, XXVI, pp. 535 - 544.

Mackie and Wiltshire (1917): J. R.A.M.C., 1917, 29, pp. 276 - 289.

Martin and Upjohn (1916): B.M.J. September 2nd. 1916.

Nichols, Simmons, and Stimmel (1919): J.A.M.A., 1919, 73, p. 680.

Pijper (1922): Polioclinico, 1922, p. 1163.

Schöne (1908): Münch. med. Woch., 1908, 55, p. 1065.

Schottmüller and Much (1908); Munch. med. Woch., 1908, 55, p. 425.

Shera (1922): B.M.J., 1922, p. 1916.

Simmons and McCarthy (1924): J. Lab. and Clin. Med., 1924. IX, pp. 513-518.

Taylor (1918): J. of Hygiene, 1918, XVIII, p. 415.

Thompson (1921): J. R.A.M.C., 1921, 37, p. 302.

Wilson (1909): B.M.J. 25th September 1909.

Wodtke (1920): Centralbl. f. Bakt., 1920, LXXXIV, p. 144.

Zupnik (1908): Münch. med. Woch., 1908, 55., p. 148.

******

CHAPTER V.

THE RELATIONSHIP OF THE CARRIER CONDITION IN ENTERIC FEVER TO CHOLELITHIASIS AND CHOLECYSTITIS, AND DISCUSSION ON THE ROUTE OF INFECTION OF THE GALL-BLADDER.

The association of gall-bladder troubles and typhoid fever was first observed by Naunyn in 1892. The discovery of the typhoid bacillus in cases of cholecystitis and cholelithiasis, long after recovery from the primary infection, was made many years before the recognition of the carrier state. The fact also that at necropsies of persons who had died from typhoid fever, the typhoid bacillus is invariably found has been known since the work of Anton and Fütterer (1888) and later Chiari (1894). To Gilbert and Girode (1893) belongs the honour of describing a case of acute purulent cholecystitis, occurring during the fifth month of convalescence. A pure growth of B. typhosus was obtained at operation from the contents of the extirpated gall-bladder. Sections of the

gall-bladder wall revealed accumulations of small cells in the mucosa with masses of typhoid bacilli in them. During the same decade numerous other similar cases were reported by Von Dungern (1897), Cushing (1898), Imhofer (1898), Miller (1898), Mixter (1899) and Hunner (1899). The original attack of enteric fever took place, however, 14 and 18 years previously in Von Dungern's and Hunner's cases respectively. The cases reported on by the other writers were operated for gall-stone colic developing during or shortly after the typhoid attack.

The first research work on carriers in this connection was made by Remlinger and Schneider in 1897 (quoted Vincent and Murilet, 1917), but it was not until several years later that these researches were generally accepted. Among the workers who confirmed the relationship to gall-bladder conditions of chronic carriers were Lentz (1905) and Forster (1908). The view that the gall-bladder, liver or bile-ducts is the source of the infection in carriers is now generally accepted. Numerous references have been made to the finding of the specific organisms in the gall-bladder and many cases have been described in the Section on Surgical treatment (see p. 255). In all three chronic excreters of the author's series

submitted to operation the bacilli were isolated from the gall-bladder bile. A piece of liver removed at operation from the B. paratyphosus B carrier (M.D.) proved sterile. All chronic carriers, however, do not appear to harbour the germs in the gall-bladder (Schievelbein 1919 and Garbat 1922) and Loele's (1909) case is illustrative. Following cholecystectomy typhoid bacilli still persisted in the faeces. At operation the cystic duct was found to be stenosed and microscopically no "typhoid nests" were detected in the gall-bladder wall. This, however, does not exclude a liver infection. Garbat described what he calls an "intestinal" carrier where no infection of the biliary passages or liver was present, though these later became infected. In the case of the "mixed" carrier of B. paratyphosus B and B. typhosus (H.T.) of the author's series, B. typhosus alone was isolated from the gall-bladder contents. Both these organisms were subsequently found in the faeces for a period. The drainage bile from the cystic duct only showed the presence of B. Typhosus.

Gall-stones are frequently found in the gall-bladders of carriers and may or may not give rise to symptoms of gall-stone colic. Forster (1908) in an analysis of several hundred carriers stated that

14 per cent. were gall-stone sufferers. Messerschmidt (1913) and Meyer (1914) associated 65-90 per cent. of carriers under their care with gall-stones. The other 10 per cent. invariably had distinct chronic cholecystitis or empyema of the gall-bladder. Meyer examined 70 cases of acute and chronic cholecystitis which came to operation in 18 months and found seven carriers out of that number. Nichols, Simmons and Stimmel (1919) performed cholecystectomy on six carriers, five of whom had gall-stones. Knauer (1920), who performed necropsies on nine carriers, found gall-stones in eight. Henes' (1920) 3 cases had no gall-bladder symptoms though calculi were present in two. Out of 21 typhoid patients who became carriers Garbat (1922) found seven (33 per cent.) suffered from gall-bladder symptoms. Only three had gall-stones but two of the most persistent carriers showed no symptoms whatever. These carriers were discovered at operation out of 174 cases of acute cholecystitis. B. typhosus can frequently be isolated from the interior of the gall-stones. This was done in the case mentioned above (H.T.). Numerous other investigators have had similar results, e.g. Grimme (1908) and Hage and Brinkman (1923), etc. Two out of the three cases of the author's series had no gall-stones present, though the gall-bladder wall in each histologically showed a subacute cholecystitis. None of the cases

suffered from symptoms related to the gall-bladder.

The importance of a routine bacteriological examination of the bile in all cases of cholecystitis and cholelithiasis cannot be stressed sufficiently, as B. typhosus as an etiological agent of these conditions is of more frequent occurrence than is generally recognised. This is shown by the work of Hamilton (1910), Meyer (1914), Brannon (1920), Garbat (1922) and numerous other workers. Hamilton examined 24 cases of chronic cholecystitis submitted to operation in a general hospital and found seven to be carriers of either B. typhosus, or B. paratyphosus A or B. Likewise Meyer found 7 carriers in 70 cases, Brannon 43 in 425 cases and Garbat 7 in 174 cases.

Route of infection of the gall-bladder by enteric organisms.

After Blachstein, in 1891, reported the important discovery that the bile of rabbits, injected intravenously with living typhoid bacilli, harboured these organisms for as long as 109 days after inoculation it opened up a new avenue for the study of enteric fever. The route by which these organisms reached the gall-bladder was immediately subjected to investigation. The following views have been advanced:- 1. that the organisms are brought to the liver by the portal circulation and from there are swept down with the bile

and thence into the intestine; 2. that they are carried directly to the gall-bladder by the blood-vessels which supply it in the form of emboli; 3. that they ascend from the bowel by the common bile duct.

The accepted view appears to be that the typhoid bacilli reach the gall-bladder by the blood-stream and not from the lumen of the bowel. In favour of this, in the first place, is the fact that the normal intestinal organisms seldom pass for any considerable distance up the common duct and the rarity of clinical gall-bladder infections with B. coli; and, in the second place, that after introduction of typhoid bacilli into the blood in rabbits they can be recovered from the gall-bladder for several months, with or without ligation of the common bile duct. The theory of ascending infection from the intestines has been, thus, practically discarded, though Sherrington (1893) and Carmichael (1902) claimed that this view is correct as they were unable to infect the bile by subcutaneous and intravenous injection of various micro-organisms into rabbits and mice, though the blood be teeming with these organisms. Their work has been subsequently disproved by numerous other investigators. The first theory has been supported by the experimental work of Blachstein (1891), Welch(1891)

Doerr (1905), Nichols (1914) and others, while the second view has been substantiated by the work of Chirolanza (1900), Forster and Kayser (1905), J.Koch (1909) and Morgan (1911). Chiari (1894) thought all three ways possible but the work of Blumenthal (1910) and later investigators would seem to prove that the first two theories are more logical. Doerr ligatured the common duct of rabbits, after which they were given intravenous injections of living typhoid bacilli. Gall-Bladder infection was, in these animals, constant. If, on the other hand, the cystic duct was ligatured, he could demonstrate no organisms in the gall-bladder. Chirolanza found typhoid bacilli in the gall-bladder when the cystic duct was closed. He pointed out, furthermore, that the gall-bladder is well supplied with blood-vessels and that typhoid bacilli were sometimes found in the folds of the mucosa near the capillaries, whereas the bile itself might be sterile. J. Koch was also able to demonstrate these clumps of organisms in cut sections. Thus the latter investigators thought that infections occur through the wall of the gall-bladder and not from the bile. Koch drew his conclusions from the histological picture in a human case of typhoid cholecystitis in

which he found emboli of bacilli in the folds of the mucous membrane. Nichol's experiments seem to prove that provided the organisms are circulating in sufficient quantities in the blood, they regularly enter the bile from the liver.

The most recent and thorough researches in this connection have been made by Meyer, Neilson and Feusier (1921) and are worth recording. From extensive experimental work on various animals, especially rabbits, they believe that the gall-bladder of about one third of rabbits injected intravenously with large doses of living typhoid bacilli, receives the infection through the terminal capillaries of the mucosa, and that chronic rabbit carriers result probably from embolic capillary invasion of the wall with subsequent transverse affection of the bile. Persistence of the bacteria is favoured by the formation of biliary calculi (60 to 80 per cent. of the cases), by the extension of the inflammatory process to the cystic duct and by a severe cholecystitis leading to a loss of contractibility of the wall followed by a state of empyema. The infection of the remainder occurs by the descending or haematohepatogenous route from the liver. This is suspected of causing a temporary cholecystitis, and a pericholangitis and hepatitis is a rather constant accompaniment, while a wall invasion results in a chronic

persistent infection.

On the other hand, from their own findings and the evidence which they have gathered from the literature, they state it is evident that an embolic infection of the gall-bladder plays an insignificant role in the human carrier state. They confirm Nichols, Simmons and Stimmel's (1919) work, namely that in the majority of cases of rabbit infections the bacteria were eliminated with the hepatic duct bile from certain intra-hepatic foci. These hepatic areas of necrosis or micro-cholangitic abscesses are probably analogous to the lesions described by Blachstein (1891). These would explain the persistence of typhoid bacilli in the stools of rabbits after cholecystectomy. Meyer and his co-workers state that the typhoid bacillus reaches the gall-bladder in human cases of typhoid fever regularly after the liver has been disabled by the poisonous products of the bacteria. The formation of bacillary foci in the gall-bladder wall supposedly by haematogenous origin, they claim to be exceptionally rare, and that the conclusion of J. Koch based on an exceedingly severe and unique form of typhoid cholecystitis, is by no means justified.

However, no explanation is given why the human chronic carrier condition should thus differ from that

of the rabbit.

It would appear more probable in human chronic ex-ureters, that infection of the bile may result from both an intrahepatic infection but more probably from capillary emboli invasion of the gall-bladder wall. Examination of histological sections made from the gall-bladders removed at operation from the three carriers (K.O., H.T., and M.D.) (see p. 152) did not reveal any direct proof of embolic focal infection, though foci of inflammatory cells, often in relation to the small blood-vessels, were identified. Part of the wall stained for organisms with polychrome-methylene blue, showed only very scattered rod-like organisms beneath the lining epithelial cells in the tissue of the villus in the case of H.T. No definite relationship could be established between them and the inflammatory foci present. In any case the bile showed a mixed infection of B. typhosus and B. coli. The specific organisms were grown in ox-bile and broth in all three cases from the gall-bladder wall after washing thoroughly in normal saline. A piece of the liver removed at operation from M.D. was also examined. Histologically, there was a slight but distinct cellular infiltration in the portal tracts and it was difficult to determine its exact relationship

to the structures in the portal tracts, but they appeared to lie around the minute blood vessels rather than the bile ducts. No organisms were found in an exhaustive search of liver sections. On cultivation of pieces of the liver tissue in ox-bile and broth, no growths were obtained. In spite of cholecystectomy being performed on this case, after a period of many months during which no B. paratyphosus B. were obtained from the faeces, these organisms were once more isolated on one occasion. It is possible that in this case there has been a slight residual liver infection but evidently the main seat of infection has been removed. The residual infection might possibly be situated in the appendix as suggested by Vosburg and Perkins (1925). In the other two cases in which cholecystectomy (H.T.) and cholecystgastrostomy (K.O.) were performed none of the specific organisms have been found in the faeces in spite of many examinations carried out for approximately two years or more. Here the infection would appear to be from the gall-bladder wall. In none of these cases was the common duct ligatured so that infection of the bile by the haematohepatous route can be excluded, especially in the latter two cases.

Thus, in the author's own experience, a transverse infection of the bile from the blood-vessels of the gall-bladder wall seems to be the main source of infectivity, though the other route of infection would appear also possible. This may be the case in chronic carriers who suffer from severe symptoms related to the gall-bladder and liver though Koch's case seems to disprove this view.

****

B I B L I O G R A P H Y.

Anton and Fütterer (1888): Munch med. Woch, 35, p.315.

Blachstein (1891): Bull. John Hopkins Hosp., 1891, p.96.

Blumenthal (1910): Centralbl. f. Bakt., I, O., 28, p.1314.

Brannon (1920): J. Am. Med. Assn., 74, p.173.

Carmichael (1902): J. Path. and Bact., VIII, p.276.

Chiari (1894): Centralbl. f. Bakt., 15, p.648.

Chirolanza (1909): Ztschr. f. Infektionskr., 62, p.11.

Cushing (1898): Bull. John Hopkins Hosp., IX, p.67.

Doerr (1905): Centralbl. f. Bakt., 39, p.624.

Dungern, Von (1897): Münch med. Woch., 1897, June 29th. Vol. 44.

Forster (1908): Münch Med. Woch., 1908, 55, p.1.

Forster and Kayser (1905): Münch. med. Woch., 52, p.1423.

Garbat (1922): Monos. of Rockefeller Instit., No.16.

Gilbert and Girode (1893): Compt. Rend. Soc. Biol. p.95.

Grimme (1908): Münch. med. Woch., 55, p.16.

Hage and Brinkman (1923): Mitteil. a. d. Grenl. d. Med. u. Chir, 37, p.25.

Hamilton (1910): J. Infect. Dis., VII, p.393.

Henes (1920): J. Am. Med. Assn., 75, p.1771.

Hunner (1893); Bull. John Hopkins Hosp., X, p.163.

Imhofer (1898): Prager med. Woch., XXIII, p.169.

Knauer (1920): Centralbl. f. Bakt., 85, p.237.

Koch, J. (1909): Ztschr. f. Hyg. u. Infektionskr., 62, p.1.

Lentz (1905): Klin. Jahrb., 14, p.475.

Loele (1909): Deutsche med. Woch., 35, p.1429.

Mayer (1914): Münch. med. Woch., 61, p1116.

Messerschmidt (1913): Ztschr. f. Hyg. u. Infektionskr, 75, p.411.

Meyer, Neilson and Feusier (1921): J. Infect. Dis., 28, p.456.

Miller (1898): Bull. John Hopkins Hosp., IX, p.85.

Mixter (1899): Boston Med. and Surg. Journ., 1899, May 25th.

Morgan (1911): Journ. Hygiene, 11, p.202.

Nichols (1914): J. Expt. Med., 20, p.573.

Nichols, Simmons and Stimmel (1919): J. Am. Med. Assn., 73, p.680.

Richardson (1897): Boston Med. and Surg. Journ., 1897, Dec. 21st.

Schievelbein (1919): Centralbl. f. Bakt., Jena, 1, Abt., O., 83, p.97.

Sherrington (1893): J. Path. and Bact., I. p.258.

Vincent and Murilet (1917): Typhoid and Paratyphoid Fevers, London 1917, p.66.

Vosburg and Perkins (1925): Surg., Gyn., and Obst., XL, p.404.

Welch (1891): Bull. John Hopkins Hosp., II, p. 121.

****

CHAPTER VI.

THE HISTOLOGY OF THE GALL-BLADDERS IN ENTERIC CARRIERS

The specimens were obtained from three female chronic intestinal excreters in whom the attack of enteric fever dated some years back and who had been under observation for many months. It is noteworthy that in none of these cases had there ever been symptoms referable to the gall-bladder. The tissues were fixed immediately after surgical removal either in Zenker's solution or in 5.0 per cent. formalin. Case I - H.T., aged 52 years; excreter of B. typhosus; on rare occasions organisms with the cultural and serological characters of B. paratyphosus B were also isolated from the faeces.

The gall-bladder was enlarged and distended but not obviously pathological from without. The mucous membrane was thickened, had a definitely honey-combed appearance and its plications were exaggerated.

Thirteen small facetted gall-stones about the size of peas were found in the gall-bladder, weighing in all 0.74 grms.

Histologically, scattered foci of inflammatory cells are seen in the submucosa both in the centre and at the base of the villi, often in relation to the small blood-vessels, with a more diffuse cellular change in other parts. There appears also to be some inflammatory change in the deeper parts of the gall-bladder wall in the fibro-muscular coat, but to a much less extent (Fig.1. x60). The cells in the submucosa are mainly round cells of the type of plasma cells and lymphocytes, with scanty eosinophiles. Some of these cellular foci have a strong resemblance to a hyperplastic nodule of lymphoid tissue with a so-called germ centre. In the foci a larger-mononuclear type of cell with a pale vesicular necleus, which is sometimes indented but not definitely lobulated, is seen. The protoplasm is pale and faintly granular. Deeper in the wall it is diffucult to differentiate these cells from fibroblasts, but in the foci they suggest an endothelial origgin. There are, in addition, a small number of polymorphs present, and not infrequently these may be seen wandering between the columnar lining epithelial cells towards the surface. The blood-vessels, both capillary and

Microphotographs of the Gall-Bladder wall of Enteric Carrier H.T.

Fig.1 x 60.

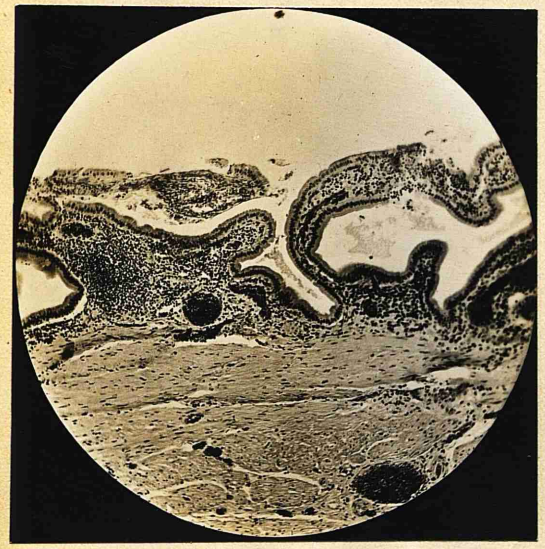


Fig.2 x 250.

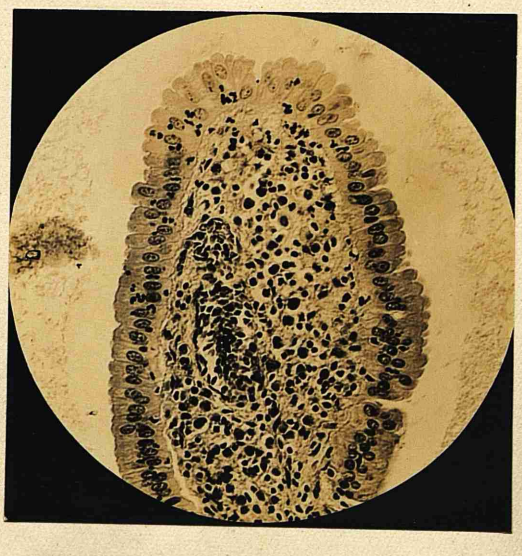
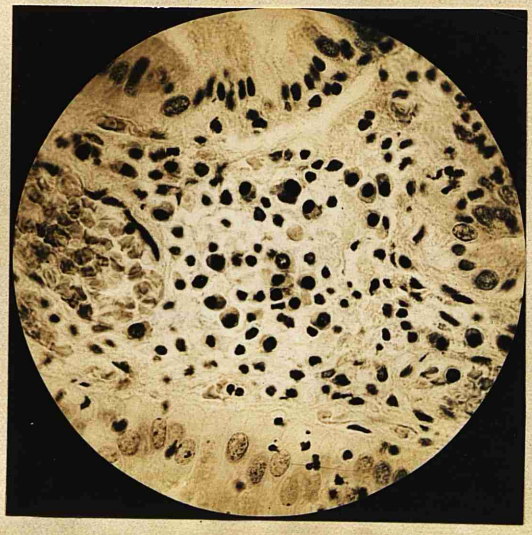


Fig.3 x 500.



venous, show a distinct degree of congestion. The lining epithelium shows little evidence of a superficial catarrh, and there are no signs of marked chronic inflammatory changes in the gall-bladder wall. (Fig.2 x 250) and (Fig.3 x 500). There is a slight tendency to a polypoid appearance of the mucosa, but this may be due, as Meyer, Neilson and Feusier (1921) suggest to the sections examined being taken from the fundus. Part of the wall was stained for organisms with polychrome-methylene blue, but only very scanty rod-like organisms were found in twos and threes immediately beneath the lining epithelial cells in the tissue of the villus. No definite relationship could be established between them and the inflammatory foci present.

The condition, thus, appears to be one of sub-acute cholecystitis affecting mainly the interstitial tissues.

Case II - K.O., aged 27 years, excreter of B. typhosus. The pathological changes in the gall-bladder wall are similar to those in the first case, but there is more diffuse inflammatory change in the substance of the wall, and there are signs of a superficial catarrh (Fig.4 x 60 and Fig.5 x 250). The gall-bladder was found at operation to be somewhat thickened and adherent to the liver towards the neck.

Microphotographs of the Gall-Bladder wall of Typhoid Carrier K.O.

Fig.4 x 60.

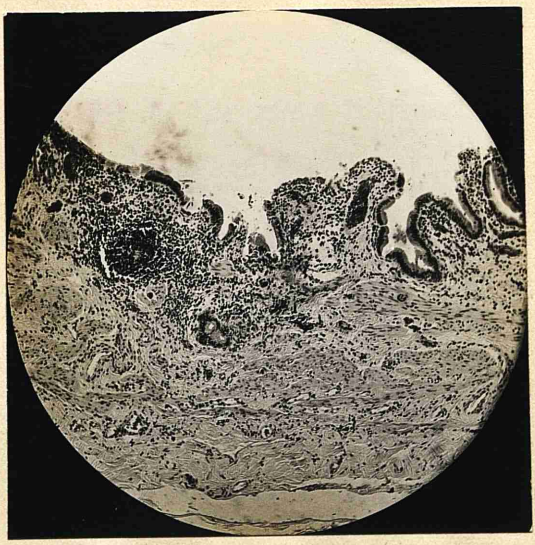
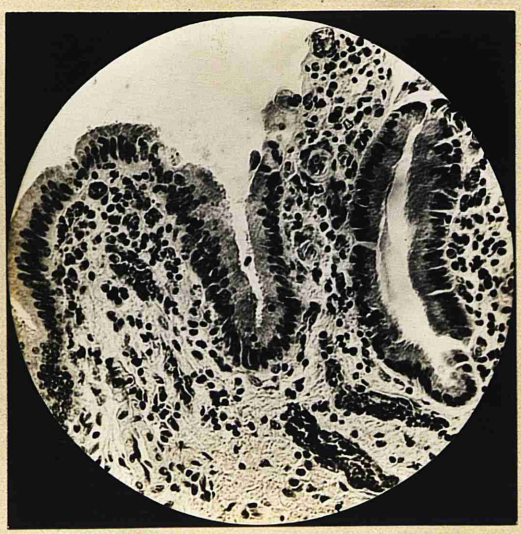


Fig.5 x 250.



Case III - M.D., aged 39 years, excreter of B. paratyphosus B. The appearances of the gall-bladder wall closely resemble those present in the second case, but here again the changes tend to be somewhat more diffuse though the focal arrangement is still met with. At operation the gall-bladder appeared small but nothing abnormal was noted (Fig.6 x 60 and Fig. 7 x 250).

A piece of the liver removed at operation was also examined. Histologically, there is a slight but distinct cellular infiltration in the portal tracts. The cells are mostly lymphocytes. Plasma cells are not apparent. The larger mononuclear type of cells observed in the gall-bladder wall is not seen. It is difficult to determine the exact relationship of this cellular infiltration to the structures in the portal tracts, but they appear to be around the minute blood-vessels rather than the bile ducts. No organisms were found in an exhaustive search of liver sections. On cultivation of pieces of the liver tissue in ox-bile and broth no growths were obtained.

For the purposes of comparison with the above three cases a series of thirty specimens of gall-bladder wall, obtained at operation in the Royal Victoria Infirmary, Newcastle-upon-Tyne, were studied. These cases all showed cholecystitis of varying degrees of

Microphotographs of the Gall-Bladder wall of Enteric Carrier M.D.

Fig.6 x 60.

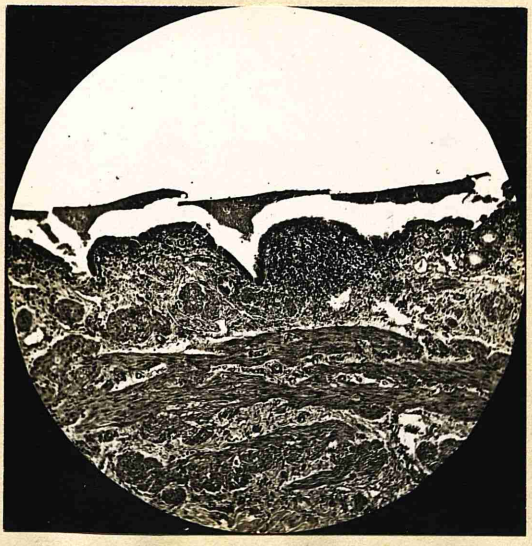
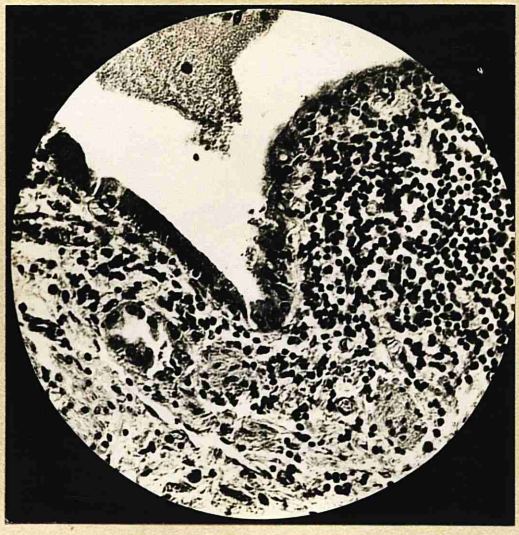


Fig.7 x 250.



severity. In only two cases were the appearances comparable to the changes seen in the carrier cases. In both were hyperplastic nodules suggestive of lymphoid tissue, which contained definite so-called germ centres. One shewed very little mucous membrane left and a thick and fibrous wall, containing focal accumulations of inflammatory cells of advanced degree throughout. The other appeared to be of an acute inflammatory type with ulceration of the villi in parts and an infiltration and accumulation of inflammatory cells in the villi and also diffusely throughout the wall.

The latter gave a history of typhoid fever nine years previously. The gall-bladder, which contained two stones, was adherent to the liver and duodenum, and was tense and distended. The wall was much thickened. The first gall-stone colic attack came on five years after the attack of enteric fever. Unfortunately no bacteriological examination of the bile was made in this case. The former was filled with numerous stones, one of which was impacted near the ampulla of Vater. The cellular infiltration in these cases was much more marked than in the gall-bladders from the typhoid carriers.

My own observations agree with those of Garbat (1922) who describes practically identical appearances

in the gall-bladder of typhoid carriers without clinical symptoms referable to this organ. He states that the gall-bladder may show only very slight pathological change; the normal glistening appearance of the peritoneal coat may be dulled; the wall slightly thickened; the entire organ may or may not be enlarged, and on being cut open the mucous membrane may present only a thickened or congested appearance. Microscopically the different layers of the wall may be sharply outlined, but infiltrated by lymphocytes and a few leucocytes. In these cases, Garbat states, the gall-bladder acts purely as a test-tube, containing the bile medium, in which typhoid bacteria propagate without affecting the gall-bladder itself. In the other type, where a gall-bladder disturbance has been experienced, the entire organ may be buried in adhesions; the walls may be a quarter or a half thicker than normal, and on section the entire normal gall-bladder appearance may be obliterated and replaced by a mass of fibrous tissue, infiltrated by lymphocytes and polymorphs. Between these two extremes all degrees of pathological changes may exist.

A characteristic feature in the author's own cases is the focal cellular collections, the so-called typhoid nests of various authors.

This raises the question of the presence of

lymphoid tissue in the gall-bladder wall, for, as has been mentioned above, there is a distinct resemblance between a hyperplastic nodule and a typhoid nest. According to Maccarty (1909) and Meyer, Neilson and Feusier (1921) lymphoid tissue is present. Maccarty describes the accumulation of lymphocytes around a so-called germ centre situated at the base or top of the villi in the submucosa. Boyd (1923) on the other hand emphatically denies their presence in man though twice found in the case of dogs.

In two of the control cases these hyperplastic lymphoid nodules were seen, but were certainly larger than the cellular foci seen in the undoubted typhoid carrier cases, and moreover, they were associated with a much more widerspread cellular infiltration in the deeper coats of the gall-bladder wall. That lymphoid tissue is present in the gall-bladder wall appears conclusive both from a developmental and histological point of view.

The conclusion come to is that the gall-bladder wall in typhoid carriers, especially in carriers without symptoms referable to the organ, does not present any absolutely diagnostic appearance, but shows the presence of focal accumulations of inflammatory cells in the villi, probably of the nature of typhoid nests

which are in definite relation to the central blood vessel of the villus.

The condition is one of sub-acute cholecystitis or according to Maccarty's classification a chronic catarrhal cholecystitis.

Historical review of the condition of the gall-bladders

Among the observations made on the histology of the gall-bladder in typhoid fever the following constitute a few. J. Koch (1909) investigated the histology of the gall-bladder mucosa in a fatal human case of typhoid fever, who died from cardiac failure in the third week of the disease. The organ contained cloudy, slimy, green bile and its walls were much thicker than normal. Typhoid bacilli were recovered from the gall-bladder wall and many other sites. On microscopical examination the mucosa was very much corrugated and papillated and at the extremities of the papillae "typhoid nests" were found with necrotic areas in their vicinity. The superficial epithelium had completely disappeared and there was a marked inflammatory proliferation of the submucosal folds. A conspicious feature was the close relationship of these "typhoid nests" to the minute end capillaries of the sub-mucosal papillae, suggesting that the bacilli had reached that situation solely by way of the blood-vessels. No organisms were demonstrable in their lumen.

Histological sections of the gall-bladder walls of rabbits, intravenously inoculated with living typhoid bacilli, gave a condition essentially similar to that of the previous human case (Chirolanza 1909 and Morgan 1911). Nichols (1916) found a similar condition but no foci of infection in the bladder wall. Meyer, Neilson and Feusier (1921), in their experimental studies on the mechanism of gall-bladder infections, described the changes found in the gall-bladders of rabbits inoculated intravenously and directly into the gall-bladder with typhoid bacilli, and examined after different stated intervals of time. In all, a definite catarrhal cholecystitis was observed, but was of a more severe nature in the case of intravenous injections when localized foci of necrosis and later a diphtheritic change extending to the muscularis and serosa was present, and which developed rapidly. The formation of haemorrhage, the focal necrosis and diphtheritic lesions in the tip and base of the villi suggested an embolic invasion of the terminal capillaries. This was also supported by the fact that direct injection of living organisms into the viscus did not give rise to localized areas of necrosis in the villi. The epithelium was intact, and invariably studded with emigrating leucocytes, and the mucosa diffusely infiltrated. The cellular reactions which developed

in the course of infection of the gall-bladder wall were variable; while usually diffuse they sometimes formed mural abscesses. In chronic rabbit gall-bladder carriers Meyer and his co-workers stated that one of two findings was quite constant namely (1) empyema of the viscus with severe inflammation of the wall, or (2) biliary sand or small calculi. A detailed description of the microscopical changes found in chronic human carriers is worth mentioning. The mucosa and submucosa was thickened to 4 to 8 times its normal dimension, and the papillae are stumpy and diffusely infiltrated with lymphocytes. Occasionally nodular areas resembling lymph follicles were observed. The covering epithelium though exhibiting marked hyperplasia was intact. The cellular infiltration extended to the muscularis which was either atrophic or had its oblique fibres increased in number, and the stroma of the fibrous serous coat was enormously thickened and infiltrated. The cystic duct and frequently the extra- and intra-hepatic system were involved in the inflammatory process. A distinct cholangitis was found in 50 per cent. of ~~the~~ rabbits killed between the 50th and 100th day of the infection. ~~In the latter variety, they state,~~ In rabbits after infection over ~~30 and~~ 100 days the thick-

ness of the wall was not materially increased but the mucosa exhibited elongated proliferation of the papillae. The whole of the mucosa contained numerous glands, which frequently showed papillomatous extensions in the muscularis and the submucosa, resembling an adenomatous growth. Hypersecretion of mucus was distinctly visible. An intact but hyperplastic epithelium covered a diffusely cellular-infiltrated stroma. The connective tissue growth in the serous and even muscular coat was extensive but the lymphocytic infiltration was slight. Round cells were, however, frequent in the hepatic connective tissue. These hepatic areas of necrosis or microcholangitic abscesses, they think, are probably analogous to the lesions described by Blachstein (1891) and would explain the persistence of typhoid bacilli in the stools of some rabbits after cholecystectomy.

Meyer, in addition to his experimental rabbit work, studied several gall-bladders of convalescent typhoid patients which had been removed for gall-stones. Histologically a diffuse infiltration of the mucosa or nests of round cells covered by an intact epithelium were observed though relatively few organisms were detected in the wall.

Descriptions of gall-bladders of rabbits infected with B. paratyphosus B are given by Fränkel and

Much (1911) and Arai (1922). The former observed bacterial thrombi in the capillaries of the mucous membrane, either at the top or base of the folds. Frequently wall abscesses and bacterial masses were noted in the lymph vessels. The latter stated that by injection of the organisms into the bone marrow of rabbits, lesions closely similar to the human infection were found in the gall-bladder wall. Numerous bacilli were demonstrated in the mucosa, the epithelial cells of which were desquamated, and a few organisms were seen in a lymph cavity and the blood vessels of the submucosa.

****

REFERENCES.

Arai (1922): Scientific Rep. from Gov. Inst. for Infect. Dis., Tokyo Imp. Univ., for 1922, Vol I, p. 69.

Blachstein (1891): Bull. John Hopkins Hosp. 1891, Ii, p. 96.

Boyd (1923): British Journ. of Surg., 1923, x, p. 337.

Chirolanza (1909): Zkchr. f. Infektionskr., 1909, 62, p. 11.

Frankel and Much (1911): Ztschr. f. Hyg. u. Infektionskr, 1911, 69, p. 342.

Garbat (1922): Monos. Rockefeller Inst. for Med. Res., No 16, May 10th, 1922, p. 51.

Koch (1909): Ztschr. f. Hyg. u. Infektionskr. 1909, 62, p. 1.

Maccarty (1909): Pathology of the gall-bladder and some associated lesions. Address to Utah Med. Assoc. Sept 1909.

Meyer, Neilson and Feusier (1921): Journ. Infect. Dis., 1921, 28, p. 457.

Morgan (1911): Journ. Hygiene, 1911,11, p. 202.

Nichols (1916): J. Expt. Med., 1916, 24, p. 497.

******

CHAPTER VII.

Mixed Infections of Typhoid and Paratyphoid bacilli with particular reference to the Carrier condition.

The question of mixed infections with B. typhosus and paratyphoid or other organisms has attracted the attention of numerous observers; and there appears to be good evidence that such genuine mixed infections may occur. Conradi (1909) states that he had an attack of typhoid fever himself in the course of which "paratyphoid" bacilli were recovered from the blood. The faeces and urine gave only typhoid bacilli and his serum agglutinated B. typhosus alone. Other observers have recorded similar findings and, as suggested by Ledingham and Arkwright (1912), it would appear that in disordered conditions of the intestine following infection with the typhoid bacillus, the permeability of the intestine may be so altered as to permit the entrance of other organisms into the blood-stream. The interesting fact,

why in such cases the organisms most liable to gain entrance in this way should belong to the Gaertner-paratyphoid group, still awaits explanation. Ledingham and Arkwright put forward the suggestion that these organisms have not much power of initiating infection unless present in considerable numbers, and that the path of infection has to be made easy for them by some antecedent disturbance of the intestine.

Illustrative examples suggesting the occurrence of mixed infections of B. typhosus and B. paratyphosus B are described by Conradi (1904), Gaehtgens (1907), Castellani (1907), Beckers (1908), Popp (1911) and others.

Conradi was able to isolate "paratyphoid" bacilli from the faeces and urine of four chronic typhoid carriers. As a rule these organisms were only got on one occasion. Gaehtgens, like Conradi, obtained positive findings of "paratyphoid bacilli on single occasions in the excreta of a few typhoid convalescents and typhoid carriers. In the former the occurrence of "paratyphoid" organisms in the stools took place in the sixth week from the onset of infection. Gaehtgens believed that in two of the cases there was a genuine mixed infection, and in two others the "paratyphoid" organism was acting as

a mere saprophyte, while in the remaining two the suggestion was that a paratyphoid infection had succeeded the typhoid infection. Conradi, however, regarded the "paratyphoid" infection as being caused by ingestion of infected food and that the organisms were invariably saprophytes since they could occur in healthy men. This has not been confirmed by many other writers; thus Sobernheim (1910) examined 1000 samples of faeces or urine of healthy men without once finding B. paratyphosus B; similarly Seiffert (1909) examined 600 specimens of faeces with negative results; O. Mayer (1909) also performed 1000 examinations in the vicinity of individuals suffering with enteric fever but in only two cases did he recover "paratyphoid" bacilli from the stools; Morgan (1906-1907) and Savage (1908-1909) likewise had negative results.

During the investigation of a large series of typhoid convalescents and carriers Ledingham and Arkwright collected a large number of non-lactose-fermenters which produced acid and gas from mannite. None of these colonies proved to be B. paratyphosus B, although several possessed cultural characters agreeing to some extent with those of the Gaertner-paratyphoid group. The examinations were performed by Bainbridge and O'Brien who in 1911 procured from

various sources - mostly German - a number of strains (25) of "paratyphoid" bacilli. Many of these proved to be identical with B. suipestifer, and others with standard strains of B. paratyphosus B. The latter proved to have been isolated from genuine cases of paratyphoid fever or from paratyphoid carriers, whereas the former had been derived from food-stuffs or cases of food-poisoning. Hence it should be borne in mind that no distinction is drawn by many German writers between B. suipestifer and B. paratyphosus B, so that some cases of so-called true mixed enteric infections may be a mixed infection with B. suipestifer. Castellani described a case of true mixed infection with B. paratyphosus B and B. typhosus. Both these organisms were isolated together on the seventh day of illness from the stools. A similar result was obtained twice, later in the course of the disease. The urine was positive alone for B. paratyphosus B. The blood gave a positive Widal reaction, e.g., on the 46th. day of the disease the serum agglutinated B. typhosus in a dilution of 1:800 and B. paratyphosus B at 1:1000. The absorption test showed that the blood contained specific agglutinins for each of these bacteria and Castellani thus concluded that this excludes the possibility of the case being one of typhoid fever with the

presence of B. paratyphosus B as a saprophyte.

Among the more recent reference to mixed infections are those of Robinson (1915), Gautier and Weissenbach (1916), Achard (1916), Gérard and Fenestre (1916) Zironi (1917), Hébert and Bloch (1917) and Etienne (1918). Robinson refers to two cases of possible mixed infection; B. typhosus was isolated from the faeces of one, and B. paratyphosus B from the other; the serum agglutinated both organisms in a dilution of 1:200. No definite proof is given and his findings are basedon the Widal reaction. No absorption tests were performed, so that they cannot be regarded as true cases. In Gautier and Weissenbach's case B. typhosus and B. paratyphosus B were both isolated from the faeces but the latter organisms alone from the blood. The serum agglutinated both up to 1:500 dilution. There is no strict evidence to support the contention of Gérard and Fenestre that their case showed a true mixed infection of B. paratyphosus A and B. typhosus. They state that B. paratyphosus A were isolated from the blood in the first and third weeks and later from the faeces. They appear to base their contention on the fact that the serum agglutinated B. typhosus in a dilution of 1:500, though this organism was never isolated from the blood or faeces, and they claim

that the absence of typhoid bacilli was due to the latter being overgrown by B. paratyphosus A. Experiments in which bile was used as culture medium showed that one or other organism takes the upper hand and with three different species tested all showed disappearance of B. typhosus. That this may occur in test-tube experiments was proved by the author himself in a peptone water culture inoculated with approximately equal quantities of 24 hour peptone water cultures of B. paratyphosus B and B. typhosus (3 platinum loopfuls of each). At the end of a week's incubation at 37°C even with the aid of the Brilliant-green enrichment method, only the former could be isolated. Numerous colonies were tested. Achard inoculated rabbits intravenously and guinea pigs intraperitoneally with mixtures in the proportion of $\frac{1}{2}$ or $\frac{1}{4}$ of B. paratyphosus B to $\frac{1}{2}$ or $\frac{3}{4}$ of B. typhosus respectively. B. paratyphosus B was isolated from the heart-blood of the rabbit on killing in 1 - 2 hours and of the guinea-pig in 20-24 hours. B. typhosus was not recovered. Achard thinks it is perhaps different in man who is less susceptible to paratyphoid than typhoid bacilli, and that perhaps one finds in these cases only B. typhosus as the septicaemia due to it appears to be much more prolonged than paratyphoid septicaemia.

Both Zironi and Etienne found paratyphoid and typhoid bacilli contemporaneously in the blood of acute cases as shown by blood cultures, but Herbert and Blach found B. typhosus and B. paratyphosus B successively in the blood of a typhoid patient.

Interesting findings in carriers were reported by Pribram (1912) and Arnd (1923); the former performed cholecystectomy on an intestinal excreter of B. typhosus and found that the typhoid bacilli had disappeared from the faeces, but that later B. paratyphosus B was isolated and continued to be excreted for four years afterwards; the latter had a similar experience in two persons who had been typhoid carriers before cholecystectomy and who ceased to be so after it, but were found then to be carriers of B. paratyphosus B. In one case B. typhosus was isolated from the faeces and gall-bladder, from the latter in pure culture. In both cases the paratyphoid bacilli subsequently disappeared. Arnd, however, gives no dates to show how long his examinations were made before or after operation. As far as one can judge from his article the cases were convalescent ones when first observed.

Description of Author's Case of a mixed carrier of B. typhosus and B. paratyphosus B.

During the course of these investigations B.

paratyphosus B was isolated from the faeces of one of the excreters of B. typhosus (H.T.) on five occasions. Four times before excision of the gall-bladder and once afterwards.

She came under observation for the first time on 14.4.23 and B. typhosus was isolated from the faedes on many occasions. On 13.6.22 an organism which gave the cultural and fermentative reactions for B. paratyphosus B appeared in the Brilliant-green peptone water tubes while on the same day B. typhosus was present on the direct MacConkey agar plate. Again on 15.6.22 B. paratyphosus B was isolated this time by direct plate and through the Milk Enrichment method (see p. 58). On 20.7.22 a similar result was obtained by the Brilliant-green method. B. paratyphosus B was not then found until 24.5.23, although in 78 bi-weekly examinations of the faeces B. typhosus was recovered 48 times until Cholecystectomy was performed on 4.5.23 and subsequently twice out of 5 examinations to 24.5.23. Here B. paratyphosus B appeared on the MacConkey agar plate from the tube of faeces treated by Benzene (see p. 67), while B. typhosus was isolated from the same specimen of faeces by brilliant green.

On three of these occasions the cultural characteristics and fermentative reactions of the organism were fully tested, two or three organisms being

put through. Unfortunately the cultures were destroyed accidentally on the other two occasions before exhaustive tests were made. These cultures, however, agglutinated by stock rabbit antiparatyphoid B. serum, and tested once for their sugar reactions. They gave a similar result.

TABLE TO ILLUSTRATE CULTURAL AND FERMENTATIVE REACTIONS OF B. PARATYPHOSUS B. ISOLATED FROM TYPHOID CARRIER (H.T.).

Gram.	Motility	Lactose	Glucose	Mannite	Dulcite
Negative	+ +	o	A.G.	A.G.	A.G.

Maltose	Saccharose	Inosite	Litmus Milk		Indol
A.G.	o	o	1st. day Slight Ac.	3rd. day Alk.	o.

Gelatine.
No gas.

The colonies on MacConkey agar were rounded, distinctly larger than colonies of B. typhosus, and

TABLE XII.

TABLE TO ILLUSTRATE THE AGGLUTINATIVE CHARACTERS OF THE SAME ORGANISM

Serum Employed.	Dilution up to which agglutination occurred.	Titre of serum.	Remarks.
Made by Author.			
Stock Rabbit Antityphoid.	No agglutination in 1:100	1:12,800	
" " antiparatyph.B.	1:25,000	1:25,000	Repeated usually with three colonies
Oxford Laboratories.			
Standard Antiparatyph.B. (series 3, No.1210).	1:200 (slight agglutination 1:400).	1:200	
Standard B. Aertrycke (series 3, No. 1229).	1:25 (very slight agglutination).		None with 1:50 dilution.
Standard B. Gaertner (series 4, No. 1229).	No agglutination in 1:25.		

gave the characteristic lipping or heaping up of their margins after the 2nd or 3rd day.
Table XII gives the agglutinative characters of these organisms. See p. 174.

Stock B. paratyphosus B (Primrose strain) and Oxford Standard B. paratyphosus B. emulsion (Factor 5.7) gave precisely similar results when tested against the same series of sera.

The patient's blood-serum was examined on seven occasions and a full description of the findings is given on p. 106 (section on Biological reactions.) It suffices here to state that a positive Widal reaction was obtained throughout for both B. typhosus and B. paratyphosus B. Unfortunately absorption tests were not made. On one occasion (19.6.22) there were 39 standard agglutination units per c.cm. of patient's serum for B. paratyphosus B and 32 for B. typhosus, but as a general rule the agglutinin content of the serum was lower for B. paratyphosus B than for B. typhosus. On this same carrier McKendrick (1923) performed four cutaneous hypersensitiveness tests. The first on 7.5.22 was negative for B. typhosus, B. paratyphosus A and B; in the second on 23.6.22 the results were vitiated through haemorrhage; the third and fourth on 23.7.22 and 1.8.23 respectively were both strongly positive for B. typhosus and positive for B. paratyphosus B

as well.

It is noteworthy that B. paratyphosus B was isolated from the faeces after cholecystectomy had been performed, as B. typhosus alone was grown from the bile obtained at operation and on subsequent drainage of the cystic duct.

Discussion.

While it is possible that contamination of the cultures may have occurred this possibility seems to be remote as the paratyphoid bacillus was isolated on five separate occasions with the one case and not with any of the seven other carriers, while on two occasions both B. typhosus and B. paratyphosus B were isolated from the same specimen of faeces. Again on one occasion the faecesof the "mixed carrier" werepositive for B. paratyphosus B when the only source of contamination, the prolific excreter of B. paratyphosus B (M.D.) was negative.

The biological and Morphological characteristics of the paratyphoid organism are absolutely conclusive. The "mixed carrier" infected other seven individuals but all of these developed a B. typhosus infection only. The conclusion regarding this case is that there is a true mixed infection of B. typhosus and B. paratyphosus B, a fact which is supported by

the serum containing agglutinins for both the organisms as well as McKendrick's independent findings in the Cutaneous Hypersensitiveness Skin tests. Thus it seems more probable that the condition is one of true mixed infection, similar to that described by Gaehtgens rather than of the nature of the saprpphyte theory described by Conradi. During the two years stay in hospital this carrier was in close contact with a B. paratyphosus B carrier and it is possible that the secondary infection with B. paratyphosus B was derived from that source. The absence of any virulence of the infection could easily be explained by the heterologous immune bodies derived from the B. typhosus infedtion.

****

B I B L I O G R A P H Y.

Achard (1916): C.R. Soc. Biol., LXXIX, p. 751.

Arnd (1923): Schweiz. med. Woch., April 26th. 1923. p.423.

Bainbridge and O'Brien (1911): Journ. of Hyg., XI, p. 68.

Beckers (1908): Hyg. Rundsch., 1908, p.313.

Castellani (1907): Lancet, 2nd February, 1907, pp. 284-6.

Conradi (1904): Deutsch. med. Woch., 1904, pp.1165-7.

Conradi (1909): Klin. Jahrb., XXI, p.421.

Etienne (1918): Presse Méd., Paris, 1918, XXXVI, p.503.

Gaehtgens (1907): Arb. a.d. Kaiserl. Gesundh., XXV, p.203.

Gautier and Weissenbach (1916): Bull. Soc. Med. Hôp., Paris, 1916. Mar. 24th., p.450.

Gérard and Fenestre (1916): Progrès médicale, Paris, February 1916, XXXII, No.8, p.65.

Hérbert and Bloch (1917): Bull. Soc. Med. Hôp., Paris, 1917, July 27th., p.37.

Ledingham and Arkwright (1912): The Carrier Problem in Infectious Diseases, London 1912, pp.144-145.

McKendrick (1923): Journ. Path. and Bact., XXVI, pp. -535-544.

O.Mayer (1909): Klin. Jahrb., XXII, p.254; ibid, XXI, p.171.

Morgan (1906-07): Brit. Med. Journ., 1908, i, p.908; ibid 1907, ii, p.16.

Popp (1911): Munch. Med. Woch.,1910. p.584.

Pribram (1912): Wein. Klin. Woch., 1912, p.1344.

Robinson (1915): Journ. Med. Research, 32, p.399.

Savage (1908-09): Local Govt. Boards Reports, App. B, No.4, p.316.

Seiffert (1909): Zeitsch. f. Hyg., LXIII, p.273.

Sobernheim (1910): Centralbl. f. Bakt., orig., LX, p.2.

Zironi (1917): Riforma. med., Roma, 1917, 33, p.771. Ref. Abstr. Bact., 1918, 2, No.76, p.25.

****

CHAPTER VIII.

THE TREATMENT OF TYPHOID CARRIERS.

Ever since the discovery of the carrier state by Vom Drigalski in 1904, innumerable substances and methods have been employed in the effort to effect a cure. The importance of chronic bacillus excreters from the epidemiological point of view may be somewhat over-rated in stating that if we can find a cure for carriers we can get rid of typhoid fever as Forster (1908) stated. Nevertheless they present a very serious problem since carriers appear to be principally responsible for maintaining the disease in the periods between epidemics. Much confusion has been introduced into the literature by authors reporting cures both on insufficient data and on cases in the early convalescent or temporary carrier state.

Ledingham and Arkwright (1912) illustrate this in their account of a so-called female temporary carrier, who in late convalescence suffered from an attack of gall-stone colic, following on which specimens of urine and faeces were negative for five months; accordingly the case was looked on as belonging to the category of temporary carriers. But a further examination six months later revealed enormous numbers of typhoid bacilli in the faeces, so that thereafter this patient was regarded as a chronic carrier.

Reports of spontaneous cures after gall-stone colic must also be treated with reserve. Prigge's results in 1910 in examination of his chronic intestinal carriers during the previous three years showed that only one out of twenty-eight could be struck off the list and even this patient again excreted typhoid bacilli after three years' intermittence. The cases were examined monthly as far as possible. Of the 28 cases 6 had negative periods of one year, 2 of 1½ years, 2 of 2 years, 2 of 2½ years and one of 3 years.

The author examined a bacillus isolated from a chronic typhoid carrier, which had been reported to have been successfully treated with vaccine by Watt (1923) and found it to be an atypical B. typhosus which was somewhat deficient in agglutinin-binding

power. This organism was first isolated on three successive occasions in June and July 1922, and again in February 1923, or the twenty-first examination after a negative phase lasting between five and six months shortly after the reported cure.

Many authors, unfortunately, do not distinguish between the chronic persistent carrier and the temporary or convalescent carrier and report cures in the latter type of case by various treatments when these may have spontaneously cleared up without medicinal or operative interference. Some do not even mention the length of time during which their patients have been excreting the organisms nor for how long afterwards observations were continued.

From the numbers and variety of substances tried the amount of success can be gauged, and although from time to time various workers have claimed success by one means or another their results have never been thoroughly substantiated apart from surgical cures.

CHEMOTHERAPY.

Drug treatment of intestinal carriers has been entirely ineffective in ridding these persons of their typhoid bacilli. A priori, the use of antiseptics is not hopeful, since such bodies have in general as great an affinity for the tissues as for the typh-

oid bacilli, so that though they may act powerfully in vitro on a pure culture of organisms, or in a non-albuminous fluid like urine, their action is abolished in the presence of protein substances, hence their ineffectiveness when the organisms are in the tissues. The pathology of the carrier state makes it difficult to conceive that any therapeutic treatment would be radical enough to influence old typhoidal lesions in the gall-bladder and bile-ducts. The fact that it is possible to reproduce the "carrier" condition in rabbits as shown by Blachstein (1891), Blumenthal (1910), and numerous other workers, has opened up a new field for chemo-therapeutic research which may have some practical application for the human carrier, but the success so far attained in rendering the rabbit carrier typhoid-free cannot be considered in any way striking.

Among the measures which have been tried both in human and rabbit bacillus excreters, the following may be mentioned:-

Cholangogues, especially dried bile and bile salts, were among the first agents suggested by Forster (1907), who hoped thereby to increase the secretion of bile and thus to wash out the organisms from the gall-bladder. The administration of these substances for months has proved ineffective in the hands of

various workers.

Sodium Salicylate, (five grammes daily for weeks), was claimed to effect a cure by Hilgermann (1923) but no success has been obtained by others.

Küster (1923) tried Copper Acetate, thymogen, helmitol, urotropine, thymol-charcoal, saliformin and iodine-tincture-charcoal without success.

Cystin-mercury and "cystinal" have been employed by numerous German workers, who claim the combination to be non-toxic and to allow bactericidal action of the mercury in the bile. Stuber in 1918 got good results in rabbit carriers; he also treated twenty human carriers "within three to five months of onset of infection" with soluble cystin-mercury, using 0.2 gramme three times daily for fourteen days or for twenty-one days in obstinate cases; he states that in all cases B. typhosus disappeared from the stools. The only complications met with during the administration were stomatitis in two cases, and in some others slight diarrhoea and abdominal discomfort which disappeared in a few days. In a further article Stuber states that he has treated twenty-one typhoid, thirty-four B. paratyphoid B and three B. paratyphoid A. carriers who had been carrying the bacilli from periods of two months to three years and were observed for eight weeks after the finish of treatment. He claims ninety per cent. of

cures, since fifty-three out of fifty-eight who were treated completely lost the bacilli, but he found four series of treatments were necessary in established carriers with anatomical change in the gall-bladder, these being more resistant.

Kuster and Wolf (1918) state that the cystin taken by mouth passes into the bile and produces an increase in the taurocholic acid secreted. The mercury is retained in part in the liver and is then eliminated by the bile and by giving heroic doses one can accumulate sufficient mercury in the bile. Messerschmidt (1918) treated eighteen cases with cystin-mercury and in only two did the bacilli disappear, while Geiger (1918) had only one cure out of eighteen cases and he states that his cases were all chronic, persistent carriers while those of Stuber's were only of a few months' duration. Stuber's later article seems partly to refute this atatement as some of his patients were carriers of up to three years standing. These, however, appear to have been resistant to the cystin-mercury treatment. This substance would appear worthy of a more extensive trial.

Salvarsan, Neosalvarsan and neoarsphenamin have also been employed. Uhlenhuth and Messerschmidt (1912) had no success with salvarsan and arsphenamin in rabbit carriers; Haibe (1921) had a similar result with neoarsphenamin in the case of dogs; Leitner (1918)

treated twelve human B. paratyphosus B. carriers with neosalvarsan (0.3 grm. and 0.6 grm.). In three cases the elimination of the bacilli stopped after one injection and in seven cases after two injections, while two others were not influenced: Stokes and Clarke (1916) and Geiger (1918) also tried neosalvarsan but without any successful result.

Uhlenhuth and Messerschimidt in 1912 tried separately in rabbit-carriers iodoethyl-hexamethylene-tetramine, monochlor-acetylcholic acid, salicylate of copper, mercury atoxylate, phosphorus and colloidal mercury without success. Again in 1920 the same authors give an extensive list of substances tested on their chronic rabbit carriers, (which had received injections of typhoid cultures directly into the gall-bladder), thus calomel, "atoxyl", quinine, "quinosal","optoquine", platine", also silver, nickel, gold and copper in finely divided metallic state, colloidal arsenic, nucleinates of copper, of silver and of sodium, and thymol have been given subcutaneously, intravenously and by mouth without any consequent diminution in the excretion of the typhoid bacilli. In addition numerous dyes were tried e.g. silver, mercury and copper salts of fluorescein and safranin, and were found ineffective <u>in vivo</u>. The effect was found to be the same with substances whibh

have the reputation of being selective in their action on the biliary system e.g. camphor, emetine, phosphorus and the salts of the bile acids. Methyl violet and fuchsin intravenously and intramuscularly were found to stain the bile and penetrate into the gall-bladder. The authors claim to have had partial success with these, especially methyl violet, having obtained fourteen cures out of twenty-two carrier rabbits; while ten others not similarly treated remained infected. They state that only three per cent. of carrier rabbits undergo spontaneous cure. Diphenylmethane dyes like auramine are more toxic and less bactericidal, and furthermore are not excreted in the bile. Dyes of the fluorescein series are excreted by the kidneys and are therefore useless for the present purpose. In addition they state brilliant-green and malachite-green are less bactericidal than methyl violet and fuchsin, but the latter are, somewhat toxic and produce necrosis at the site of injection and though their leuco-bases are less toxic the bactericidal property is absent. The proportion of spontaneous cures of carrier rabbits has been found by various others authors to be much higher, even as high as sixty per cent. according to Bully (1911).

Knick and Pringsheim (1911) found that the bile

of dogs treated separately per os with large doses of menthol, methylene blue, hippuric acid and urotropin was bactericidal or strongly inhibitory to the growth of B. typhosus in broth. On the other hand they found that mercurous chloride, sodium salicylate, salicylic acid, oil of turpentine and methylene blue had little or no effect.

Rectal injections of a variety of substances have been tried without any convincingly favourable result. Conradi (1910) gave rectal injections of chloroform mixed with milk and cream to inoculated laboratory animals. The mixture was passed by a sound into the large intestine daily for five days as a rule, and the animals were killed not later than the first fortnight after inoculation. Of a series of twenty-one rabbits so treated, sixteen were found to be typhoid-free while all the controls (twelve) remained infected. In a second series gall-bladder puncture after laparotomy was performed before commencement and the bile bacteriologically examined. The result was that five chronic rabbit carriers were cured while three, untreated, remained positive.

Hailer and Rimpau (1912) injected rectally methyl-iodide, ethyl bromide, chloroform and iodoform in chloroform, all of these being mixed with milk and cream before injection. The first two substances

were found too toxic for use in laboratory animals, but the authors reported occasional success with iodoform and chloroform and also with bromoform, but do not recommend any of these for practical use.

These workers, using Conradi's technique found seven out of sixteen rabbits became typhoid-free. i.e. forty per cent, while five ou t of eighteen controls were also typhoid-free i.e. thirty per cent., at the same period after inoculation.

Bully (1911) had similar results with thirty rabbits treated, but a sixty per cent. of the controls were also typhoid-free.

Both Bully and Conradi have tested the therapeutic effect of chloroform in two human female typhoid carriers ("Gelodurat" capsules per os; 0.5 grm. chloroform and 0.5 grm. olive oil), but no obvious change in the excretion of typhoid bacilli resulted.

Tsuzuki and Ishida (1910) reported cases treated with iodine in the form of potassium iodide in conjunction with Fowler's solution with promising results, basing their opinion on the fact that the excreta of persons so treated cleared up more quickly than those of untreated cases.

Kalberlah (1915) gave his human carriers tincture of iodine in conjunction with charcoal, and cites apparent cures, if one is justified in judging from repeated negative cultures.

Hailer and Wolf (1915) tested phenols and ethereal oils using thymol, and pyrogallol as representatives of the former and sandalwood oil, pinene, eucalyptol and cinnamon oil of the latter, but only cinnamon oil gave any encouraging result.

Hertz (1916) suggested the injection of protein (milk) into the gluteal muscle. This caused a temperature rise, chill and disappearance of organisms, and is claimed by the author to have been successful in several instances.

Nichols, Simmons and Stimmel (1919) tried calomael, sodium sulphate, salol, hexamethylenamine without effect in human chronic intestinal bacillus excreters.

Haibe (1921) gave a series of drugs such as colloidal silver, neoarsphenamin and hexamethylenamin to dogs for the purpose of ridding them of bacteria present in the bile ducts, and states these have unfortunately not been successful but came to the conclusion that they had had some influence on the urinary elimination of bacilli. He tried arsenical preparations on human chronic carriers but none proved efficacious. Haibe believes that a strict dietetic regimen should be recommended preferably with milk as a basis. Frequent purging with calomel seemed to be useful but no noteworthy results were secured.

Stone (1919) carried out a few experiments on carrier

rabbits using dyes which were known to be excreted through or broken down by the liver in the hope that their action on the organisms localized there might be powerful enough to cause their complete destruction, but safranin, methyl violet, crystal violet, ethyl violet and Spiller's purple gave negative results. Methyl violet etc. were found too toxic for use in rabbits.

Beckwith (1921) found that "New Green 3B" injected intravenously rendered the bile bactericidal to B. typhosus in animals but that it was too toxic for use, causing emboli by changing from the sol. into the gel. state. Auramine, acriflavine and proflavine were inneffective for sterilizing the gall-bladder, since they are more excreted by the urine.

Murstad (1921) emphasizes that while the treatment by disinfectants has no effect in the case of carriers with typhoid bacilli in the bile and stools of the so-called faecal carriers - urinary carriers may be sterilized by drugs, the most effective being hexamethylenamin and other members of the formaldehyde group.

Glatard (1921) treated ninety-four acute typhoid and paratyphoid patients with colloidal metals intravenously and states that favourable results were inconstant and collapse occasionally occurred. In nineteen

per cent. there werehaemorrhagic complications, while in three hundred and seventy-nine cases not treated in this way haemorrhage only occurredin seven per cent. He emphasizes the uselessness and dangers of colloidal metals in this respect. As a result of Glatard's observations it appears that one can rule out the use of colloidal metals in the treatment of chronic typhoid bacillus excreters.

Vinegar-water and acid wines were claimed by Alvarez (1917) to have been of great benefit in the treatment of acute typhoid and paratyphoid cases which seldom suffered from complications when treated thus. If employed during the acute stages these substances might possibly help to diminish the numbers of permanent carriers but were found on trial to be useless as a curative agents. Details concerning this treatment are given later.

Nichols (1917) during the course of experimental work on typhoid rabbit carriers found that although human and ox bile generally favour the growth of B. typhosus rabbit and guinea-pig bile kill off all the bacilli in twenty-four hours. He thought this antiseptic action was due to the alkalinity of the bile, and found further that the reaction of the bile can be influenced by alkalies such as sodium bicarbonate given intravenously, which made rabbit bile more alkaline and apparently this is true also for human bile.

In support of this he quotes Crille's (1916) statement that the reaction of the bile was influenced by changes in the alkalinity of the blood. Nichols gave to early human convalescent carriers, two grammes thrice daily for two days of sodium bicarbonate and cultures from the faeces afterwards were consistently negative. In consequence he recommended its use particularly in early gall-bladder carriers.

Beckwith's results (1921 b) seem to prove that the bile from rabbit-carriers of B. typhosus is more alkaline than that of normal animals. In the first the mean was Ph 8.33 while in the latter Ph 7.41. Furthermore he found that B. typhosus is viable in rabbit bile even when Ph value is 9.4. Accordingly these results are at complete variance with those of Nichols. Another apparent fallacy of Nichols' results lies in the fact that the alkaline treatment was only employed in temporary (not chronic persistent carriers), and that these would in all probability clear up spontaneously of their own accord.

Dragstedt, Dragstedt and Nisbet (1922) in an article on Intestinal Antisepties state that direct application of solutions of the antiseptics at present known to short segments of the intestine in animals does not effect sterilization or inhibit the production of

intestinal poisons, and thus, at present, no experimental justification can be cited for the clinical use of antiseptics intended to produce intestinal antiseptisis by direct action on intestinal micro-organisms in the gastro-intestinal tract. Fantus (1923) reminds us that calomel is now generally admitted not to be an intestinal disinfectant, nor has its action as an intestinal antiseptic been definitely proved. Numerous workers have tried the effect of calomel on chronic bacillus excreters without success e.g. Uhlenhuth and Messerschmidt (1920) and Nichols (1919), but Haibe (1921) believes frequent purging with calomel to be useful.

Cummins, Fawcus and Kennedy (1910) reported an apparent cure by the use of Roentgen Rays applied over the gall-bladder region in human carriers. One carrier presented symptoms of slight cholecystitis and this form of treatment might, it was hoped, bring about an increase of phagocytosis in the walls of the inflamed gall-bladder. The outcome of this treatment as perhaps showing some inhibitory action on the discharge of bacilli, is certainly interesting and suggestive, but at present it would be premature to signalize this method as affording a prospect of permanent cure. As a matter of fact the discharge of bacilli by their patient had been intermittent

before the commencement of treatment. Later, after being negative for a period of about seven months, during which time five examinations were made, at the sixth B. typhosus was once more isolated from the faeces, so that he cannot be considered as cured. In another Army carrier the method was tried but without the slightest effect on the discharge of typhoid bacilli.

Author's Observations.

During the research on chronic typhoid and paratyphoid carriers the following substances were employed by the author.

Ox bile was given in increasing doses to a B. typhosus (K.O.), a B. paratyphosus B (M.D.) and a mixed B. typhosus and B. paratyphosus B. carrier (H.T.), over a period of three months. Burroughs, Welcome and Company's four grain ox bile tabloids were given by mouth, commencing with 10 grains and gradually increasing to 80 grains per day. The patients preferred swallowing the tabloids whole to taking them in the crushed condition; no clinical disturbance or purgative effect was experienced. The faeces remained more or less positive throughout administration as shown by bi-weekly bacteriological examinations on direct MacConkey agar plates and by the Brilliant-Green fluid Enrichment method.

Various proprietary intestinal antiseptic preparations were given in almost heroic doses without any effect whatsoever. Dimol ("Dimethylomethoxyphenol in combinationwith the tri- and tetra- methylophenols") which is stated to have a Rideal-Walker coefficient of 35 and is prepared by the Anglo-French Drug Company was administered to two chronic bacillus excreters (K.O. and H.T.) in increasing doses until four tablets were taken thrice daily for 14 days. One (H.T.) remained negative during administration but this probably corresponded with one of her negative phases as within a week her faeces were once again positive. She was a very intermittent excreter. The other (K.O.) remained positive all the time. The following substances were tested, in every case without affecting the excretion of the specific organisms in the faeces:

Felamine, (prepared by the "Sandoz" chemical works, Basle, Switzerland, such that each tablet contains 0.225 grms. of hexa-methylenetatramin and 0.075 grms. of glycocholic acid), four tablets thrice daily given to (H.T. and J.M.) for repeated periods of fourteen days;

Kerol, prepared by Kerol Limited, Newark, ?England, stated to be composed of an oxygenated compound with a di-phenyl nucleus, and claimed to have carbolic acid co-efficient of 23 against B. typhosus; four

intestinal capsules given to J.M. thrice daily for sixteen days on two separate occasions;

Acetozone, prepared by Park, Davis & Company, and containing fifty per cent. benzoyl-acetyl-peroxide was also given to a carrier (J.M.) commencing with four ounces seven times daily and rising to sixteen ounces seven times daily of a watery solution with ten grains to the pint dissolved in it, until a quarter of an ounce of the powder was used;

Kaolin, (native Chinese white aluminium silicate), 30 grains increased to sixty grains thrice daily, given in the form of a suspension with mucilage of acacia for several months to the B. paratyphosus B. carrier (M.D.).

Yadil ("Tri-methanal allyl carbide") 1 drachm increased to 2 drachms, three times a day for two weeks, given to a lunatic chronic typhoid bacillus excreter (J.M.).

Naptholated Charcoal, prepared by Fraudin, Boulogne, France, such that each teaspoonful contains exactly twenty centigrammes (about three grains) beta-napthol seemed to give hopeful results in doses of six teaspoonfuls three times a day as the faeces of the first chronic bacillus excreter (M.M.) remained negative during the fortnightof administration. Immediately previous to the commencement of treatment a negative examination was got in the faeces on 6.11.22 which continued to remain typhoid-free till

4.12.22 when a positive result wasagain obtained. During this period four negative examinations were made. The charcoal was given on 9.11.22 and stopped on 23.11.22. Unfortunately these hopes were not sustained on its being given to a less intermittent excreter (K.O.) whose faeces examinations proved positive all the time. A repetition in the first case gave a similarly poor result, proving that the previous occasion was only an intermittent period in excretion.

Sutcliffe's coal-dust with acriflavine adsorbed (kindly prepared by Dr. David Campbell, Materia Medica Department, University of Glasgow) such that nine grammes contained 1.0 gramme acriflavine = one dose. Three doses were given in the day (to K.O.). Here again failure was met with, but it is important to note that only a small quantity of acriflavine was able to be extracted from either the urine or the faeces, showing the firmness of the absorption by the coal dust.

Naphthaline, given in gelatin capsules, commencing with 3 grains thrice daily and gradually increasing the dose up to 50 grains thrice daily for 14 days did not influence the excretion of B. paratyphosus B. by the chronic carrier (M.D.).

Sodium salicylate (30 grains thrice daily to J.M.), salol (20 grains thrice daily to J.M.), bismuth

salicylate (30 grains thrice daily to H.T.), and acid sodium phosphate (60 grains thrice daily given in the form of powders to K.O. and also given in gelatin capsules to J.M.) for periods of three weeks in each case, had no good result either in diminishing or stopping the excretion of the typhoid organisms. "Baptisia", a homoeopathic drug, used in the treatment of acute typhoid fever, was also given a trial on two carriers (K.O. and J.M.). Vinegar, in doses up to 6 drachms thrice daily was given in milk to K.O. for 1 week with a negative result. Raw Onions have been stated to be a specific by some authors for various types of intestinal worms. These were given to the demented lunatic typhoid carrier (J.M.) starting with one a day. The administration had to be suspended when the number was increased to four a day owing to the highly excited condition of the patient.

Frequent purging by calomel and elaterin had no curative effect and aided if anything the facility in isolating the typhoid bacilli.

Alkaline therapy: An attempt was made to corroborate the results of Nichols (1916 and 1917) in the case of a chronic bacillus excreter (H.T) who had a cholecystectomy with drainage of the cystic duct and who still excreted B. typhosus both in the bile and

faeces for some time after operation.

Two grammes of sodium bicarbonate by mouth increased after two days to three grammes was administered thrice daily for four days without any effect whatever as the bile and faeces still remained positive.

The Hydrogen Ion concentration of the bile was tested roughly by means of the Indicator Dyes.

The bile was diluted with neutral distilled water and the same technique followed as described on p. 121 for the faeces by Cannon and McNease (1923). The following indicators were tried in addition; phenol-sulphonal phthalein and phenolphthalein.

Before administration the Ph value was 7.2 - 8.4 probably about 7.8: two days after commencement it appeared to rise slightly to about 8.0 but again, in spite of the increased dose of alkali fell back toabout 7.8, being if anything nearer 7.2 than 8.4; after administration had ceased the bile gave the same reaction as before.

An attempt was made similarly to "flavinise" the bile and acriflavine was given by mouth to the same patient (H.T.) in increasing quantities (2 ccs. of 1% aeriflavine solution thrice daily, increased to 5 ccs - 0.05 grm. t.i.d.).

The administration had to be discontinued owing to the sickness which higher doses induced in the patient. The bile showed the presence of the dye only in

minute quantities, a concentration between 1:750,000 and 1: 1,000,000 being obtained when 0.05 grm was being given. The bile was first alkalinised by a 10% solution of sodium hydrate and then treated with methylated ether and alcohol to extract the acriflavine. A fluoresence was looked on as a positive result. Control experiments were made previously with varying dilutions of acriflavine in sterile human bile.

Another patient (K.O.) who had a cholecystgastrostomy performed was able to take 9 ccs. of the 1% Acriflavine solution (0.09 gram) thrice daily, though 10 ccs made her sick.

No bactericidal effect was secured, both the bile and faeces specimens still remaining positive for B. typhosus. The conclusion come to was that acriflavine given in a solution of distilled water by mouth, had no influence on the excretion of typhoid bacilli, and the quantity which reached the bile in these doses was negligible, even with the maximum quantity which the patient could tolerate.

The alteration of the intestinal flora by diet and carbohydrate feeding is fully discussed in the chapter on the transformation of the intestinal flora with special reference to the implantation of B. acidophilus (p. 367). Both Barker (1914) and

Torrey (1915) reported favourably on the use of lactose and milk in the treatment of enteric fever, while Haibe (1921) recommended a strict dietetic regimen also for chronic carriers with milk as a basis. The ordinary hospital routine diet, the valuesof which were worked out by Dr. G. Paterson, was employed. The proportion of its constituents were altered till they corresponded with those recommended by Torrey (1915).

Protein	= 50 - 100 grams.
Fat	= 75 - 100 grams.
Carbohydrate	= 250- 300 grams.

The increased quantity of carbohydrate was given in the form of arrowroot at the mid-day meal and at bed-time. The arrowroot was weighed before cooking and contained 82% starch. An allowance was made for the amount lost in cooking, as cooked arrowroot contains only 30% of carbohydrate.

The diet then constituted a high calory carbohydrate diet and was employed later with a view to increasing the aciduric flora in the intestine in conjunction with the giving of B. acidophilus milk.

Lactose in large quantities (6 ounces per day for periods of over a month) were given to two carriers (K.O. and M.D.) and caused a partial transformation from a mixed intestinal flora to an aciduric one in the case of K.O. while no marked effect was observed

in the other. No beneficial or curative effect was obtained on the excretion of the enteric organisms by milk soured with B. acidophilus either in conjunction with the high calory carbohydrate diet or with lactose nor did lactose alone have any inhibitory action on the excretion of the specific organisms.

In conclusion to this chapter it can be stated that so far the attempts by chemotherapy to cure intestinal carriers have not yielded results affording much evidence of their success. On consideration of the nature of the lesions met with in carriers, the problem of effecting a cure is an extremely difficult one. In the case of carriers in an early stage of this condition, there may be some hope of effecting a cure by one or other of the methods already tried and quoted above, but in long-standing chronic cases the prospect of success of this kind would seem to be extremely remote.

Though it may prove impossible to render the chronic carrier free from the specific bacilli, the prospect of diminishing the output of bacilli by carriers by means of some modification of the regime employed in the treatment of enteric cases is perhaps more hopeful.

Tsuzuki and Ishida (1910) recommend the use of arsenic and iodide of potassium in convalescent typh-

oid cases and claim that the bacilli disappear from the excreta at a somewhat earlier period under such treatment. Such a result cannot be considered more than encouraging.

Mayer's (1910) observations led him to believe that an exclusive milk diet during the disease, and an abundance of milk during convalescence, were most favourable in diminishing the number of carriers. With a mixed diet, on the other hand, the carrier state was more likely to supervene. The data on which he founded this opinion, however, were not sufficiently extensive to admit of reliable conclusions being drawn.

TABLE OF SUBSTANCES EMPLOYED BY AUTHOR IN TREATMENT OF ENTERIC CARRIERS.

Substance.	Maximum Dose.	Duration of treatment.
Ox Bile.	80 grains per day.	Three months.
"Dimol"	4 tablets thrice daily.	Repeated for two periods of 14 days.
"Felamine"	4 tablets thrice daily.	Repeated periods of 14 days.
"Kerol"	4 intestinal capsules thrice daily.	Two periods of 16 days each.
Acetozone	-	-
Kaolin	60 grains thrice daily.	Several months.
"Yadil"	2 drachms thrice daily.	Two weeks.

Naphtholated Charcoal.	6 teaspoonfuls three times a day.	Repeated periods of 2 weeks.
Sutcliffe's coat-dust with acriflavine adsorbed.	9 grms. containing 1 grm. of acriflavine thrice daily.	3 days.
Naphthaline	50 grains thrice daily.	74 days.
Sodium Salicylate.	30 grains thrice daily.	3 weeks.
Bismuth Salicylate.	30 grains thrice daily.	3 weeks.
Acid sodium Phosphate.	60 grains thrice daily.	3 weeks.
Salol.	20 grains thrice daily.	3 weeks.
"Baptisia"	-	4 days.
Vinegar	6 drachms thrice daily.	1 week.
Raw Onions	4 per day.	-
Calomel	4 grains.	Repeated occasions.
Pulv. Elaterin Co.	4 grains.	Repeated occasions.
Sodium bicarbonate.	3 grammes thrice daily.	Four days.
Acriflavine	0.09grm. thrice daily in 1% solution.	-
Lactose	6 ounces	1 month.
High calory Carbohydrate diet.	-	-
Acid sodium sulphate.	50 grains three times daily.	Three weeks.

Treatment aiming at alteration in the hydrogen-ion concentration of faeces.

Since with members of the colon group a P h value of about 5 is the limiting value, and any higher degree of acidity prevents growth, as Michaelis and Marcora (1912) and Clark and Lubs (1915) have pointed out, a means wereby the typhoid organisms in the intestinal tract of carriers might be killed of in vivo suggested itself, if only the reaction of the intestinal tract could be rendered sufficiently acid.

Preliminary experiments were made to confirm the findings of the above workers by growing a mixture of B. coli and B. typhosus in broth, which had been rendered markedly acid by the addition of dilute hydrochloric acid. The P h concentration of the medium was tested before inoculation with the organisms, and it was found that a value of 5 was sufficient to cause growth to cease.

Attempts were next made to alter the normal Ph value of the faeces by the administration of various drugs and feeding with carbo-hydrates and milk soured by various bacteria.

The normal faecal reaction of apparently healthy men on mixed diets lies between Ph 7.0 and 7.5 i.e. slightly alkaline to litmus, though temporary var-

iations may occur without giving rise to any unusual symptoms on the part of the subject.

Certain factors may cause a variation in the reaction and these are dietary, physiological and biological; bile insufficiency with non-absorption of the fatty acids causes the stool to be acid; pancreatic insufficiency causes alkalinity; milk diet with non-breaking down of the lactose associated with diarrhoea and premature caecal evacuation with carbohydrate fermentation also causes acidity. Robinson (1922) studied the effect of laxatives on faecal reaction and found the usual result to be a lowering of the Ph value of the faeces and the production of an acid stool. He states diarrhoea is accompanied by acidity and constipation by alkalinity of the excreta. The administration of the alkali, magnesium oxide, does not differ from others in this respect, but the faecal material passed after the cessation of the laxative action is usually acid. Robinson's view is that the physiological factor is the predominating one in influencing faecal reaction, no effect being noticeable as the result of introducing acidophilic bacteria into the intestine. The intestine apparently exerts a regulating influence which prevents the development of acidity by micro-organisms. Hull

and Rettger (1917) stated that acidity of the intestinal tract is highest in the duodenum as a rule, and lowest at the ileo-caecal valve, and claimed that diet is the main factor, and that the reaction of the intestine remained independant of the character of the intestinal flora. Rettger and Cheplin's (1921) findings support this statement as they found no increase in the acidity of the faeces where the flora had been changed to one consisting of 90% B. acidophilus. On the other hand, Cannon and McNease (1923) disagree with the conclusions of Rettger and Cheplin, who, they state, assume the actual acidity of the contents of the higher regions of the large intestine to be the same as that of the faeces themselves. Cannon and McNease (1923) found a marked difference in the actual acidity of the contents of the caecum and lower colon in rats when the determination is made separately, the contents of the colon being much less acid than those of the caecum. This fact is analogous in some respects to the statements of McClendon, Shedlov and Karpman (1918) that in rabbits with long ileums the acidity decreased on the way down. McClendon suggested that this was probably due to the progressively greater absorption of carbon dioxide. If this is the case, anything which would

hasten the passage of the contents along the tract would tend to prevent absorption of neutralization. Thus the acidity of the caecum is in all probability either absorbed or neutralized as the contents pass down the tract. Obviously then the hydrogen ion concentration of the faeces cannot tell us what is occurring higher up in the tract in the region of maximum bacterial activity, where most probably the intestinal transformation takes place. This would indicate that Rettger and Cheplin are incorrect in minimizing the importance of the actual acidity in eliminating the ordinary mixed flora.

Kendall's (1921) interpretation of the factors involved in the transformation of the intestinal flora are that an available carbohydrate is acted on by both proteolytic and fermentative types of organisms and as a result an acidity developes which is distinctly unfavourable for the former group.

Cannon and McNease's experiments in white rats indicate that the actual acidity of the intestinal contents of the caecum and colon is an important factor in the simplification of the intestinal flora. This varies directly with the hydrogen ion concentration, a Ph value of 7.0 being characteristic of a gas producing proteolytic type, whereas an increasing acidity is characterized by a diminution of the proteolytic types and their replacement of

aciduric types dominated mainly by B. acidophilus.

The effect in any case is a simplification of bacterial types with the elimination to a large extent of the usual colon group.

Methods of testing the Reaction of the faeces.

Goeffon's technique: Litmus paper both blue and red is laid on fresh faeces. The moisture soaks through and the reaction is read from the side which is not in contact. Needless to say the stools must be absolutely free from contamination with urine or the reaction is useless.

Robinson found a large percentage of results with litmus as an indicator were inaccurate and consequently he advises the use of the electro-metrical method. The procedure of Cannon and McNease is as follows:-

500 milligrams of faeces are suspended in 20 c.c. of practically neutral distilled water, centrifuged at high speed to eliminate turbidity and the indicator then added. Brom-thymol-blue, brom-cresol-purple and methyl red were the indicators used. The hydrogen-ion concentration was first determined roughly by adding a drop of the indicator to a few drops of the emulsion in a porcelain dish, then the exact acidity was determined by diluting the emulsion with an equal volume of neutral water, adding appropriate

indicator and comparing in comparator with La Motte buffer mixtures ranging from Ph 4.4 to 7.2.

For the purposes of the work in hand litmus paper as an dndicator was deemed quite sufficient and the technique of Goeffon was employed throughout.

Author's Observations.

The substances in the follow ing list were given to the enteric carriers with a view to causing a cessation of excretion or elimination of the specific organisms. It was hoped by the administration of large doses of certain of these substances to render the faeces sufficiently acid to inhibit or destroy the organisms in vivo.

Effect of Chemical Substances.

Acid sodium sulphate or sodium bisulphate ($NaHSO_4 + H_2O$), the so-called antityphoid tablets of Rideal, (who stated that it required fifteen grains to the pint of water to kill B. typhosus in vitro in fifteen minutes, though Martindale and Westcott in their Extra Pharmacopeia found the time to be two minutes) was given to the carrier (H.T.) in doses increasing from 10 to 50 grains three times daily for a period of three weeks without the desired result.

(This substance was formerly the army water steriliser and was administered ~~withou~~ by mouth in 10 grain

gelatin capsules).

The reaction of the faeces was altered from alkaline to litmus to acid when forty and fifty grains were being given thrice daily.

A note of the reaction of the faeces was kept during the administration of all the substances previously described under chemotherapy and the subsequent table illustrated their effect on the reaction. The doses are given in the earlier chapter.

Substance.	Patient.	Reaction.			Remarks.
		Before	During	After.	
Acetozone.	J.M.	Var.	Alk.	Var.	
Acid Sodium phosphate.	K.O.& J.M.	Alk.	Alk.	Alk.	
Acriflavine.	H.T.	Alk.	Alk.	Alk.	
Acriflavine absorbed with Sutcliffe's Coal-dust.	K.O.	Alk.	Alk.	Alk.	
Bismuth Salicylate.	H.T.	Alk.	Alk.	Alk.	
Charcoal (Naptholated)	M.M.	Alk.	Alk.	Alk.	
Dimol	K.O.	Alk.	Alk.	Alk.	
	H.T.	Alk.	Slightly Alk.	Alk.	
Fehamine.	J.M.	Var.	Var.	Var.	
	H.T.	Var.	Mostly Alk.	Var.	
Kaolin.	M.D.	Var.	Mostly Alk.	Var.	

Substance.	Patient.	Reaction.			Remarks.
		Before	During	After.	
Kerol.	J.M.	Var.	Mostly Alk.	Var.	
Naphthaline	M.D.	Alk.	Ac. (slightly so. alk).	very	45 grs. tid.
Ox Bile.	H.T. M.D.	Var. Var.	Var. Var.	Var. Var.	Slight occasional variations to neutral and acid.
Salol.	J.M.	Alk.	Alk.	Alk.	
Sodium Bicarbonate	H.T. & K.O.	Alk.	Alk.	Alk.	
Sodium Salicylate.	H.T.	Alk.	Alk.	Alk.	
Vinegar	K.O.	Alk.	Alk.	Alk.	
Yadil.	J.M.	Alk.	Alk.	Alk.	

Note. In the above table the following abreviations are used.

Ac. = Acid.

Alk. = Alkaline.

Var. = Variable.

Thus of the chemical substances employed only Naphthaline in doses of 45 grains thrice daily and Sodium bisulphate were effective in rendering the faeces acid. This, however, as has been seen, was ineffective in destroying the enteric bacteria.

Effect of Purgation.

Calomel in doses of 1 to 4 grains given at bedtime, and occasionally followed by a drachm of

magnesium sulphate in hot water the next morning, was given on various occasions to cases K.O., M.D., and H.T. Invariably the resulting fluid or semi-solid faeces were acid on these occasions. It was noticeable that when a hard, formed, stool was sent for examination the reaction tended almost invariably to be alkaline. This would, thus appear to agree with Robinson's findings.

Elaterin, given in the form of 4 grains Pulv. elaterin co., which contains one-tenth, of the active principle, at bedtime to the patient (H.T.) did not act so uniformly, as on a fair number of occasions neutral stools were obtained, even when the faeces were in a semi-fluid state.

Effect of milk soured with various organisms.

The reaction of the faeces of two of the patients (M.D., and K.O.) throughout the B. acidophilus milk and lactose feeding was alkaline or slightly alkaline to litmus. In the case of the third carrier (H.T.) the faeces reaction was much more variable, being alkaline, neutral, and acid on various occasions, but no relationship could be established during the acid periods with the taking of the milk or the consistency of the faeces. The character of the faeces was particularly noticeable in all the carriers fed with milk soured by B. acidophilus, as

they remained throughout more or less semi-pulaceous and light yellow in colour. This was very evident in comparison with the faeces of other carriers of the series, whose stools were frequently hard formed, thus rendering the isolation of B. typhosus and B. paratyphosus B. much more difficult, if not impossible.

Milk, infected with B. lactis aerogenes was administered to the paratyphoid B. carrier (M.D.). When one and a half fluid ounces of the milk were being taken the faeces were alkaline, but when the quantity of was increased to four ounces and a vaccine of the same organism had been administered the reaction became neutral and on occasions slightly acid. This was particularly evident when the flora had been almost entirely converted into colonies of B. lactis aerogenes.

Feeding with milk infected with B. coli (inosite fermenting) in doses of one and a half fluid ounces in the case of the same carrier gave an acid stool throughout though no absolute predominance of this variety of B. coli could be demonstrated in direct MacConkey agar plate cultures from the faeces.

Further details of the treatment by milk soured by these organisms is given on page 406.

Milk, infected with certain varieties of coli, appears to have the effect of altering the reaction

of the faeces from alkaline to acid. The acidity produced had no permanent effect on the excretion of the specific organisms, though during the intensive periods of administration these were much diminished in numbers.

Methods of treatment which aim at altering the intestinal flora.

The administration of milk infected with various types of lactic organisms is a natural complement to a high calory carbohydrate diet with a view to the simplification of the intestinal flora to one in which the aciduric organisms predominate.

Torrey (1915) reported that typhoid bacilli were isolated less frequently from the stools of typhoid patients on a high calory carbohydrate diet than from those in another series in which feeding was less liberal. The character of the faeces was altered, being almost always moist and soft, and the type of flora was one in which the aciduric organisms predominated. In a later article (1919) he reports that 250 grammes of lactose daily, principally in the form of milk and bread, was sufficient to give a positive B. acidophilus flora in the faeces.

Hull and Rettger's (1917) experiments on the influence of lactose feeding on man show that eight pounds of milk sugar taken during eleven days

produced a very marked aciduric flora, consisting particularly of B. acidophilus and B. bifidus, while four pounds over the same period caused the simplification of the flora to be much slower. In quantities less than this there was little or no alteration. They emphasize the value of laxatives in addition, so that by hurrying the lactose through the intestine a sufficient amount reaches the lower parts where the aciduric bacteria multiply most abundantly under the right conditions of environment and nutriment. Without the use of the laxative or some other agent which saves the lactose from rapid absorption in the small intestine, in which most marked bacterial changes putrefactive and otherwise, take place, little encouragement is given to the aciduric types.

According to Morris, Porter and Meyer (1919) in the absence of a proper pabulum of carbohydrates B.acidophilus can only lead a very limited intestinal existence, but when conditions are favourable to the presence of overwhelming numbers of B. acidophilus and B. Bifidus a very simple flora results.

Bass (1923) stated that 300 grammes of lactose per day was necessary in some cases in order to afford conditions suitable for their growth.

From the findings of the above workers it would appear necessary when feeding with milk infected with

sour-milk organisms to secure a proper pabulum by giving large quantities of lactose of a suitable high carbohydrate diet or to give large quantities of the milk.

The use of sour milk in relation to treatment and cure of typhoid carriers has been tried on numerous occasions, and milk soured with lactic acid bacilli has been recommended by some as affording an apparently permanent cure. Thus Liefmann (1909) gave Yoghurt in quantities of one third to two thirds of a litre daily to two insane female carriers and observed that one week after the commencement of the treatment the stools in both cases were negative. After seven weeks the stools of one of the cases were again positive but later examinations were negative. The last examination (there were only eight in all in each case) was made at the end of the eleventh week. Evidence from such a short period of observation proves nothing, not even that the temporary cessation was attributable to the treatment employed. Yoghurt or B. bulgaricus milk has been employed by Nichols (1919) without any beneficial results. Thomson and Ledingham (1910) report that three asylum carriers who were under bacteriological supervision for about two and a half years, received sour milk (half a pint three times a day) daily for ten or eleven months without any appreciable effect on the

excretion of typhoid bacilli in the faeces. Captain Fawcus, of the Army Medical Department, (1909 - 1910) reported fully on the treatment of army carriers by lactic acid bacilli. No improvement took place except in one case where a temporary diminution in the numbers of bacilli excreted was noticed. A second case appears to have reached more favourably to treatment (250 ccs. of a B. bulgaricus culture in diluted malt extract being given daily). The faeces remained free from B. typhosus for three months when the man was dismissed back to duty. This case may have been one of the temporary variety or a very intermittent type of excreter, but one is sceptical of the Bulgarian bacilli having any curative effect. At Netley Major Cummins (1910) had no success with this method of treating intestinal carriers.

Zweig (1910) has employed Lactobacillin in two carriers, and is inclined to support Liefmann in his advocacy of this mode of treatment. In Zweig's cases the bacilli disappeared after the commencement of the treatment and were not demonstrable during the succeeding three months. Only six examinations were, however, made during that period.

Kaiser (1921) reported that some strains of B. paratyphosus A and B will resist over two per cent. of lactic acid in Yoghurt milk for eighty-four to a hundred and twenty hours though most strains are killed

in a short time, and that the rapid production of acidity is most harmful to the organisms. His findings do not look encouraging for the success of some milk treatment in ~~chronic~~ chronic paratyphoid excreters.

Author's Observations.

In the case of two chronic B. typhosus (K.O., and H.T.) and one B. paratyphosus B. (M.D.) excreters, a proprietary preparation called Lactobacilline was first fried. Lactobacilline is prepared by "Le ferment", Paris according to Metchnikoff's formula it is stated. The tablets were ground up and given in milk commencing with three five grain tablets daily and increasing up to nine per day over a period from two to four months. In no case was the excretion of the organisms affected and they were found as regularly in the faeces as before. Bass (1923) warns against the use of commercial tablets of B. adidophilus and allied organisms stating that none of the tablets examined had as many as a thousand viable bacteria present of any kind, and that a thousand million tablets would be necessary to contain as many bacilli as are contained in a litre of acidophilus milk, which is the quantity found by most observers to be necessary to transform the flora. Bacteria were more numerous in the commercial liquids examined but here again seven or eight gallons would be necessary to get as many as in a litre of acidophilus milk. He

emphasized that only fresh cultures produced according to the proper bacteriologic methods should be used and warns against the mistake that was made with B. bulgaricus in employing only a teaspoonful dose, which is only a fraction of the amount of culture that others have already found necessary to change noticeably the intestinal flora. Unfortunately the quantities of sugars required are too large to be continued over long periods of time, hence the practical application of this method must necessarily be limited.

Milk soured with B. acidophilus was also given to the same three chronic carriers for periods extending over a year. B. acidophilus milk was used in preference to milk soured by B. bulgaricus because the former is a normal inhabitant of the human intestinal tract while the latter is not (Rahe 1915 and 1918) and in addition the transformation of the normal mixed intestinal flora can be easily obtained by feeding with B, acidophilus and not with Yoghurt. B. acidophilus soured milk is also more palatable. A full description of the methods employed in the isolation of aciduric organisms from the faeces of the carriers, the preparation of the milk soured by B. acidophilus, and a discussion on the differences in their morphology and cultural characters will be found in the chapter devoted to this subject (see p. 367).

The carriers drank approximately two and a half pints per day taken in three doses between meals. Smears, both from milk and faeces, were made daily and stained by Gram's method with a view to determining the relative numbers present. In addition cultures on liver glucose agar, whey agar, and two per cent. lactose agar were made from time to time to ensure the purity of the acidophilus growth in the milk and to isolate the organisms from the carrier's faeces. The aciduric organisms by this means became the predominating organisms in the faeces, but in spite of this there was no noticeable diminution of the typhoid andparatyphoid bacilli excreted and certainly no cure.

Various experiments were tried during the period of administration. Alteration of diet to a high calory carbohydrate one, and the giving of lactose as well as purgation with laxatives and cathartics. None of these procedures had curative effect; their action on the intestinal flora is more fully discussed in the chapter on B. acidophilus.

A point worth noting here is that the Ph value of the faeces was never altered by the sour milk therapy, as ascertained by litmus, following the method described by Goeffon.

Thus it appears quite conclusively that no curative result in chronic bacillus excreters is

obtained from the use of various types of sour milk, but whether the administration will prove of value during the convalescent stages in diminishing the numbers of carriers remains to be seen. Up to the present time most of the observers who have studied this problem appear to have gained encouraging results, but the subject needs further and more extensive study before any reliable conclusions can be drawn.

Fletcher (1920) in the course of his investigations on capsulate mucoid forms of paratyphoid and dysentery bacilli hoped that if some particular organisms frequently or always overgrew and crowded out the paratyphoid bacilli during convalescence such an organism might be employed with benefit in the treatment of carriers. During his investigations on typhoid carriers the author (1923) noticed that certain types of B. coli derived from the faeces of carriers had the effect of overgrowing or inhibiting the growth of B. typhosus and causing their disappearance from mixed cultures <u>in vitro</u>, to a greater extent than a stock B. coli strain had. These appeared to be the cause of the comparative failure in the brilliant-green fluid enrichment method of isolation (see p. 49). Among the other varieties of B. coli which tend to have this property

are those of the lactis aerogenes type and inosite-fermenters.

Thus it appeared that milk cultures of such varieties of B. coli might be employed with a view if not to curing the carrier state at least to suppressing the pathogenic organisms during the period of administration. So milk, infected with B. lactis aerogenes, and incubated for twenty-four hours was given first to a B. paratyphosus B. faecal carrier (M.D.), commencing with teaspoonful doses three times daily and increasing every second day by teaspoonful doses till twelve were being given thrice daily. This was subsequently increased until 4 ounces were being given thrice daily. There was no marked alteration of the intestinal flora and no predominance of the "heavy opaque-spreading margined" B. coli of the lactis aerogenes type, though a few were present. The patient's serum at no period showed the presence of agglutinins for this variety of B. coli.

Raubitschek's (1912) work on animals suggested the basis for the experiment described in the succeeding paragraph. He found that in feeding animals with bacteria, not normally present in their faeces, these foreign bacteria could be found with difficulty or not at all in the faeces afterwards, but that

in animals, previously immunised by subcutaneous or intraperitoneal injections of the same organisms until agglutinins to these bacteria appeared in their serum, the organisms appeared in the faeces and persisted for many weeks afterwards.

A vaccine was next prepared of B. lactis aerogenes killed by heating in water bath at 56°C. for one hour in the usual way and injected into the same patient subcutaneously commencing with five millions and rising to one thousand millions. Only a very slight agglutination was got with 1:50 dilution of patient's serum a fortnight after the vaccine was discontinued. The feeding experiment was continued at the same time as the vaccine was being given. **(See Table XIII, p. 226).**

After stoppage of the administration by mouth of B. lactis aerogenes, only small numbers of these organisms were found for the next two or three weeks, showing that no permanent implantation had taken place.

The number of colonies of B. paratyphosus B. excreted, as shown by direct plating, returned to approximately the same as before administration.

Another patient (J.M.), a chronically demented typhoid bacillus excreter, was fed with milk

TABLE XIII.

TABLE TO ILLUSTRATE THE EFFECT OF VACCINATION BY B. LACTIS AEROGENES ON THE IMPLANTATION OF THE SAME ORGANISMS IN THE INTESTINE BY FEEDING EXPERIMENTS, AND THE EFFECT ON EXCRETION OF B. PARATYPHOSUS B. IN THE FAECES OF CARRIER (M.D.).

Date.	Dose of Vaccine.	Dose of milk infected with B. coli, lactis aerogenes.	Direct Plate (MacConkey Agar, B. Paratyphosis B. colonies.	Colonies of B. coli, lactis aerogenes.	REMARKS.
12/4/23.	5 millions	4 ounces thrice daily.			Vaccine of B. lactis aerogenes was given from 12/4/23 to 2/5/23. while milk infected with same organism was given per os from 12/4/23 to 20/5/23 inclusive
13/4/23.	-	" " "			
14/4/23.	-	" " "			
15/4/23.	-	" " "			
16/4/23.	10 millions.	" " "			
17/4/23.	-	" " "			
18/4/23.	20 millions.	" " "			
19/4/23.	-	" " "			
20/4/23.	40 millions.	" " "			
21/4/23.	-	" " "			
22/4/23.	80 millions.	" " "			
23/4/23.	-	" " "	20	None.	
24/4/23.	125 millions.	" " "			
25/4/23.	-	" " "			
26/4/23.	250 millions.	" " "	10	Many.	
27/4/23.	-	" " "			
28/4/23.	500 millions.	" " "			
29/4/23.	-	" " "			
30/4/23.	750 millions.	" " "	3	Very numerous.	

Date.	Dose of Vaccine.	Dose of milk infected with B. coli, lactis aerogenes.	Direct Plate MacConkey Agar, B.paratyphosus B colonies.	Colonies of B. coli lactis aerogenes.	Remarks
1/5/23.	-	4 ounces thrice daily.			
2/5/23.	1000 millions.	" " "			
3/5/23.	-	" " "	5	Very numerous	
4/5/23.	-	" " "			
10/5/23.	-	" " "	1	Practically pure culture.	
14/5/23.	-	" " "	2	Fairly numerous (note purgative given previous evening).	
17/5/23.	-	" " "	2	Mostly.	
20/5/23.	-	" " "			
21/5/23.	-	— — —	4	Mostly	
24/5/23.	-	— — —	64	A few.	

similarly infected and was also given a vaccine of the same organism subcutaneously in the same manner as described for the B. paratyphosus B. carrier. The dose of the milk was increased till six ounces were being given thrice daily. Unfortunately this experiment proved inconclusive as the patient became unmanageable, and frequently when he was supposed to be getting the full dose of milk overturned the glass or refused to take it. His serum did not develop any agglutinins for B. lactis aerogenes and his faeces only during the middle of treatment showed a preponderance of these organisms. No reliable conclusions could be drawn from the results in his case.

Again during the interval in giving the B. lactis aerogenes milk to the paratyphoid carrier (M.D.), milk similarly infected with a stock strain of inosite-fermenting B. coli (Peat strain) was given for a period of fourteen days, commencing with a teaspoonful and increasing to twelve teaspoonfuls thrice daily. There was no appearance of agglutinins for the inosite-fermenters and only a small proportion of the colonies tested from the MacConkey agar plates proved to be inosite-fermenters. There was little or no reduction in the numbers of B. paratyphosus B. excreted.

Milk infected with B. lactis aerogenes was given in gelatin capsules to the carrier (H.T.), and again in formalinised gelatin capsules in doses of five thrice daily, but here also there was no alteration of the intestinal flora.

Difficulty was experienced in preparing the gelatin capsules for administration owing to softening.

The experimental feeding of these patients by bacteria not normally present in their faeces without any previous immunisation by vaccines failed to cause their implantation in the faeces but after vaccination implantation occurred for a time. Thus this result agrees with that of Raubitschek (1912), whose experiments shewed that the bactericidal action of the faeces was not responsible, and that the organisms could be recovered from the stomach contents easily 5 hours after feeding, but that cultures from different parts of the intestine were negative prior to, but not after immunisation. Immunisation would appear to be the decisive factor in implantation. Schütz (1909) thought that living intestinal epithelium was responsible for the bacteridical effect normally, but as has been shewn neither the author's nor Raubitschek's experiments support this view.

Shortly after cholecystectomy and cholecyst-gastrostomy respectively had been performed on two of the chronic carriers (H.T., and K.O., respectively B. proteus was noticed for a time in their urine and faeces. In the case of (K.O.) B. proteus appeared for the first time in the urine on 10.5.23. six days after operation and later on 17.5.23 in the faeces. On 28.5.23 the colonies were not quite so numerous and B. typhosus was isolated from the same MacConkey agar plate, but on 31.5.23 the colonies were so numerous that they obscured any other growth. Since the latter date the faeces have remained consistently negative for the typhoid organisms, nor has B. proteus been found again. In the other case (H.T.) colonies of B. proteus did not appear until ten days after operation. They appeared first in the urine and later on 21.5.23 in both urine and faeces. B. proteus was not isolated after 31.5.23. Since that time B. typhosus has been once present in the faeces on 28.6.23. At times this organism thus completely dominated the intestinal flora, as shown by direct MacConkey plate and via brilliant-green, to the exclusion of B. typhosus, and thus would appear to be worth trying as an additional organism to those already employed by the author, as these two cases are probably cured.

It is noteworthy that brilliant-green in a dilution of 1:1,000,000 causes a definite inhibition of the growth of B. proteus and in some instances causes a complete inhibition. The cultural characters of the bacillus isolated in both these cases are shown in the following table:-

Table to show cultural characters of B. proteus isolated from Carriers.

Motility.	Lactose	Glucose	Mannite	Dulcite	Maltose
+	o	A^{1} $A\&G^{4}$	o	o	o

Sarrharose	Inosite	Litmus milk	Gelatin	Indol	Peptone Water.
o	o	V.sl.A.	o^{1} liquefied^{4}	o	

A = acid
V.sl.A. = Very slight acid.
G = gas.
Numerals = day of appearance.

VACCINE THERAPY.

Opinions regarding the value of vaccine therapy in enteric carriers are varied. Numerous observers claim to have obtained cures, especially with autogenous vaccines, while others, equally numerous, are as emphatic that it is useless. Soon after the value of prophylactic vaccination became established, vaccine therapy as a means of clearing

up carriers was inaugurated and for a time considered successful. R. Koch as early as 1902 suggested typhoid vaccines as a remedy for the carrier state. Houston (1906) stated that vaccination with a killed culture of the patient's own bacilli has had a favourable effect in only a proportion of cases. Irwin and Houston (1909) reported a typhoid urinary carrier, cured by an autogenous vaccine, and Meader (1910) also reported a case successfully treated. Following these many other apparent cures were reported from time to time, and thus this treatment was thought to promise a solution of the carrier problem. Johnston (1912) reported his work on experimental rabbit carriers, and claimed success from vaccine treatment, but as certain of his results are so divergent from those obtained by Stone (1919), Doerr (1905), Bull (1915-1916), Francke and Parker (1919) and others, one cannot but question their validity. Whatever may have been the success of these earlier experimenters, repetition of their work and the subsequent use of vaccine in both human and animal carriers has given practically negative results. Thus two chronic urinary carriers, invalided from the Indian Army have been treated with typhoid vaccines (Fawcus, Kennedy and Cummins - 1910) without definite improvement in their condition. Browning and Gilmour (1910) reported a failure with a

mixed autogenous vaccine used for several months in the case of a chronic gall-bladder carrier, who had cholecystostomy performed and who was found to be excreting typhoid bacilli from the fistula two and three-quarter years after the acute illness. Walker Hall and Roberts (1911) reported their observations on a female urinary carrier who earlier failed to react to autogenous vaccine treatment but who later (quoted from Ledingham and Arkwright - 1921) after nephrotomy with removal of renal calculi and another course of vaccine treatment was typhoid-free during a period of four months. As this same case had a previous negative phase of nine months one cannot say whether a cure was obtained or not, or whether this constituted another intermission. Thomson and Ledingham (1910) have treated five female carriers in this way with entirely negative results, whether stock or homologous vaccines were employed. A report by the Director-General of the Army Medical Service (1909) gives an account of the vaccine treatment of an intestinal carrier, both with stock and autogenous vaccines, and a temporary cessation in the exeretion of the germs in the stools lasting for three or four months, was noted during the course. Later this case received X-Ray treatment and was reported by Cummins, Fawcus, and Kennedy (1910), but

did not benefit materially by it as only a temporary cessation of excretion of B. typhosus took place. Stokes and Clarke (1916) claim, by means of a stock typhoid vaccine, to have benefitted two chronic urinary carriers. Two injections were made subcutaneously of 500 and 1000 millions and urotropine given at the same time. In both cases a definite focal reaction was experienced and after this had passed off the typhoid bacilli disappeared from the urine. As they base their findings on six negative examinations over a period of 15 days in one of their cases too much reliance cannot be put on the conclusions. Recently Watt (1923) published a cure in a chronic bacillus excreter by means of a detoxicated vaccine. Shortly after this report had appeared an organism which had been found earlier and which gave the typical fermentative and sugar reactions for B. typhosus, but did not agglutinate with any of the stock antisera, was again isolated from the faeces on the twenty-first examination after a negative phase lasting almost six months. This organism was investigated by Professor Browning and the author and found to be a typical B. typhosus which was somewhat deficient in agglutinin-binding power, an extensive series of absorption tests being carried out and an animal inoculated with the organism in order to prove this (see p. 347).

Haibe (1923) stated that as a general rule autogenous vaccines did not prove officacious in his series of cases. Nichols, Simmons and Stimmel (1919) tried both stock and autogenous vaccines without any success. Uhlenhuth and Messerschmidt (1920) found vaccines useless in their typhoid rabbit carriers. Kach (1920), on the other hand, reports that two chronic bacillus carriers seemed to be permanently freed from bacilli by an autogenous vaccine. But in the case of a urinary carrier the typhoid bacilli were still found in spite of a series of twenty-two injections. Murstad (1921) advises that vaccine treatment of carriers should be abandoned bacause it is ineffective.

In cases where there is a chronic suppurating process or fistula due to B. typhosus numerous observers have claimed that vaccine treatment is of great value in aiding the healing. Arnd (1923) quotes Bureau and Marchard, who had a case of a woman, suffering from herpes zoster eight months after typhoid fever. She developed a fistula in Scarpa's Triangle and under Poupart's ligament, the pus from which gave a pure culture of B. typhosus. The fistula healed and broke down six times in two and a half years and was finally cured following sixteen injections of a homologous vaccine. Weil

(1917) reports a case of a typhoidal suppurating osteomyelitis which had dragged on for two years, during which time seven futile operations were performed, being cured in three weeks by vaccine therapy. In another case after eleven months with three operations cure was complete in one week. improvement amounting to cure was obtained in two weeks in suppurating lesions of six and twelve months' standing in other cases. It failed only in one case which was cured after a sequestrum had been removed.

Pensuti (1917) noted that acute typhoid cases which were refractory to vaccine treatment showed late or deficient formation of agglutinins and that in over fifty per cent. they developed a suppurating process. The logical conclusion to obviate suppurative complications would then appear to be to raise the agglutinin content of the patient's serum by vaccine therapy.

Hindmarsh (unpublished), however, in a personal communication to the author reports a case of a Brodie's abscess of the tibia occurring eighteen years after an attack of typhoid fever, which gave a growth of B. typhosus and which healed following an operation without any vaccine treatment.

The author studied the case of a man (M.M.) aged 48 years who developed a swelling over the

junction of the cartilage and bone of the fourth rib six months after enteric fever and from which a pure culture of B. typhosus was obtained. The case was operated on by Dr. Scoular Buchanan who detected no connection between the lesion and bone or cartilage. This case practically healed but within 6 or 7 months after operation broke down once more. Vaccine therapy might possibly prove of value, and is worth a trial in this type of case.

Whether preventive inoculation or vaccine therapy will lesson or prevent the occurrence of bacillus excreters among those who do not clinically become affected with enteric fever or lesson the number of chronic excreters from convalescents is a question which deserves attention. Good (1923) suggests that preventive vaccination proved very efficient in preventing the development of carriers. On the other hand Simmons and McCarthy (1924) found four chronic typhoid carriers (4.8%) and one B. paratyphosus B. carrier (1.1%) among 84 convalescents cases of typhoid fever, all of whom had received triple typhoid vaccine previous to their infections. They conclude that this result is not surprising as the vaccinations ~~that th~~ were incapable of preventing the oc currence of the fever in these subjects It has been observed by Gay and Claypole (1913) that

immunized rabbits do not become carriers after intravenous injections of a dose of living micro-organisms of the enteric group that produce the condition in normal animals. They may, however, still be locally infected by injections directly into the gall-bladder, as has been pointed out by Uhlenhuth and Messerschmidt (1912), and Emmerich and Wagner (1916) or even by intravenous doses when sufficient to overcome the increased bactericidal effect of the blood (Nichols, 1916). Preti (1917) claims by means of vaccine given intravenously and subcutaneously to have abolished the carrier state in ten days. The majority of the cases upon which he bases his contention appear to belong to the category of convalescent or temporary carriers. As he states, the carrier state lasts for about two months, occasionally four months, and rarely for a year or more as in the case of chronic excreters. Hebert and Bloch (1922) concluded that vaccination seemed to have an effect upon the incidence of typhoid bacilli in the urine and faeces, on the slender basis of 43 cases of which only 13 were completely vaccinated, as the bacilli were found most often and most abundantly in the excreta of the non-vaccinated patients. Goubau (1917) has shown that by vaccination with an autogenous strain of

B. typhosus, convalescent or temporary carriers of the bacteria clear up much sooner than when they are left untreated. However, as Reibmayer (1918) points out, twenty-five per cent of the so-called convalescent carriers free themselves spontaneously of infection i.e. the typhoid and paratyphoid bacilli disappear from the stools within three months after apparent recovery from the acute fever. Meyer (1921) advises that in all typhoid cases an attempt should be made to prevent by proper treatment (high calo ry diet instead of starvation which favours biliary stasis) the development of the carrier state and that carriers should be treated in the earliest possible stages by intensive vaccination with an autogenous strain of B. typhosus as a prolonged carrier state leads to severe lesions which cannot afterwards be influenced by such procedures. The actual method by which vaccination should affect the excretion of typhoid bacilli, especially in the case of the chronic bacillus excreters is still somewhat obscure. The serum of man and animals, injected with antityphoid vaccine till they are highly immunized, has been found by the majority of observers not to be bactericidal to B. typhosus either in vivo or in vitro. Johnston (1912) claims it to be bactericidal, but Teague and McWilliams (1917) who corroborate the work

of Buxton and others, showing that normal rabbit serum is capable of killing large numbers of typhoid and paratyphoid bacilli and that serum of rabbits highly immunized against typhoid and paratyphoid bacilli ~~and that serum of rabbits highly immunized against typhoid and paratyphoid bacilli~~ do not kill these organisms, disagree with Johnston's findings. They state that the agglutinin titre of an immune serum is not indicative of bactericidal power of blood plasma in vivo. Francke and Parker (1917) found no difference in the bactericidal powers of normal and immune rabbits on intravenous injection of living typhoid bacilli. Their observations are in accord with those of Bail (1905) and with those of Meyer, Neilsonand Feusier (1921). Stone's (1919) results agree with those of Teague and McWillians, but, in addition, this worker claims to have demonstrated that typhoid bacilli disappear more quickly from the organs of immune animals than from normal animals, which would seem to indicate that the destruction is due either to some interaction between the tissue cells and plasma with the bacilli in vivo, or to some other factor which has been over- looked. From the varied opinions expressed above preventive inoculation would appear to have some value in lessening the number of carriers

produced, but more extensive research will need to be carried out before any definite opinion can be given.

Another method by which vaccine treatment may be given is by the mouth, as employed by Besredka (1919) for prophylactic purposes in dysentery, typhoid and paratyphoid fevers. He bases his hypothesis on the fact that the guinea-pig cannot be protected against anthrax except by the intradermal route (apparently the skin lesion is the only natural means of infection of this animal). In addition, he recommends ox bile by mouth to stimulate biliary secretion and states that the combined effect of the administration of foreign bile and increased secretion of the subject's own bile favours desquamation of theintestinal mucosa and therefore absorption of the virus. The bile sensitises the animal so that no matter by which route the virus is introduced typhoid infection developes in the intestine. Vailliant (1922) who gave a bile pill and a T.A.B. tablet in the morning on an empty stomach on three consecutive days for prophylactic purposes claims to have obtained a greater degree of immunity by this method of vaccination than by the usual subcutaneous route. Trémolières, Loew and Maillart (1915) in their researches on antityphoid

vaccination by the alimentary route found that vaccine given by mouth in the form of pills had no effect in producing agglutinins, precipitins, or raising the opsonic index in the patient's serum, though these were produced by the subcutaneous method.

To test the value of this method of treatment in the case of a chronic intestinal typhoid bacillus excreter, Besredka's vaccine tablets (pastilles antityphiques biliées) were obtained from Villette, Paris, and were given to a chronic carrier (K.O.), without having any effect on the excretion of the typhoid bacilli. A bile pill and a T.A.B. tablet were given on an empty stomach on three consecutive days. No details were given as to the numbers of organisms in each T.A.B. tablet. There was a very slight increase in the agglutinins of the patient's serum for B. typhosus and a marked rise for B. paratyphosus A.

TABLE TO ILLUSTRATE THE EFFECT OF PASTILLES ANTITYPHIQUES BILIEES BY MOUTH ON WIDAL REACTION OF A TYPHOID CARRIER (K.O.).

TIME.	STANDARD AGGLUTININ UNITS PER C.C. PRESENT IN PATIENT'S BLOOD.		
	TYPHOID.	PARATYPHOID A.	PARATYPHOID B
Before.	16.0	13.0	11.0
After (7 days).	18.0	41.0	8.0
After (11 days).	22.0	49.0	10.0
After (3 months).	12.0	10.0	4.0

Again, a typhoid vaccine consisting of a twenty-four hours bouillon culture of a stock B. typhosus (R.L.L.), killed at 60°C. for one hour in a water bath, was administered by mouth to another carrier, a male asylum typhoid excreter (J,M.) commencing with 100 millions and increasing to 20,000 millions.

Date.	Dose.	Remarks.
15/1/23.	100 millions.	
17/1/23.	200 "	
21/1/23.	350 "	Following the second
23/1/23.	500 "	dose the patient's tem-
25/1/23.	700 "	perature rose to 99.3°F.
27/1/23.	1000 "	and some nausea and
29/1/23.	1500 "	diarrhoea was experienced
31/1/23.	2000 "	This passed off in a few
1/2/23.	3000 "	days, but the motions
3/2/23.	45000 "	were somewhat loose for
5/2/23.	6000 "	about a week later.
7/2/23.	8000 "	No other ill effects
9/2/23.	10,000 "	resulted throughout the
11/2/23.	15,000 "	treatment.
13/2/23.	20,000 "	

TABLE TO ILLUSTRATE THE EFFECT OF TYPHOID VACCINE BY MOUTH ON WIDAL REATION OF CHRONIC CARRIER (J.M.).

TIME AND DATE.	STANDARD AGGLUTININ UNITS PER C.C. OF PATIENT'S SERUM.		
	TYPHOID.	PARATYPHOID A.	PARATYPHOID B.
Before. (14/1/23).	29.0	-	-
During. (1/2/23).	29.0	-	-
(5/2/23).	25.0	-	-
After (22/2/23).	59.0	-	-
(5/3/23).	29.0	-	-

It is thus seen that a definite rise in the agglutinin content of the patient's serum took place shortly after the vaccine had been given, but seen fell once more to the normal level for this patient. The vaccine was tested by inoculating a rabbit intravenously in increasing doses and caused agglutination in a dilution of 1:6,400 of its serum in a few weeks. The serum before vaccination failed to agglutinate with a dilution of 1:50. The vaccine had no effect on the excretion of B. typhosus, nor was any diminution in the numbers of bacilli in cultures of the faeces noticeable.

The same patient (J.M.) was next inoculated subcutaneously with a"residual"vaccine of stock B. typhosus (R.L.L.)(Jenkins B.M.J. 11/6/21.), commencing with 500 millions and rising to 5,000 millions during a period of fourteen days. No reaction occurred

with any of these doses. The Widal reaction for B. typhosus in the patient's serum which contained 29.0 standard agglutinin units per c.c. before inoculation fell to 12 units a week after treatment and then during the course of the next month gradually rose to 25 units. The faeces still continued to show the presence of B. typhosus in the same quantity as before the vaccine therapy was employed. The residual vaccine was prepared as follows:- the growth was emulsified with 1% solution of phenol in sterile tap water, and poured into a tall glass, and volume noted and number of organisms per c.c. present estimated: 40% NaOH was added until the suspension contained ½% NaOH; set aside for one hour until opacity of suspension was lost; HCl added drop by drop until it was neutral to litmus and an additional drop for every 10 c.c. of volume, the fluid becoming opaque again; Hydrogen peroxide (10 vols.) in the proportion of 1 c.c. for every 50 c.c. of suspension then added and glass placed in oven at 55°C. for 24 hours when sedimentation occurred; supernatant fluid removed and volume made up to original figure with 1% phenol in sterile tap water; again 1 drop of HCl was added for every 10 c.c. of volume and sedimentation allowed to take place (usually in 3 to 6 hours); Supernatant fluid

decanted and sediment neutralised with 4% NaOh; original volume was once more restored with normal saline containing no phenol and the resulting solution autoclaved without destroying its properties

Beckerich and Haudurey (1922) inaugurated treatment of acute cases of typhoid fever by means of Bacteriophage, given by mouth (2 ccs.) and subcutaneously (1 cc), in nine patients of typhoid and two of paratyphoid B. and had favourable results in five of the former and both of the latter. In the future the Bacteriophage may come more and more into use as a therapeutic agent and from its lytic powers against the various organisms should be worth a trial against the typhoid bacilli chronic excreters. The author had no success with lytic experiments on B. typhosus in vitro with filtrates of carrier and stock varieties of B. coli.

****

B I B L I O GRR A P H Y.

Alvares (1917): Liglo medico, Madrid, LXIV, No 3291, p. 1. Ref. J.A.M.A., 1917, 68, p. 885.

Arnd (1923): Schweiz. Med. Woch. 53, Jahrb, 423,

Bail (1908): Arch. f. Hyg., 52, p. 272.

Barker (1914): J.A.M.A., 63, pp. 929-931.

Bass (1923): Southern Med. Journ., Birmingham Ala., 26, Ref. J.A.M.A. 1923, 80, p. 508.

Beckwith (1921): a. J. Infect. Dis., 29, p. 495.

Beckwith (1921) b: Soc. Exp. Biol. and Med., XVIII, p. 36.

Besredka (1919): Par. médicale, 12, p. 460.

Blachstein (1891): Bull. John Hopkins Hosp. ii, p.96.

Beckerich and Hauduroy (1922): Compt. rend. Soc. de Biol., Par., 86, pp. 168-170.

Blumenthal (1910): Centralbl. f. Bakt., I, orig, 28, p. 1314.

Browning and Gilmour (1910): Glasgow Med. Journ., August 1910.

Bull (1915-16); J. Expt. Med., 1915, 22, p. 475; ibid 1916, 23, p. 419.

Bully (1911): Zeitschr. f. Hyg., 59, p. 29.

Cannon and McNease (1923): J. Infect. Diseases, 32, p. 175.

Clark and Lubs (1915): J. Biol. Chem., Balt., 22, p. 87.

Conradi (1910): Ztschr. f. Immunitätsforch, 1910, 7, p. 158.

Crile (1916): J. A.M.A., 16, p. 1830.

Cummins, Fawcus and Kennedy (1910): Journ. R. A.M.C., XIV, p. 351.

Doerr (1905): Centralbl. f. Bakt., I, orig, XXXIX, p. 624.

Dragstedt, Dragstedt, and Nisbet (1922): J. Lab. and Clin. Med., Chicago, 1922, 8, No 3, p. 190.

Drigalski (1904): Centralbl. f. Bakt., abt. orig, XXXV, p. 776.

Emmerich and Wagner (1916): Med. Klin., 12, p. 819.

Fantus (1923): J. A.M.A., 80, p. 556.

Fawcus, Kennedy and Cummins (1910): Journ. R.A.M.C., XV, p. 351.

Fletcher (1920): J. R.A.M.C., XXXIV, p. 219.

Forster (1907): Verhandlung der Deutschen. Path. Gesellschaft.

Forster (1908): Münch. med. Woch., 55, p. 1.

Francke and Parker (1919): J. Med. Research, 39, p. 301.

Garbat (1922): Monos. Rockefeller Instiți No. 16. May 10th 1922.

Gay (1918): Typhoid fever considered as a problem of Scientific Medicine, New York, 1918.

Gay and Claypole (1913): Arch. Int. Med., 1913, 12, p. 613.

Geiger (1918): Deutsche med. Woch. 1918, May 2nd., p. 486.

Glatard (1921): Bull. de la Soc. Méd. Hôp., Par., 45, p. 990.

Geoffon: Manuel de Coprologie Clinique. Masson et Cie, Editeurs.

Good (1923): Schweiz. med. Woch., Basel, 1923, 53, p. 421.

Goubau (1917): Arch. Méd. Belges, Paris, 1917, 70, p. 590.

Haibe (1921): Comm. Roy, Soc. Belg., quoted J.A.M.A., 1921, 77, p. 136.

Hailer and Rimpau (1912): Arb. a. d. Kais, Gesundh., 36, p. 409.

Hailer and Wolf (1915): Arb. a. d. Kais, Gesundh, 48, p. 80.

Hertz (1916): Wein. Klin Wchnschr., 29, p. 1290.

Hilgermann (1923): quoted Arnd (1923):

Hall, Walker and Robberts (1911): J. Path. and Bact., 1911, XV, p. 120.

Hébert and Bloch (1922): Ann. de l'Inst. Past. Par., 36, p. 157.

Houston (1906): Brit. Med. Journ., Oct. 6, 1906,

Hull and Rettger (1917): J. of Bact., 2, p. 47.

Irwin and Houston (1909): Lancet, 1, p. 311.

Johnston (1912): J. Med. Research, 27, pp. 177-187.

Kach (1920): Zeitschrift, f. Klin. Med., Berl, 89, p. 87.

Kalberlah (1915): Med. Klin., 11, p. 581.

Kaiser (1921): Centralbl. f. Bakt., I, orig, 80, p. 554.

Kendall (1921): Text Book on Bacteriology, 1921, p. 625.

Knick and Pringsheim (1911): Deutsch. Arch. Klin. Med., 101, p. 137.

Koch (1902): "Die Bekämpfung des Typhus, Nov. 1902; Litzung des wissenschaftlichen Senats bei der Kaiser Wilhelms - Academie Nov. 28, 1902.

Kuster (1923): quoted Arnd (1923).

Kuster and Wolf (1918): Münch. med. Woch., 1918, Oct. 22, p. 1182.

Ledingham and Arkwright (1912): The Carrier Problem in Infectious Diseases, London 1912.

Leitner (1918): Wien. Klin. Wchnschr., 1918, No. 26.

Lentz (1905): Klin. Jahrb., XIV, p. 475.

Liefmann (1909): Munch. Med. Woch., 56, pp. 527-551.

McClendon, Shedlov and Karpman (1918): J. Biol. Chem., Balt., 34, p.1.

Mayer (1910): Centralbl. f. Bakt., Abt. I, orig., 53, pp. 236-279.

Meader (1910): Mod. Med., 2, p. 244.

Messerschmidt (1918): quoted Küster and Wolf (1918):

Meyer (1921): J. Infect. Dis., 28, p. 381.

Meyer, Neilson and Feusier (1921): J. Infect. Diseases, 28, p. 40; ibid. p. 458.

Michaelis and Marcora (1912): Ztschr. f. Immunitätsf., O., 14, p. 170.

Morris, Porter and Meyer (1919): J. Infect. Dis., 25, p. 349.

Murstad (1921): Med. Rev., 1921, 38, p. 215. Ref. J. A.M.A., 1922, 79, p. 561.

Nichols (1916): J. Expt. Med., 24, p. 497.

Nichols (1917): J. A.M.A. 68, p. 958.

Nichols, Simmons and Stimmel (1919): J. A.M.A., 79, p. 680.

~~Nichols (1919): J. Am. Med. Assoc., 73, p. 680.~~

Pensuti (1919): Policlinico, Rom., Med. Sect., XXIV, No 2, p. 83. Ref. J. A.M.A., 1917, 68, p. 1296.

Preti (1917): Riforma, Med., 1917, 33, No 41. Ref. Abstr. Bact. 1918, 2, p. 1266.

Prigge (1909): and (1912): Arb. a. d. Kais. Gesundheits, 1909, 22, p. 245. ibid, 1912, 41, p.276.

Prigge (1910); Klin. Jahrb., 1910, 22, p. 245.

Rahe (1915): J. Infect. Dis., 16, pp. 210-221.

Rahe (1918): Journ. of Bact., 3, p. 407.

Raubitschek (1912): Virchow's Arch., 209, pp. 209;220.

Reibmayer (1918): Munch. med. Woch. 65, p. 670.

Rettger and Cheplin (1921): Treatise on Transformation of the Intestinal Flora with special reference to B. acidophilus. New Haven 1921.

Robinson (1922): J. Biol. Chem., Balt., 52, p. 445.

Schutz (1909): Münch med. Woch. 33 quoted Raubitschek (1912).

Simmons and McCarthy (1924): J. Lab. and Clin. Med., IX, pp. 513-518.

Stokes and Clark (1916): J. R.A.M.C. XXVI, pp. 461-470.

Stone (1919): J. Infect. Dis., 25, pp. 284-298.

Stuber (1918): Munch. med. Woch., 1918, p. 201 and p. 790.

Teague and McWilliams (1917): J. of Immunol., Balt., II, No. 2. pp. 167-185.

Thomson and Ledingham (1910): Loc. Govt. Board, Report of Med. Off. 1908-09, Appendix B.

Torrey (1915): J. Infect. Dis., 16, pp. 72;108.

Torrey (1919): J. Med. Research, 30, p. 415.

Tremolières, Loew and Maillart (1915): Bull. Acad. Méd. 1915, Oct 26, p. 477.

Tsuzuki and Ishida (1910): Deutsch. med. Woch., 36, p. 1005.

Tonney, Caldwell and Griffen (1916): J. Infect. Dis., 18, p. 239.

Uhlenhuth and Messerschmidt (1912): Deutsch. med. Woch., pp. 2397-99.

Uhlenhuth and Messerchmidt (1920): Deutsch. med. Woch., p. 1293.

Vailliant (1922): Annal. de l'Instit. Past., Par, Feb. 1922.

Watt (1923): Lancet, No. 519, CCIV, p. 378.

Weil (1917): Bull. de l'academic de Med., Par., LXXVII, p. 108.

Zweig (1910): Deutsch. Med. Woch., 1910, p. 1803.

****

CHAPTER VIII CONTINUED.

SURGICAL TREATMENT OF ENTERIC CARRIERS.

Operative treatment has proved the most efficacious means of relieving the carrier condition up to the present time, and has been directed against the gall-bladder. The association of gall-bladder complaints and typhoid fever was observed by Naunyn in 1892, but Lentz (1905) first directed attention to their association with the carrier condition. It was, however, left to Forster (1908) to develope this view more completely. Thus since it has been proved that the gall-bladder is the main site of vegetation of the typhoid organisms in intestinal carriers, operative interference suggested itself as a probable remedy for the carrier state.

Cholecystotomy, cholecystostomy and cholecystectomy, alone and with appendectomy, have been tried,

and lastly, as suggested by Macrae, cholecystgastrostomy (see p.291). Long continued drainage through the hepatic duct with cholecystectomy is recommended by Garbat (1922), as physiologically bile, though formed continuously, is excreted only intermittently into the duodenum at the time of digestion. None enters when the stomach is empty. Hepatic drainage, he claims, allows a continual flushing of the bile ducts. Vosburg and Perkins (1925) recommend the removal of the appendix at the same time as the gall-bladder since the lymphoid tissue in its wall is often affected during an acute attack of typhoid fever. They cite Gilman's work on "amoebiasis" in favour of their suggestion as an example of how the intestinal tract may be temporarily cleared by drug treatment but how on removal of the appendix a permanent cure results.

Lack of success is attributed in early operations to the failure of the surgeon to excise completely the cystic duct. The operation should be performed, if possible, within six months of the onset of the disease, but if gall-bladder symptoms be present it should be performed at once. Of course, this applies to carriers who have been watched throughout the course of the disease.

In making an extensive review of the literature on surgical treatment difficulty was found in finding

evidence to substantiate many so-called cures. No definite criterion has been established as to what actually constitutes a cure. In many instances the bacteriological examinations of the faeces were only carried out for a few weeks after operation.

Criterion of cure of carrier condition and for the discharge of convalescents.

Henes (1920) recommends three negative consecutive duodenal cultures, and claims that more accurate and dependable results are obtained from allowing the duodenal tube to remain in position all day. Garbat (1922) states that an absolutely reliable indication of the complete absence of typhoid bacteria in the intestinal tract is two consecutive negative duodenal cultures and cultures from the faeces on the same days. No special interval of days between these examinations is specified. The U.S. Army rule is if three consecutive negative stool and urine examinations made at six day intervals prove negative only then are the typhoid patients allowed to be discharged.

Faeces examinations alone are thought by Garbat to be useless owing to the intermittency of excretion by many carriers. Browning and Gilmour (1910) describe a rational procedure for examinations to be carried out before typhoid convalescents should be

discharged; the faeces and urine are examined at intervals of 6 days on five occasions during convalescence and again a similar series after an interval of two months. If the results are all negative then and only then may it be safely assumed that the subject is free from typhoid bacilli.

Before a carrier may be judged to be cured in the author's experience the periodicity of excretion must first be determined by bi-weekly examinations of the faeces and urine over a period of several months. Duodenal cultures could be made to shew if a gall-bladder infection existed. After operation the examination of the faeces should be continued at weekly intervals for one to two years, (Ledingham and Arkwright 1912). In the case of one of the series examined, the B. paratyphosus B. excreter (M.D.), after operative treatment the faeces were negative for 8 months, and at the end of that period the organisms were again isolated on one occasion, but again have been negative for a further 8 months. Thus, if the examinations had not been continued for many months this case would have been considered as cured, though it may be cured now.

Definition of the chronic carrier condition.

Another difficulty arises as to what constitutes a chronic enteric excreter. Unfortunately many observers describe cures in cases which have suffered

from an acute attack of typhoid fever only a few months before. These cases might quite reasonably have proved to be of the temporary or convalescent variety and undergone a spontaneous cure.
An excreter should not be deemed chronic until at least a year has elapsed since the attack of enteric fever.

Review of literature on Surgical treatment directed against the gall-bladder in chronic B. typhosus excreters.

The association of gall-bladder disease and typhoid infection is described by Hunner (1899) in a case of acute suppurative cholecystitis with isolation of B. typhosus 18 years after an attack of typhoid fever. He presents an interesting table of 9 other cases operated on for cholecystitis and cholelithiasis in both recent and old typhoid infections, in all of which B. typhosus was isolated from the gall-bladder bile or from the contained gall-stones. To Dupré (1891) who operated on Chantemesse's case is given the honour of being the first to operate for this condition. None of these cases were known to be carriers before operation. It was not until 1907 that surgical interference was directed towards cure of the carrier state by Dehler, who operated on two female asylum carriers. These had

given rise to four cases of typhoid fever, including one fatal case. In his first case cholecystostomy was performed on 20/8/06 with drainage of the cystic duct which was dilated. Before operation 37 out of 39 specimens of faeces examined contained abundant typhoid bacilli. Two cherry-sized gall-stones were removed at operation. The bile was positive for the first twenty-one days and so long as the bile flowed freely from the fistula B.typhosus was scarce and was indeed for four weeks not demonstrable in the bile, but as the fistula narrowed and the bile flowed more slowly the bacilli got time to multiply and were very numerous. Later when the fistula closed and the gall-bladder cicatrized, the bacilli disappeared from the fistula secretion. The fact that the faeces remained negative for the specific organisms in 187 examinations over a period of more than six months except on three occasions, 17/10/06, 9/3/07, and 8/4/07, which were thought to be due to the mixing of specimens, suggested that any bacilli carried directly down from the bile passages into the intestine were overgrown by other bacteria.

It was considered not improbably that the discharge of the bacilli from individual portions of the liver and their bile-ducts would gradually cease completely and to hasten this action cholagognes were given for two months. The second case gave a doubtful

history of typhoid fever 19 years before, and here again cholecystostomy with drainage was performed on 10/4/07. 39 out of 55 specimens' of faeces examined before operation were positive for B. typhosus. The bile that drained away from the fistula contained typhoid bacilli which disappeared as the flow diminished. The gall-bladder contained several small stones which were sterile. After the ninth day B. typhosus was found only twice (25/5/07, and 3/7/07) out of 30 samples of faeces and since then 80 further specimens were negative. Grimme (1908) reported that he had the operation of cholecystectomy performed on a female asylum carrier who never suffered from symptoms pointing to implication of the gall-bladder. No inflammatory changes were found in the gall-bladder but there were 30-40 small stones, the size of peas, present and the interior of the stones contained typhoid bacilli. No typhoid bacilli were seen in sections of the gall-bladder wall. B. typhosus was found 15 days after operation but not at a later period. The period of observation after operation was only 55 days. Fromme (1910), however, states that the excreta of Grimme's patient were examined twice at an interval of 4 days two years later with negative results.

A female lunatic chronic bacillus excreter in

whose faeces during convalescence no typhoid bacilli were demonstrable but who had an attack of gall-stone colic about 11 weeks later and who was operated on, is reported by Loele (1909). The gall-bladder was very adherent and difficult to remove and between it and the stomach was an abscess containing a stone. The cystic duct was stenosed, but the hepatic duct was drained. The gall-bladder contained pus and 21 stones of the size of hazel-nuts which were sterile internally. The bile contained B. typhosus and on the 3rd. day after operation, the faeces as well. Death occurred six weeks later and at autopsy typhoid bacilli were found in the small intestine, transverse colon, liver, spleen, and heart blood, but the smallest bile ducts in the liver and the urine proved negative. Loele concluded that the typhoid bacilli did not come from the gall-bladder as it was cut off by the stenosed cystic duct, and contained pus but not bile. The bacilli may have grown in the dilated choledochus or in the intestine. He does not think removal of the gall-bladder in this case would have done the slightest good. It is of interest to note that he found no typhoid "nests" microscopically in the bladder wall. His conclusions are that the stones were present before the typhoid attack and that a fresh attack caused new inflammatory

changes and empyema; that cholecystectomy can be of no use if the seat of vegetation is in the bile tracts or in diverticula of the upper intestine; and that in no cases is operation justifiable in carriers without some palpable swelling or pain of the gall-bladder before operation. Cholecystectomy was performed by Fromme (1910) for gall-stone disease on four adult females, not known to be carriers, but for gall-stone colic. The first case was a woman aged 46 years who had typhoid fever 14 years previously. The bile at operation contained typhoid bacilli and cocci. Three faecal examinations were made with negative results a year later. The second case was also a woman, aged 46 years; she gave no history of typhoid fever but her husband suffered from that disease 5 years previously. An attack of gall-stone colic was experienced one or two years before the operation for cholecystectomy at which the gall-bladder was seen to be full of pus and gall-stones, and bound down by adhesions. The bile gave a pure culture of B. typhosus. Death resulted 3 days after operation. No typhoidal bacilli were isolated from the faeces in the caecum at post-mortem but the bile from the liver contained B. coli and abundant typhoid bacilli. No history of a previous typhoid attack was given by the third case, a woman aged 45 years. At operation the common bile duct was discovered to be distended

by gall-stones, but the gall-bladder only contained bile which gave an abundant growth of B. typhosus. The common bile duct was drained after the cholecystectomy for 10 days, and typhoid bacilli were isolated from the drainage bile. The faeces were positive for B. typhosus for 20 days after operation, but became negative and remained so in four examinations during the next six weeks. In Fromme's last case, a woman, aged 38 years, following cholecystectomy the faeces were free from typhoid bacilli in many examinations made during the subsequent 55 days, and also in a further two examinations a little over a year afterwards.

Browning and Gilmour (1910) describe as illustrative a case of typhoid cholecystitis; a female aged 40 years had an attack of enteric fever on May 1907. In July severe pain occurred in the right hypochondrium, and a swelling was felt in that situation, which subsided and the patient remained well except for occasional attacks of pain in the gall-bladder region till November 1907, the pain again became severe and the swelling reappeared. In December the gall-bladder was opened and stitched to the abdominal wall. The gall-bladder contained muco-pus but no calculi. On several occasions cultures were made which yielded a pure growth of B. typhosus. In July

1908 the typhoid bacilli ceased to be found in the discharge from the gall-bladder. By March only a small fistula remained which persisted to November 1908, when the wound healed. In August 1909 a normal child was born after an easy labour. Two months later severe pain began again to be felt in the right hypochondrium and the old scar broke down. The fistula was opened up and a pure culture of B. typhosus was again obtained from the discharge on 4 occasions. In December bile began to discharge freely and the typhoid bacilli were still in pure culture. On the last examination in January 1910 typhoid bacilli were still present in the passage 2¾ years after the acute illness.

Dehler (1912) reports two cases of cure by cholecystectomy:

Case I. A woman aged 64 years who was detected as a carrier in 1904, but gave no history of enteric fever; from June 1904 to May 1907 36 out of 42 examinations on the faeces were positive for B. typhosus. On 21/5/07 she experienced a sudden onset of gall-bladder symptoms and was operated on 23/5/07. The operation consisted in evacuation of a large abscess in the gall-bladder and removal of gall-stones - cholecystotomy - and was attended by recovery. There was a scanty discharge of bile for a few days from the incision. The faeces were found to be pos-

itive f rom 26/5/07 to 30/7/07 and 6 subsequent examinations till 3/10/07 were likewise positive. Since that time negative results have been consistently obtained in further examinations on the faeces; 36 in 1907; 63 in 1908; 34 in 1909; 33 in 1910.

Case II: A nurse aged 25 years, who suffered from enteric fever on 20/9/08 to 27/1/09, and was treated with "pyramidon and cholagogues to prevent a residual typhoid infection." Systematic control of urine, and faeces gave negative findings for B. typhosus from the 14th week. In September 1909 she had an attack of fever with jaundice and tenderness over the gall-bladder region and once more on 10/10/09 typhoid bacilli appeared in the faeces just as the first severe attack of icterus was disappearing. Several similar attacks occurred. The urine was constantly negative throughout. On 4/5/10 extirpation of a moderately adherent gall-bladder was performed. The wall was thick and contained many small abscesses. The contents consisted of turbid bile from which B. typhosus was isolated in pure culture. Drainage of the hepatic duct and administration of "Sodium choleinicum", caused an external flow of 425 c.cms. of bile per day, which always contained typhoid bacilli and in the first few days streptococci as well. The faeces were colourless and free from the typhoid

organisms from the 15th day after operation. The drain was removed after 5 weeks. The bile continued to flow through the fistula which gradually healed. The bile also became negative for B. typhosus on 9/6/10. The faeces slowly regained their normal colour but still remained typhoid-free in 90 examinations. During this period two admixtures or contaminations occurred from other patients which the patient had nursed. How Dehler comes to this conclusion is not made clear but one may conclude that an apparent cure was obtained by cholecystectomy in this case. Dahler, in conclusion, remarks that cholecystectomy with long drainage of thehepatic duct is both justified and indicated.

Two cures of ~~typhoid~~ chronic typhoid bacillus excreters are reported by Daeschler (1912). In one the acute attack of enteric fever occurred one year previously and in the other 3 years before. Both excreted B. typhosus in the faeces continuously and these organisms were also found in pure culture in the extirpated gall-bladders. Repeated observations were made after operation on the faeces for several weeks on the first case and for some months on the other and showed the absence of typhoid bacilli. Schultze (1913) had two failures with cholecystectomy in chronic typhoid carriers, who suffered from typhoid fever 4 and 5 years before. Apparently B. typhosus

was observed in the faeces for many months afterwards. Leary (1913) reports a cure following cholecystectomy, with complete excision of the cystic duct in two intestinal typhoid carriers.

The first case was a man aged 27 years who gave no history of typhoid fever or similar illness, but came under observation in August 27th 1912 suffering from a chronic biliary fistula following an operation for the removal of gallstones and empyema of the viscus The wound closed for a few days at intervals, but broke down again. During these periods he suffered great abdominal pain and fever which was relieved on drainage. The discharge from the gall-bladder was mostly clear glairy mucus, and on November 19th and 27th 1912, B. typhosus was isolated from the contents gall-bladder. Positive cultures of B. typhosus were obtained from the faeces on November 27th, and 30th, December 3rd, 5th 7th and 10th, but the urine was sterile. On December 12th 1912, the gall-bladder and the entire cystic duct were removed, and a calculus - a little larger than a hazel-nut was found inpacted in the cystic duct, the stump of which was canterized with phenol.

After operation on examination of the faeces B. typhosus was isolated on December 16th, but negative results were obtained subsequently on December 18th

1912, January 23rd, and 25th 1913. The discharge fromthe wound was negative for B. typhosus on 23rd. December and the wound healed on the 30th of the same month.

The second case was a man who had a suspected attack of typhoid fever in June 1910 and who did not report sick till September 18th 1910. B. typhosus was discovered in the stools on November 20th 1910 and in weekly examinations till March 20th 1911, in spite of vaccine treatment. In the summer of 1912, a second course of vaccine, calomel, buttermilk, soldium sulphite, urotropine, salol and ipecacuhana failed to effect a cure, consequently he was operated on ~~in~~ January 21st 1913. The gall-bladder which was large and distended and the cystic duct were removed, and B. typhosus isolated from their contents in pure culture. The faeces were positive for B. typhosus on January 20th and 21st. 1913. Subsequent to operation the faeces contained the specific organisms on Januray 25th and 27th, but not thereafter. ~~were not isolated.~~ Negative examinations were made on January 29th and 31st, February 3rd, 5th, 7th 8th, 10th, 13th 17th and 20th. Neither case suffered any ill-effects as a result of operative interference.

The examinations carried out after operation on these two cases are too few to base any definite opinion as to a cure being obtained. The second case, however, seems to have been a constant excreter of B. typhosus prior to operation, but subsequently became negative for a period of a month and may be accepted as cured.

Mayer (1914), acting on the assumption that the biliary passage are the chief resting places of persisting typhoid bacilli, examined bacteriologically the gall-bladders obtained at operation in a large series of cases not previously known to be typhoid excreters. From the large number of persistent carriers attending his laboratory (95 in all) he suspects that this method may reveal many others which have hitherto escaped detection. He examined 70 cases which came to operation in 18 months. In 6 typhoid bacilli were found and one B. paratyphosus B i.e. 10 per cent were positive for B. typhosus or B. paratyphosus A or B. Of the positive cases, 5 were empyemas of the gall-bladder, and 2 shewed severe cholecystitis. 4 of the cases gave a previous history of severe attacks of typhoid fever, which were proved clinically. Of the other three, only one gave a history which could be relied upon. In all the cases with known history the infections occurred from 1 to

14 years previously. One of the cases, a healthy 18 year old lad, was infected by contact with a chronic typhoid intestinal excreter. He suffered a protracted illness for over a year with complications. An acute cholecystitis supervened on a chronic one, and he became a chronic excreter of B. typhosus. Cholecystectomy was performed and a large cholesterin stone was found present in a greatly thickened gall-bladder from the bile of which B. typhosus was isolated in pure culture. The operation was performed on 16/9/13. Stool and urine samples thereafter on 27/9/13, 28/9/13 and on 9 occasions in October 1913, and also on 2/12/23 were negative. In another case in which the bile was positive for B. typhosus the bacilli were found in the faeces for the first three months after operation, but not subsequently. No further details are given.
In one further case 6 months after cholecystectomy, typhoid bacilli were found in the stools. There were no obsefvations made prior to operation on these cases so that their nature was not determined.
In the remainder of Mayer's series no details are given regarding the length of time the cases were under observation after operation nor the number of examinations made.

The most recent observations on surgical treatment of typhoid carriers have been recorded by

Dubs (1919), Nichols, Simmons and Stimmel (1919), Henes (1920), Haibe (1921), Murstad (1922), Garbat (1922), Barilari and Rodrigez (1922), Arnd (1923), Hage and Brinkman (1923), and Vosburg and Perkins (1925). Spelthalm, according to Dubs, in his review (up to 1916) on typhoid carriers, who had been operated on successfully mentions 5 and 8 cures both clinically and bacteriologically, following cholecystostomy and cholecystectomy respectively. Dubs mentions 3 cures following cholecystectomy in female carriers by Bircher and one by Bleuler. Unfortunately the author has been unable to trace these cases in literature. No references are given to the original articles by Dubs. The latter's own case was a man, aged 53 years, who suffered from Dementia Praecox since 1915 and the date of his attack of typhoid fever was not certain. He had never been ill since his 26th year. The Widal reaction was positive for B. typhosus. Typhoid bacilli were isolated from the faeces, but not from the blood or urine. Cholecystectomy was performed and the gall-bladder found to be large and partially adherent to omentum, colon and duodenum. The cystic duct was divided near the neck and a short stump left. The bile from the extirpated gall-bladder yielded B. typhosus in pure culture. After operation the stools

shewed a slight diminution of the numbers of typhoid bacilli excreted and became negative for 3 weeks, but once again the organisms were present in large numbers up to three months. Nichols, Simmons and Stimmel give a detailed description of 4 cures and 2 failures with cholecystectomy in the case of chronic bacillus excreters. None of the cases presented symtoms referable to the gall-bladder. In every case cholecystectomy was performed. This is best shown in the following table from their article.

Case.	Date of typhoid fever attack.	Interval between infection and operation.	Condition found.	Result.
2.	1918	6 years.	Cholecystitis.	Cure.
3.	1918.	5 months.	Stone.	Cure.
4.	1911.	7 years.	Dilated; Stone.	Cure.
5.	1911.	7 years.	Cholecystitis with stone.	Failure.
6.	1910.	18 years.	Cholecystitis with stone.	Failure.
7.	1910.	3 years.	Cholecystitis with stone.	Cure.

In all B. typhosus was isolated from the gall-

bladder bile. These authors employ the duodenal culture method as their criterion of cure. Case 3 is too recent to be counted a chronic bacillus excreter. Henes described 3 cases of acute typhoidal cholecystitis with extirpation of the gall-bladder. Two were cured and one still persisted in excreting typhoid bacilli. In addition cholecystectomy was performed on 3 patients who had continued to excrete the specific organisms for about 8 months after the acute attack. In all the gall-bladder contained stones and bile which gave B. typhosus in pure culture One died 3 days after operation. In another the stools became negative on the 40th day after operation but were once more positive for B. typhosus on the 293rd. day and thereafter. This case is stated to have been subsequently cured but no data are given, as the case had passed out of the author's hands. It is difficult to understand how this case became cured. The last case is stated to have been cured but here again no details are given. In Haibe's case, cholecystectomy resulted in curing the condition after failure by autogenous vaccines and purging. B, typhosus was found inthe gall-bladder but the bile from the hepatic duct was sterile.

Murstad's analysis of the results of gall-bladder operations in24 carriers supports the view

that typhoid bacilli, though isolated from the liver, disappear after drainage or on removal of the gall-bladder. Of 8 cases upon whom cholecystostomy, and of 16 cases who had cholecystectomy performed, in 4 and 15 respectively the bacilli disappeared. In Murstad's own experience, however, cholecystectomy failed to eliminate the bacilli from the stools, indicating that a focus or foci of infection persisted in the intestinal tract. He had 2 cases cured by cholecystectomy and one failure. The gall-bladder of the latter case was found upon removal to be completely free from typhoid bacilli.

Garbat described cholecystectomy without drainage on 4 chronic typhoid carriers. In two the carrier condition disappeared immediately. In a third duodenal cultures remained positive for 4 months after operation, although the stools were negative; the duodenal cultures, however, became negative 8 months later. In the last case, both duodenal and stool cultures continued positive for B. typhosus after operation. Garbat counts two negative consecutive duodenal cultures and faeces cultures ample evidence of the carrier being rendered typhoid-free. A chronic typhoid carrier who had recurrent acute gall-bladder symptoms is reported by Barilari and Rodrigez. No typhoid bacilli were found in the stools following

removal of the gall-bladder.

An attempt was made by Arnd to stamp out an enteric epidemic in an asylum by performing cholecystectomy on certain of its in-mates. Althgether 15 typhoid or paratyphoid carriers were thus treated. He states that 8 out of 10 typhoid carriers were found no longer to harbour typhoid bacilli in the stools after the operation. None of the paratyphoid carriers were sterilized by this operation, and, curiously enough, two persons who had been typhoid carriers before operation and who ceased to be so after it, were found to be paratyphoid carriers. An observation which he states somewhat detracted from the reputation of cholecystectomy for this condition was the finding that the gall-bladders of some of the typhoid carriers were perfectly sterile. This was also the case with three of the five paratyphoid carriers. Arnd finds it difficult to account for the disappearance of typhoid bacilli from the stools of a person whose gall-bladder has been found to be sterile on removal and suggests that patients may often cease to be carriers spontaneously and that in a certain number of cases in which the same result would have occurred, had no operation been undertaken. Cholelithiasis was found in 8 of the cases in one of which the bile was sterile. On examination of Arnd's

observations in detail in only one case are any dates given (case 9). In this case the operation took place only 4 months after the onset of the acute illness. One can completely discount any value in Arnd's work since no dates are given as to how long the chronic carrier condition was present, and how long after operation the examinations of the faeces were continued. The explanation of his peculiar results would appear to be that the operations were performed early in convalescent cases. Arnd quotes Blumenthal, as having 5 cures in typhoid carriers by cholecystectomy; Küsser, Spelthahn and Sauerbruch two each; Pendl and Reisinger one each, but Taylor as having two failures. As the references to these authors are not given and could not be traced, no details can be given as to the length of the period before and after operation during which bacteriological examinations were carried out and whether these successes can be regarded as reliable or not.

Hage and Brinkman describe two cases of chronic typhoid excreters, in one of which a cure by cholecystectomy was obtained. The first case was a woman, aged 36 years, who suffered from typhoid fever in 1918, and subsequently had occasional attacks of diarrhoea, but latterly was quite well

and free from gall-bladder symptoms though she was supposed to have had an attack of appendicitis. B. typhosus was discovered in the faeces and the gall-bladder was extirpated on 15/8/22. Typhoid bacilli were isolated from the bile in pure culture, and also from the interior of a single hazel-nut sized gall-stone with a soft crumbling centre. By 23/9/22 20 faeces and urine examinations proved always free from B. typhosus. On 14/11/22 both faeces and urine were negative and also a duodenal specimen was negative. On 25/11/22 an operation for a hernia through the scar was performed. Provocative administration of podophyllin followed by 5 examinations of the faeces and urine and a duodenal specimen proved negative for B. typhosus. Again on24/3/25 the faeces and urine were still free. In all 30 examinations were made with negative results. The second case had a supposed pleurisy in March 1922 and was found to be a carrier in October 1922, but had no gall-bladder symptoms. On January 22nd 1920 the gall-bladder was extirpated and B. typhosus isolated from the bile in pure culture. The interior of a solitary gall-stone was sterile. As the examination of the faeces after operation was carried out only for a short time though negative for B. typhosus the authors deem the time too short to be of value.

TABLE XIV.

THE FOLLOWING TABLE GIVES A SYNOPSIS OF HAGE AND VOSBURG AND PERKINS' CASES.

Case	Sex	Age	History of attack of Enteric Fever.	Type of Carrier.	Operation.	Date of operation	Organism in		Condition of gall-bladder.	RESULT.
							Gall-Bladder	Appendix.		
1	M	60	Unknown	B.typhosus	Cholecystectomy.	June1921			One small stone.	
					Appendectomy.	Feb.1921	+	-		Failure
2	F	41	Unknown	Paratyphoid	Cholecystectomy and Appendectomy.	Jan.1921	-	-	Nil.	Success
3	F	45	Unknown	B.typhosus	ditto.	1921	+	-	Many stones	Success
4	F	51	22 years previously	Paratyphoid (supposed).	ditto.	Feb.1921	Pos. for B.typhosus.	Pos. for B typhosus.	Many stones	Success
5	F	41	3½ years previously	B.typhosus	ditto.	Feb.1921	+	+	One stone.	Success
6	F	45	Unknown	Paratyphoid	ditto.	March 1921	+	+	Many gall-stones.	Success
7	F	34	11 years previously	B.typhosus	ditto	Sept. 1921	+	+	Several gall-stones.	Success

In a recent article Vosburg and Perkins describe the results of cholecystectomy with appendectomy in 7 insane typhoid and paratyphoid carriers for cure of the condition. It is interesting to note that Haibe (1921) mentions a case of appendicitis after an attack of typhoid fever, and when the appendix was removed B. typhosus was obtained from it in pure culture. It was not proved, however, that the patient did not harbour the bacilli in the gall-bladder as well and this appears very likely to have been the case. Table XIV on p. 277 gives a synopsis of Vosburg and Perkins' cases.

The stools of all these cases have been fairly extensively examined and again re-examined in the summer of 1922 many times and all were negative except those of case 1. Cathartics were used before some of the specimens were collected. In all cases a small rubber drain was inserted into the stump of the amputated duct and removed on the third day. Out of the 7 carriers six can be reckoned as cured; 5 out of 6 B. typhosus carriers and one paratyphoid carrier (the type is not given).

Meyer, Neilson and Feusier (1921) found that in experimental rabbit carriers with infection of the gall-bladder by B. typhosus cholecystectomy was not

always followed by cure of the carrier state.

Review of literature on surgical treatment directed against the gall-bladder in chronic B. paratyphosus A and B excreters.

As regards gall-bladder infections in chronic paratyphoid carriers, especially B. paratyphosus B excreters, which have been operated upon for cure of the condition, cases have been recorded by Lorey (1908), Forster (1908), Evers and Muhlens (1909), Pribram (1912), Mayer (1914), Jordan and Irons (1915), Kuster, Blumenthal and Spelthahn (quoted Arnd 1923) and Vosburg and Perkins (1925). In a case described by Forster and Kayser (1905), B. paratyphosus B was isolated from the gall-bladder at autopsy in Eckersdorff's (1908) case, the existence of cholecystitis was determined on clinical grounds and a paratyphoid bacillus, agglutinating like the Schottmüller strain, was isolated from the faeces. Neither of these cases were submitted to operation.

Lorey's case was that of a sailor, aged 22 years, who had an attack of enteric fever 2 years before, following which he had numerous attacks of gall-stone colic. Cholecystectomy was performed, and at operation the cystic duct was found to be closed by adhesions. The gall-bladder contained 4 pea-sized stones and a trace of bile from which B. paratyphosus B

was isolated. The mucous membrane was red and swollen and at one place was an ulcer, the size of a pea, penetrating all the layers of the wall to the serosa. Sometime after operation a fistula developed from which a bile-stained pus issued and from which B. pyocaganeus grew but no B. paratyphosus B. After operation repeated examinations of the faeces are stated to have been made which shewed the absence of the specific organisms. No details are available, however. Forster (1908) recorded a failure with cholecystectomy in a B. paratyphosus B. chronic excreter. The case reported by Evers and Mühlens was more carefully studied; during the six weeks following operation for gall-stones (cholecystostomy) B. paratyphosus B. was isolated from the faeces in 4 out of 8 examinations, and there was no evidence that the operation was successful in removing the patient from the ranks of carriers. It is, however, possible that had observations been carried out over a much more extended period a cure might have been found as the specific organisms may be excreted from many weeks after operation before ceasing. This is illustrated in two cases of the author's own series which excreted for 27 and 55 days respectively before finally ceasing.

Pribram describes an interesting case in which

B. paratyphosus B. was found to be excreted 4 years after removal of the gall-bladder. In Mayer's (1914) paratyphoid B case (see p. 268) up to 15 days after removal of the gall-bladder the specific organisms were found in the stool and in the biliary fistula contents. Thereafter they were no longer found. Jordan and Irons' case is noteworthy for the occurrence of a paratyphoid B. infection in a person who for a month previously had suffered from recurrent attacks of cholecystitis after convalescence, associated with persistence of paratyphoid bacilli in the stools, for the isolation of the organisms from the bile at operation and for the complete cessation of gall-bladder symptoms accompanied by disappearance of the B. paratyphosus B from the stools during more than one year following cholecystotomy. At no time was there any evidence that the carrier was giving rise to contact cases.

Mention has already been made to Arnd's cases (see p. 274). As the details of his results are so scanty and the previous histories of the cases not dealt with no reliance can be put on them. Küster, Blumenthal and Spelthahn are recorded by him as having obtained cures in paratyphoid carriers by cholecystectomy. The two latter are stated to have had one "cure" each and the former five by this means.

Vosburg and Perkins' paratyphoid case is described on (p. 277) and appears to have been rendered free from the carrier condition by means of cholecystectomy and appendectomy.

TABLE XV.

Table to illustrate results of operative treatment on Enteric Carriers (Chronic intestinal bacillus excreters).

Author.	Cholecystectomy.		Cholecystostomy		Cholecystotomy		Cholecystgastrostomy.	
	Success	Failure	Success	Failure	Success	Failure	Success	Failure.
Arnd.	4	3						
Barilari & Rodrigez.	1							
Bircher	3							
Bleuler	1							
Blumenthal	1		6					
Browning & Gilmour.				1				
Daeschler	2							
Dehler	1		3					
Doerr			1					
Dubs		1						
Eyers & Muhlens.						1		
Forster				1				
Fromme	3	1						
Garbat	3	1						

Author.	Cholecystectomy.		Cholecystostomy		Cholecystotomy		Cholecystgastrostomy	
	Success	Failure	Success	Failure	Success	Failure	Success	Failure.
Grimme	1							
Hage and Brinkman.	1							
Haibe	1							
Henes	2	1						
Holmes	1							
Irons and Jordan					1			
Kuster	7							
Leary	2							
Loele		1						
Lorey	1							
Mayer	5	2						
Murstad	2	1						
Nichols, Simmons & Stimmel.	4	2						
Pendl	1							
Pribram		1						
Reisinger	1							
Sauerbruch	2							
Schultze		2						
Spelthahn	3							

Author.	Chole-cystectomy		Cholecyst-ostomy.		Cholecy-stotomy		Cholecyst-gastrostomy.	
T	Suc-cess	Fail-ure	Suc-cess	Fail-ure	Suc-cess	Fail-ure	Suc-cess	Fail-ure.
Taylor		2						
Vosburg & Perkins.	5	1						
Smith (R.P)	2						1	
TOTAL.	60	19	10	2	1	1		

From the literature 60 cases (75 per cent.) have been obtained by cholecystectomy and 19 failures; 10 cures (83 per cent.) and 2 failures by cholecystostomy; 1 cure and 1 failure by cholecystotomy and the author's own success in two cases and failure in another by cholecystgastrostomy. Thus the operation which gives the greatest amount of success would appear to be cholecystectomy, ~~having~~ taking into account the much greater number of cases dealt with.

The review of the literature would not be complete, unless only cases which have been proved to be chronic carriers and which have been shewn to be cured beyond any doubt were recorded in a further table, as many of the cases in the foregoing table have not been substantiated.

TABLE XVI.

Table to illustrate operative treatment on chronic carriers who have been proved to be such and who have been observed for a reasonable time after operation.

B. typhosus excreters.

Author.	Cholecystectomy.		Cholecystostomy		Cholecystgastrostomy.	
	Success	Failure	Success	Failure	Success	Failure.
Barilari & Rodrigez.	1					
Browning & Gilmour.				1		
Daeschler	1					
Hehler	2		2			
Bubs		1				
Hage and Brinkman.	1					
Haibe	1					
Henes		1				
Leary	1					
Loele		1				
Mayer	1	1				
Murstad	2	1				
Nichols, Simmons & Stimmel.	3	2				
Schultze		2				
Vosburg & Perkins.	4	1				
Smith	1				1	
Total.	24	12	2	1	1	

B. paratyphosus B. excreters.

Author.	Cholecystectomy.		Cholecystotomy.	
	Success	Failure.	Success	Failure.
Evers and Mühlens				1
Forster		1		
Jordan and Irons			1	
Pribram		1		
Vosburg and Perkins	2			
Smigh	1			
TOTAL	3	2	1	1

From the foregoing tables of cases which have been authenticated it is thus seen that 24 cures (69 per cent.) and 12 failures in chronic B. typhosus excreters have ~~by cholecystectomy~~ been recorded by cholecystectomy. 2 successes and one failure by cholecystostomy and the author's own success by cholecystgastrostomy. The results are not so convincing in the case of chronic excreters of B. paratyphosus B, but the numbers are too small to permit of definite conclusions. Three successes and 2 failures by cholecystectomy, 1 success and 1 failure by cholecystotomy.

Author's own Observations.

The three chronic bacillus excreters '(H.T., K.O., and M.D.) who had been under observation for some considerable time and in whom various remedies were tried without success, voluntarily consented to operation though they had no symptoms referable to the gall-bladder. They were operated on by Mr. Farquhar Macrae in the Western Infirmary, Glasgow. Case I. (H.T), female aged 52 years. She had an attack of enteric fever in October 1913, and was proved to be a carrier in 1915. She was under observation from April 4th 1922 till May 4th 1923 prior to operation. Between these dates 110 bi-weekly examinations were made of the faeces and urine. Of the 110 faeces examinations 52 were positive for B. typhosus. An examination on June 13th 1922 gave a positive result for both B. typhosus and B. paratyphosus B and on three separate occasions between June 15th 1922, and July 31st 1922 B. paratyphosus B. alone was detected. The urine was positive on only three occasions for B. typhosus with long intervals between.

The longestest negative period in the faeces during the course of the examinations was from April 27 to June 8th 1922. In this period 13 negative faeces examinations were made. Another prolonged negative

phase was noted from August 28th 1922 to September 25th 1922 when 8 consecutive negative results were obtained.

On May 4th 1923, cholecystectomy was performed with drainage of the hepatic duct for twenty-seven days, after which the drainage tube was removed and the fistula gradually healed. B. typhosus and B. coli were isolated from the bile in the gall-bladder, and B. typhosus was also recovered from two of the gall-stones present. A count of the total organisms in the bile from the gall-bladder was made by means of plating a shake culture in MacConkey agar using 0.001 c.c. of the bile; 10,032,000 B. coli and 7,560,000 B. typhosus were found present per c.c. of bile. On the twenty-fifth day after operation a count of the organisms present in the bile obtained from the cystic duct shewed 4,650,000 B. typhosus and 303,000,000 B. coli per c.c. To demonstrate the presence of typhoid bacilli in the interior of the gall-stones, they were first washed with saline and treated with 1 - 1000 corrosive sublimate for two minutes to dissolve any mucus adhering and washed again with saline. The stones were dried, ground up in saline and cultures made from them. After operation the discharging bile from the hepatic duct was examined daily for twenty-seven days till

the drain was removed. During the whole of this period the same organisms were found constantly present. From the time of operation bi-weekly examinations of the faeces and urine were made until 6/12/23 and subsequently weekly specimens up to her death on 28/2/25. The faeces frequently yielded positive results for B. typhosus till June 28th 1923 and on one occasion May 24th 1923 for both B. typhosus and B. paratyphosus B. From June 28th 1923 till her death 91 further examinations of the faeces were made with constantly negative result. Previous to surgical treatment the longest negative period was one of two months as noted above; subsequently negative results were obtained for twenty-one months. The patient apparently suffered no ill effects from the cholecystectomy but some months later developed a ventral hernia, which caused her discomfort. Her general health remained good until the beginning of February 1925, when she complained of flatulence and dyspepsia, but under treatment the condition improved. On 27/2/25, she suddenly collapsed, suffering with acute abdominal pain. A large hernia was found protruding through the operation scar. This was easily reduced but symptoms of cardiac failure developed and she died on 28/2/25. Unfortunately no post-mortem examination was obtained.

Case II (K.O.), female aged 27 years, suffered from typhoid fever in August 1914, and was proved to be a carrier in 1915. She was under observation from October 29th 1921 and up to the date of operation 155 and 156 biweekly examinations were made of the faeces and urine respectively. The faeces were positive for B. typhosus on 132 and negative on 23 occasions; the urine was positive 17 times and negative in 139. On May 4th 1923, an anastomosis was made between the gall-bladder and the stomach, (cholecystgastrostomy) with a half-inch opening. The common bile duct was not ligatured. Examination of the bile from the gall-bladder yielded B. typhosus in pure culture. There were no gall-stones present. The bile shewed the presence of 13,760,000 of the specific organisms per c.c. Before operation the longest negative phase in the faeces was from May 25th to June 29th 1922, when six consecutive negative results were obtained. Since operation the faeces and urine have been examined bi-weekly for 9 months and subsequently the faeces alone once a week for 22 months. Up till May 31st 1923, the faeces were positive for typhoid bacilli on seven occasions and the urine once, and since that date till 19th December 1925, the results have been consistently negative on 162 occasions.

Thus before operation the longest negative period was five weeks; the faeces have now given a negative result for the pathogenic bacilli for about 32 months.

Case III (M.D.) female aged 39 years; had an attack of enteric fever in December 1918, and was proved an intestinal carrier of B. paratyphosus B. in 1920. In 178 continuous bi-weekly examinations from October 27th 1921, till her operation the faeces and urine were positive for B. paratyphosus B in 168 and 41 occasions respectively. Cholecystgastrostomy was performed on July 17th 1923. The common bile duct was not ligated. The bile from the gall-bladder gave a mixed culture of B. paratyphosus B and B.coli, about 40 per cent. of which were of the B. lactis aerogenes variety. Both the mucoid and normal B. paratyphosus colonies were present. There were 25,866,700 B. coli and 1,800,000 B. paratyphosus B. present per c.c. of bile. There were no gall-stones present.

A failure resulted in this case, as the faeces were still positive for the pathogenic bacilli in 67 out of 85 examinations till 19/7/24 when a second operation, cholecystectomy, was performed by Mr. Macrae. At operation the gall-bladder was found to be distended and the opening into stomach exceedingly minute. There was no free drainage and no mixing of the gastric contents with the bile. The gall-bladder

was removed and the cystic duct drained for twenty-five days. There was only a small quantity of thin golden-yellow bile (about 1 c.c.) present and no gall-stones or concretions. On examinations of the bile no B. paratyphosus B. colonies were isolated by the direct MacConkey agar plate but 3 by brilliant-green enrichment. There was a mixed infection with B. coli, 5,760,000 organisms in all being present per c.c. Only a coliform growth resulted from a culture of a piece of the gall-bladder wall. Bile from the cystic duct drainage tube was negative for B. paratyphosus B. Following cholecystectomy there have been many periods on which numerous examinations of the faeces have been made without isolation of B. paratyphosus B. From 1/8/24 to 10/11/24 26 negative examinations were made. On 13/11/24 B. paratyphosus B. appeared once more and in 14 further examinations till 15/1/25 was isolated 6 times by aid of brilliant green. The number of organisms present were scanty however. Since 22/1/25 in weekly examinations 35 negative examinations of the faeces were made until 24/9/25 when a few B. paratyphosus B. colonies appeared again. From that date till 16/5/26 a further 30 weekly examinations have been made with completely negative results.

The finding of B. paratyphosus B. colonies on 24/9/25

after a negative phase of eight months followed by a further negative period of eight months would appear to point to a possible cure having been obtained in this case, as she was a constant excreter of the specific organisms prior to operation. The longest negative period prior to operation was from 27/3/22 to 7/4/22, when three negative findings for B. paratyphosus B. were recorded in examination of the faeces. The colonies discovered on 24/9/25 may possibly be saprophytic in nature and not from any residual infection at all. Whether this patient is cured or not the diminution of the numbers of organisms excreted may possibly render her less of a menace to the public.

It is noteworthy that all three carriers continued to excrete the pathogenic bacilli for sometime after operation and then became free from them. The first two cases can be judged to have been cured of their carrier conditionby operative treatment, and in all probability the third case as well. Table XVII, p.294 illustrates the result of operative treatment on the foregoing chronic carriers.

The idea regarding the employment of cholecystrostomy which has never been tried before, presented itself to Mr. Macrae, on the basis of Scheers' work (1919). The latter found that the gastric juice had a bactericidal action on bacilli of the colon-typhoid

TABLE XVII.

TABLE TO ILLUSTRATE RESULT OF OPERATIVE TREATMENT ON AUTHOR'S OWN SERIES.

Name.	Sex.	Age.	Date of original infection.	Gall-Bladder Symptoms.	Operation.	Interval between original infection and operation.	Condition found.	Remarks and Results.
H.T.	F.	52 years	Oct. 1913	None.	Cholecystectomy on May 4th 1923	9 7/12 years.	Subacute ch cholecystitis. 13 small pea sized gall-stones. Bile & stones positive for B. typhosus. Bile also positive for B. coli.	CURE.
K.O.	F.	27 years	Aug. 1914.	None	Cholecystectomy on May 4th 1923	8 5/6 years	Subacute cholecystitis, no gall-stones. Bile positive for B. typhosus in pure culture.	CURE.
M.D.	F	39 years	Dec. 1918	None	Cholecyst-gastrostomy on July 17th 1923 Cholecystectomy on 19th July 1924	4½ years	Subacute cholecystitis, no gall-stones. Bile positive for B. paratyphosus B. & B. coli.	? Cure.

and dysentery group such that after two minutes exposure to its action these organisms were killed. Also, then was the prospect that by effecting such a communication continuous drainage of the gall-bladder would be effected. No ill effects result from this form of surgical treatment (Gatewood and Poppens 1922). Neither of the carriers of the present series suffered in any way from subsequent ill-effects. By joining the stomach to the gall-bladder the bile passes directly into the stomach and a mixture of the gastric juice and gall-bladder contents takes place. Ligature of the common bile duct simultaneously with cholecy-st-gastrostomy would prevent the passage of B. typhosus from an infection of the liver into the intestine and is suggested as an improvement in the technique of the operation; though in the case of K.O. she appears to have been cured without this being done. There is, of course, always the danger of jaundice to be considered should the stomach-gall-bladder drainage be ineffective. Arass' (1922) experiments shewed that B. paratyphosus B. is more resistant to the action of gastric juice than the other members of the group. 15 - 45 minutes exposure in the case of the gastric juice of rabbits was found necessary to kill them. Although Ledingham and Arkwright in a review of the literature up to 1912, and Browning and Gilmour(1910),

in view of the severity of gall-bladder operations and the fact that the bacilli are not always confined to the gall-bladder but may have infected the bile ducts within the liver feel that these facts exclude operative this measure from general use. Later researches on this important subject have proved its efficacy (and Professor Browning is convinced of its value). Indeed up to the present, surgical treatment appears to be the only method at our disposal which has afforded any definite hope of cure (Meyer 1921). Admittedly failure will be met with when the typhoid infection is situated in the liver and cholecystectomy is performed, but cholecystgastrostomy with or without ligature of the common bile duct will afford relief even in event of this contingency. Should failure result from cholecystgastrostomy, cholecystectomy as was done on the paratyphoid carrier M.D., can be performed at a later date. Failure in this case appears to have been due to the opening between the stomach and gall-bladder becoming narrowed so that free intermingling did not take place. Alleviation of the condition is worth any risk involved as carriers from brooding over their condition and when under constant supervision tend to become morbid and feel pariahs and a danger and menace to society.

Conclusions.

1. The gall-bladder, bile ducts and liver are the main seats of the infection in chronic intestinal bacillus excreters although the intestinal tract also harbours the specific organisms as seen in the case of the "mixed" excreter of B. typhosus and B. paratyphosus B described. In this case typhoid bacilli alone were isolated from the gall-bladder, while after operative treatment both organisms were found in the faeces. In all three cases the specific organisms were isolated from the gall-bladder bile. In the B. paratyphosus B carrier, M.D., however, only a few specific organisms were isolated by means of brilliant-green enrichment, after cholecystectomy following cholecyst-gastrostomy.
2. Surgical treatment is the best means available for the cure of the carrier condition.
3. Cholecystectomy with drainage of the hepatic duct would appear to be the most successful method practised, but a cure has been effected by cholecystgastrostomy, in a chronic typhoid excreter of the author's series. In a review of the literature of genuine chronic bacillus excreters upon whom cholecystectomy has been

performed (24 cases) 66 per cent. have been cured by this measure.

4. Cholecystgastrostomy with or without ligation of the common bile duct is suggested as an additional curative measure.

5. One success with cholecystectomy and another by cholecystgastrostomy was obtained in two chronic typhoid carriers. A chronic B. paratyphosus B. carrier upon whom both of these operations was performed is still under observation, but appears to have been cured as well by means of cholecystectomy.

BIBLIOGRAPHY.

Arai (1922): Scientific Rep. from Gov. Inst. for Infect. Dis., Tokyo. Imp. Univ., for 1922, Vol I, p. 69.

Arnd (1923): Schweiz. Med. Woch., 53 Jahrb., p. 433.

Barilari & Rodrigez (1922): Prenza med., Argentina, Buenos Aires, 1922, 9, p. 500, ref. J. A,M.A. 1923, 80, p. 589.

Brwoning and Gilmour (1910): Glasgow. Med. Journ., August 1910.

Daeschler (1912): Centralbl. f. Bakt., I, O., 1912, 52, p. 283.

Dehler (1907 and 1912): Münch. med. Woch., 1907,LIV, pp. 779, 2134; ibid, 1912, 59, p. 857.

Dubs (1919): Münch. Med. Woch, 1919, 66, p. 178.

Dupré (1891): Paris Thése. quoted Hunner.

Evers and Mühlens (1909): Deutsch. militararzt. Ztschr., 1909, 38, p. 366.

Forster and Kayser (1905): Münch. Med. Woch, 1905, 52, p. 1423.

Forster (1908): Münch. Med. Woch, 55, p. 1.

Fromme (1910): Deutsch. Ztschr. f. Chir., 1910, 107, p. 578.

Garbat (1922): Monos. Rockefeller Instit., No 16. May 10th 1922.

Gatewood and Poppens (1922): Surg., Ggn, and Obst., 1922, 35, p 445.

Grimme (1908): Münch. med. Woch., 1908, 55, p. 445.

Hage and Brinkman (1925): Mitteil. a.d. Grenl. d. Med. u. Chir., 1923, 37, p. 25.

Haibe (1921): Commun. Roy. Soc. Belg., quoted J. A.M.A., 1921, 77, p. 136.

Henes (1920): J.A.M.A., 1920, 75, p. 1771.

Hunner (1899): Bull. John Hopkins Hosp., 1899, x, No 101 and 102, p. 163.

Jordan and Irons (1915): J. Infect. Diseases, 1915, 17, p. 234.

Leary (1913): J.A.M.A. 1913, 60, p. 1293.

Ledingham and Arkwright (1912): The Carrier Problem In Infectious Diseases, London 1912.

Lentz (1905): Klin. Jahrb., XIV, p. 475.

Loele (1909): Deutsch. Med. Woch., 1909, 35, p. 1429.

Lorey (1908): Münch. Med. Woch., 1908, 55, p. 15.

Mayer (1914): Münch. Med. Woch, 1914, 61, p. 1116.

Meyer (1921): J. Infect. Diseases, 28, p. 381.

Meyer, Neilson and Feusier (1921): J. Infect. Dis., 28, p. 457.

Murstad (1922): Med. Rev., 1921, 38, p. 215. Ref. J.A.M.A., 1922, 79, p. 561.

Nichols, Simmons & Stimmel (1919): J.A.M.A.. 79, p. 680.

Pribram (1912): Wien. Klin. Wehnschr., 1912, 25, p. 1344.

Scheer (1919): Arch. f. Hyg., 1919, 88, p. 130.

Schultze (1913): Centralbl. f. d. Grenzgeb. d. Med. u. Chir, 1913, 49, p. 1892.

Vosburg and Perkins (1925): Surg., Gyn., and Obstetrics, 1925, XL, pp. 404 - 6.

****

CHAPTER IX.

URINARY CARRIERS.

In the course of investigations on the carrier problem urinary carriers have been met with much less frequently than intestinal carriers. In approximately 25 per cent. of typhoid patients large numbers of bacilli suddenly appear in the urine, usually late in the course of pyrexia or in early convalescence but the typhoid bacilluria of early convalescence soom disappears spontaneously. The great majority of bacillus excreters pass typhoid bacilli in the faeces only, and not in the urine. Stokes and Clarke (1916) in the examination of 810 convalescents found 4 per cent temporary and 1.6 per cent. chronic intestinal carriers; 4 per cent. temporary and 0.24 per cent. chronic urinary carriers. Tanon and Dumont (1917) during their examinations of the urine of cases of enteric fever, which occurred in the 5th Army Corps of the U.S. Army

during 1915 - 1917 found that out of 1500 urines examined during convalescence 29 were positive (2 per cent.) - 10 for B. typhosus; 15 for B. paratyphosus A and 4 for B. paratyphosus B. All the cases became negative within a month except one who became definitely a chronic carrier.

Table to illustrate frequency of occurrence of typhoid bacilli in urine from acute stage to early convalescence

Author.	Number of Cases Examined.	Percentage Positive.
Petruschky (1898)	50	6
Horton-Smith (1900)	39	28
Schuder (1901)	22	23
Cole (1901)	49	35
Buchan (1908)	30	56.7
Connell (1909)	621	24.2
Patrick (1914)	58	29
McCall (cf. Patrick)	100	26
Tanon and Dumont (1917)	1500	2
Garbat (1922)	164	49.

This gives a heterogeneous average of 23 per cent. which agrees with the averages of reviews of the literature by Richardson (1903) and Osler (1913).

As has been seen the bacilli persist in the urine for a variable period, often several weeks, and then

commonly disappear spontaneously usually within three months (Garbat 1922); but in some instances they persist for years - nine years in a case recorded by Liebetrau (1906); five years in a case mentioned by Gwyn (1899) and in cases under the Glasgow Public Health Authorities (quoted Patrick 1914) for four to six years.

Typhoid bacilli have been isolated very occasionally from the urine of the female intestinal excreters which we have examined. In most cases typhoid bacilli have been recovered from the specimens of faeces examined at the same time and so it is possible that they reached the urine as a contamination. In favour of this view is the fact that catheter specimens never yielded culture of B. typhosus, but only a few such specimens were examined. On the other hand in two cases B. typhosus was obtained from the urine when the accompanying specimen of faeces was negative both by direct plating and after brilliant green enrichment. Again in another patient B. typhosus was constantly obtained from the urine as well as the faeces for a period and thereafter was found only in the faeces. Therefore it is not improbable that in carriers bacteremia occasionally occurs which leads to the presence of the bacilli in the urine. Ledingham and

Arkwright (1912) have never found the urine positive in chronic intestinal excreters of the male sex, a finding with which the author's scanty investigations agree. Thus the true urinary carrier passes typhoid bacilli in the urine only, and it is fortunate that such cases are so infrequently met with as the danger of spreading infection by urine is much greater than in the case of intestinal carriers. The actual number of typhoid bacteria in the urine is usually high - 172,000,000 per c.c. (Petruschky 1898), 500,000,000 per c.c. (Gwyn 1899), 43,000,000 (Garbat 1922), - but they also have been found in a few cases in small numbers in apparently clear urines (Buchan 1908; Connell 1909).

Typhoid bacilluria is commonly unassociated with the occurrence of any special symptoms and there may or may not be pus or albumen present in the urine. Petruschky (1898) found no relation to acidity, specific gravity or turbidity but a close association with albuminuria. The irregularity in the excretion of the specific organisms in the urine was thought to be dependent on the technique employed i.e. the use of solid media alone, but the studies of Garbat showed that there was an actual intermittency in excretion, as one specimen from a carrier voided directly into broth was sterile and another a few hours later was full

of bacilli, and that the numbers varied from 10,000,000 colonies to 200 in the course of six hours in the same case.

The earliest cases of urinary carriers were reported about the same time by Rousing (1898), Houston (1899) and Young (1900). Rousing's case was a male aged 53 years who had typhoid fever eighteen months previously. Typhoid bacilli were present in large numbers in the urine which was acid and contained pus but no blood. The patient died a month after supra-pubic cystotomy had been performed. The bladder mucosa had numerous ulcerations present separated by swollen rugae and at autopsy both kidneys were studded with small abscesses and there was a stone about the size of a walnut in the right pelvis. Rousing believed that the caloulus was present sometime before the original typhoid attack and that the trauma caused by it determined the localisation of B. typhosus in the kidney. Houston's case was a female of 35 years, who gave no definite history of a primary typhoid infection. She suffered from cystitis and her urine was turbid, opalescent, strongly acid and contained a small amount of albumin. The sediment contained numerous squamous epithelial and pus cells, and B. typhosus was repeatedly recovered from it. Young's case was a male of 39 years, who had enteric fever 6 years

previously. He suffered from a chronic ulcerative cystitis, both gonococci and typhoid bacilli being isolated from the bladder. In 1906 Napier and Buchanan reported a case of typhoid fever in which the action of the typhoid virus was directed mainly to the kidney (Nephro-typhoid). There was a pyelitis and chronic nephritis present and the urine contained typhoid bacilli for 4 months after the onset of the infection. They concluded that the patient in all probability suffered from an ordinary acute catarrhal nephritis, on which the enteric element was grafted. Greaves (1907) reported a typhoidal pyonephrosis in a patient aged 36 years, whose original attack of enteric fever occurred six years before. At operation a phosphatic calculus was found in the left ureter and from the pus in the kidney B. typhosus was recovered in pure culture. Adrian (1908) and Meyer and Ahreiner (1909) recorded a similar case of pyonephrosis treated surgically with success, in a girl aged six years. While under treatment in hospital for this condition she contracted typhoid fever - the pyonephrosis apparently ante-dating the enteric attack. After a period of 10 years during which she remained perfectly well she developed acute pains in the left kidney region with swelling of the left side of abdomen and thighs and passed cloudy urine. A communication

had evidently been established between the pyonephrotic sac and the bladder, so that typhoid bacilli vegetating in the sac were continually being discharged in the urine. Nephrectomy was performed and typhoid bacilli were isolated in pure culture from the purulent contents of the sac. The bacilli were found in the urine for 8 days after operation but not subsequently. Before operation the patient's serum gave a Widal reaction of 1:1000 but after operation the titre fell to 1:150. Irwin and Houston (1909) and Walker Hall and Roberts (1911) both record cases of chronic female urinary carriers whose primary infection occurred 7 and 6 years previously. In 1922 Dittmar described the case of a urinary carrier who was admitted to the City Hospital, Aberdeen, suffering from typhoid fever in September 1914 and was discharged after three successive examinations of his stools and urine had proved negative. Shortly after dismissal from hospital he complained of bladder ir ritation and as a result his urine was once more examined and B. typhosus discovered to be still present. He was re-admitted to hospital for a further course of treatment and once more discharged in April 1915. Three more examinations of his urine during April and June 1915 revealed numerous typhoid bacilli present. In January and December 1916, bacilli were still demonstrable in the urine but about

a year later the urine showed no organisms and a further sample in February 1921 was also reported as free from B. typhosus. The patient had a course of urotropine but owing to the small number of examinations one cannot regard a "cure" as having been effected. Numerous other workers have described cases of chronic urinary carriers but it may be of interest to record a recent case described by Sick and Deist (1923) of a servant girl aged 20 years who took typhoid fever on 15.10.20. The temperature chart showed a typical typhoid picture but the intestinal symptoms were lacking. On the other hand albuminuria, leucocytes and hyaline tube casts were present in the urine. An early abortion occurred at the height of the fever which disappeared on 2.11.20 and the temperature thereafter remained normal. Typhoid bacilli were found in both urine and faeces but they disappeared from the faeces during November 1920 but remained in the urine. Frequent examinations by cystoscope and ureteral catheterization showed that there was no noteworthy cystitis and that the bacilli came from the right kidney only. From the left kidney typhoid bacilli were never obtained. Treatment with urotropine, salol, infusion uvi ursi had no effect; washing out of pelvis of right kidney and ureter with hydrarg perchloride 1 in 5000 solution every third

day from the beginning of March 1921 did not affect the excretion of bacilli in the urine. Similar lavage with ½ per cent. urotropine was tried and the next examination showed fewer leucocytes, and at the end of March for the first time typhoid bacilli were absent. Thereafter the patient was discharged from hospital on 19.3.21 in the hope that these measures had got rid of the persistent excretion. In May 1921 B. typhosus again appeared in the urine and the patient was taken back to work as a servant-maid in the hospital so as to be under observation. Her condition preyed on her mind so much that she developed hysterical symptoms. On 12.2.22 she returned to hospital suffering from "angina" and "aphonia". After the disappearance of angina typhoid bacilli were still present in the urine coming from the right kidney but still no cystitis was present. As the girl tried to commit suicide by gas poisoning energetic treatment was instituted. Since washing out the pelvis of right kidney with 1 per cent. urotropine caused only a temporary freedom from bacilli, nephrectomy was performed and the ureter down to its orifice in the bladder removed. From the pelvis of the kidney immediately after operation typhoid bacilli were isolated in pure culture. The kidney showed marked anatomical changes and at first sight it seemed

as if foetal lobulation was marked. There were no gross changes in the mucosa of the pelvis and ureter. In places at the bottom of the depressions on the surface there appeared to be the remains of old infarctions. Microscopically these were seen not to be infarcts but coarse tissue with numerous small cysts lined by a low cubical epithelium, which extended into the kidney tissue in a wedge-shaped manner. Around these diseased areas were numerous areas of chronic inflammatory change with round cell infiltration, especially in the region of the pelvis. Accumulations of polymorphs and plasma cells were seen, in addition, in the functioning kidney substance. The mucosa of the pelvis showed definite inflammatory changes, but the walls of the ureter were healthy. Organisms were not demonstrable in stained sections of the kidney substance. The authors interpret the inflammatory signs localised to certain parts of the organ as of long standing and as having occurred at the time of the typhoid attack or even earlier. The inflammatory changes in the pelvis, they think, were due to the wandering or breaking through of bacteria, and that the absence of bacilli in sections was of no importance, as to be of value serial sections would be necessary and these were not made.

Bacilli were still present for eight days after operation but finally disappeared after lavage of the

Bladder with potassium permanganate solution, and the patient was discharged as cured in August 1922. A further examination on 8.12.22 was negative, and on cystoscopy the bladder was seen to be normal.

Previous to operation the bacilli found in the urine had been typical typhoid bacilli and remained so for the eight days after operation, but subsequently an abundant growth of colonies took place, the organisms from which were non-motile, did not agglutinate with antityphoid serum, and produced gas from various sugars but no indol. They appeared occasionally to resemble B. coli, sometimes B. typhosus and B. faecalis alkaligenes, and were isolated from the urine until September 1922. The authors group the organism between B. coli and B. faecalis alkaligines. They conclude that the lesion in this case is one of acute nephritis and that the kidney and ureter showed no changes peculiar to typhoid infection although numerous organisms grew from the pelvis. The persistence of the specific organisms for eight days after operation, they regard as due to a specific cystitis just as happens with a tuberculous kidney lesion, The previous disease of the kidney and pelvis would account for the lodgement of the bacilli and thus lead to persistent excretion.

Pathogenesis of typhoid bacilluria and the chronic carrier condition.

The cause of typhoid bacilluria has been variously regarded. Könjajeff (1899) held that bacteriuria indicated the presence of lymphoid nodules in the kidney, for in sections of these he had sometimes seen bacilli present. Suppurative foci have been described by Flexner (1896), Brownlee and Chapman (1906) and Patrick (1914). The small kidney abscesses are not common as Horton-Smith (1900) only found one out of 289 post-mortem examinations on cases of enteric fever. He believed the typhoid bacilli multiplied in the bladder as the walls in that case were chronically inflamed. Patrick, on the other hand, states that where abscesses have been found in the kidney it is reasonable to suppose that the bacilli came from these but pus cells would necessarily be present in the urine. In one of his patients, who died, multiple small abscesses from which B. typhosus was obtained in pure culture were present in the left kidney, and a small quantity of pus, which also gave a growth of B. typhosus in pure culture, had collected in a dilatation of the left ureter, just before its entrance into the bladder. Pus was present in the urine in considerable quantity. The ordinary cases of bacilluria, however, in which

no pus is present in the urine cannot be explained on the assumption that such a pathological condition exists in the kidney. He concluded that as bacilluria comes on comparatively late in the disease, while the bacilli are present in the blood in greatest numbers in the earliest stages, it is unlikely that they are excreted directly from the blood. This is supported by the findings of Horton Smith (1900) who found the blood sterile in four cases of bacilluria and Connell (1909) in two cases.

Blumer (1895) was of the opinion that the urine was infected by direct passage of bacilli from the rectum to the bladder. That the bacilli are sometimes confined to the bladder was shown by Horton Smith, who, at a necropsy of a patient in whom bacilluria had been present, isolated B. typhosus from the bladder and not from the kidney; and Gwyn (1899), who caused bacilluria to terminate in three cases by lavage of the bladder with a weak perchloride of mercury solution. The evidence brought forward for infection of the bladder alone without involvement of the kidney is scanty, but, so far as it goes, it shows that this may sometimes occur. The most feasible explanation is that the bacilli grow on the wall of the bladder, as they would on a solid culture medium, and that some are constantly washed off in the urine. The

commonly accepted view of the etiology of bacilluria is that the urine becomes infected from the bacteræmia at an early stage in the disease, while the organisms are numerous in the circulating blood, and that the bacilli multiply in the urine and possibly in the kidney substance later. The presence of residual urine has been looked on as of importance in favouring this proliferation.

In the great majority of chronic urinary carriers, a definite pathological lesion occurs somewhere in the urinary tract, usually in the kidneys. The absence of typhoid bacilli in the urine in Adrian's (1908) case after operation strongly suggests that there were no secondary deposits in the bladder itself.

The relation between the lesions in urinary carriers and cases of nephro-typhoid or acute typhoidal cystitis has not been definitely established from the pathological findings. Whether the focal abscesses in the kidney may act as vegetative depots of the typhoid bacillus in urinary carriers is uncertain. The lesion in the kidneys may thus be in the nature of multiple focal abscesses, or similating an acute nephritis (Sick and Deist 1923), or simply areas of focal necrosis such as are met with in the liver and other organs, or an infection of the

pelvis. It is more likely, as has been suggested by Pick (1910), that in the urinary carrier the bacilli lie in nests situated in the recesses of the urinary tract e.g. diverticula of bladder, intra-urethral and para-urethral ducts in the female, and prostatic ampullae and seminal vesicles in the male. Pick examined these organs in 32 autopsies and typhoid cases, and in two instances discovered spermatocystitis and prostatitis due to B. typhosus alone.

The American investigators of the problem, Nichols, Simmons and Stimmel, (1919) and Henes (1920) state that all urinary carriers are really kidney carriers, and when the condition does occur pre-existing pathological lesions of one or both kidneys are responsible for the continued infectiousness of the urine. Nichols and his co-workers had a cure following nephrectomy in a urinary carrier of six years standing, who suffered from a pyonephrosis. Similarly Garbat (1922) demonstrated in two cases by catheterization of the left ureter that the urine coming from the left kidney was the source of the organisms. He had one case of a pure bladder carrier as well, and puts forward the view that a filtering process from the blood through the glomeruli occurs, as he has noticed several instances in which a former negative urine began to show typhoid bacilli with the onset of a relapse.

Classification of Chronic Urinary Carriers.

On the basis of the findings of these workers chronic urinary carriers can be classified as

1. Pure kidney carriers.
2. Pure bladder carriers.
3. Mixed kidney and bladder carriers.

The first variety appear to be the commonest type described.

Treatment of Chronic Urinary Carriers.

Effect of Urinary Antiseptics.

Hexamethylenamine or urotropine has proved valuable in bacillurias occurring during typhoid fever and in early convalescence from that disease. Stokes and Clarke (1916) state that urotropine, 10 grains 6 hourly, caused the disappearance of typhoid badilli from the urine in 30 temporary carriers in 10 days. In two other cases it required twenty days treatment but had no effect on two chronic urinary carriers except to diminish the numbers of typhoid organisms excreted. Tanon and Dumont (1917), likewise, found that 1.5 grammes per day cleared up typhoid bacilluria in convalescents in 8 to 10 days and in only three out of 29 cases a month's treatment was required. In spite of its use one case did not resolve and became a chronic carrier. While urotropine has a

marked temporary effect in reducing the numbers of bacilli excreted in chronic carriers, it is unable to effect an entire cure and the bacilli return in full numbers when its administration is suspended as Niepratsck (1909) has shown. His case was given 3 grammes daily. Hetralin - a compound containing 56 per cent urotropine and 44 per cent resorcin - in doses of 8 grammes daily was tried in the same case with a similar result. Finally, Borovertin - a mixture of urotropine and boric acid - was tried with apparent success. 6 grammes were given daily for a month and no typhoid organisms could be demonstrated in the urine after the second day, the urine being still negative four months later. Ustvedt (1910) reported another cure by borovertin. After a nine weeks' course (120 grammes in all) the bacilli disappeared from the urine.

Irwin and Houston (1910), Nichols, Simmons and Stimmel, (1919), and Haibe (1921) all found hexamethylenamine ineffective in chronic urinary carriers but Murstad (1921) apparently believes that these can be sterilised by drugs, especially members of the formaldehyde group, while Tonney, Caldwell and Griffen, (1916) state that the urine is never posibive for B. typhosus when it gives the feaction for formalin. Sick and Deist (1923) failed to effect a cure in their

case by the use of urotropine, salol, and infusion uvi ursi by mouth, and lavage of the bladder, ureter and pelvis of right kidney, by means of ½ and 1 per cent. urotropine only caused a temporary disappearance and diminution in numbers of typhoid bacilli excreted. Lavage of the bladder following nephrectomy in the same case by means of a potassium permanganate solution apparently cleared away a residual typhoidal cystitis.

As regards a cure by the use of drugs it is hard to understand how a carrier of long standing, presenting in all likelihood lesions of a chronic character in the kidneys or urinary tract can be restored to a normal condition. Still from the reported cures by the use of borovertin a preliminary course of these should be given a trial in urinary carriers.

Effect of Vaccine Treatment. Vaccine therapy in urinary carriers has proved of value in some case but in the majority failures have been experienced. Irwin and Houston (1909) treated their female carrier case with a vaccine, using an autogenous strain in its preparation. Three doses of 50,100 and 200 millions at intervals of a week and a fortnight respectively were given without effect on the excretion of the specific organisms. The patient then received sodium lactate

and the urine was made alkaline. Two further doses of vaccine consisting of 300 and 1000 millions were next given at intervals of a fortnight and a month respectively. The urine became negative after commencement of the sodium lactate and remained negative in three examinations during the giving of the vaccine. The last examination, which was made five months later, was still negative. The authors believed that by rendering the urine alkaline the typhoid bacilli became more susceptible to the action of the immune bodies developed in the serum by vaccination.

Two chronic urinary carriers, invalided from the Indian Army, were treated with typhoid vaccines (Cummins, Fawcus and Kennedy, 1910) without definite improvement in their condition. Walker Hall, and Roberts (1911) reported their observations on a female urinary carrier who earlier failed to respond to autogenous vaccine treatment but who (quoted from Ledingham and Arkwright 1912) after nephrotomy with removal of renal calculi and another course of vaccine treatment, in which doses rising to 6000 millions were given, was typhoid-free during a period of four months. As this same case had a previous negative phase of nine months one cannot say whether a cure was obtained or not, or whether this constituted another intermission. The infection in this case was entirely confined

to the right kidney and ureter. The kidney at operation was found to be in a healthy condition, except for several aggregations of minute calculi, from which B. typhosus wasisolated in pure culture. Stokes and Clarke (1916) reported that two urinary carriers were benefited by a stock typhoid vaccine in doses of 500 and 1000 millioms in association with administration of urotropine. On the other hand Nichols, Simmons and Stimmel (1919) fried both stock and autogenous vaccines without success in a urinary carrier and Kach (1920) also reported failure in spite of twenty-two injections of an autogenous vaccine. Haibe (1921), who found in experimental work in dogs that autogenous vaccines seemed to exert a favourable influence on the urinary elimination of bacilli, and Hébert and Bloch (1922) concluded that vaccination exerted a beneficial effect on the excretion of these organisms. Murstad (1922), on the other hand, advises that vaccine treatment should be abandoned because it is ineffective.

Effect of Surgical Treatment.

Where the carrier has been proved by ureteral catheterization to be suffering from a strictly unilateral infection nephrectomy offers the greatest hope of cure. Nephrotomy with removal of renal calculi may prove of value as demonstrated by Walker,

Hall and Robert's (1911) case. In the operation for nephrectomy it is of importance to remove the whole of the ureter down to the bladder with the kidney, after which obliteration of the remaining stump quickly follows.

Nephrectomy was reported for the first time by Meyer and Ahreiner (1909) in their chronic urinary carrier who suffered from a pyonephrosis (see p.307). Nichols, Simmons and Stimmel (1919) reported the cure of a urinary carrier of six years standing, who had typhoid fever in 1912, and suffered from no symptoms relating to the urinary tract. Nephrectomy was performed and the kidney was found to be the seat of a marked pyenephrosis. The cystitis present resolved later. Sick and Deist (1923) had a similar success in their case (see p.309).

Technique employed for the isolation of B. typhosus from the urine of chronic carriers.

The author employed the method described by Browning (1918). The urine is voided directly into sterile bottles with rubber stoppers. No attempt was made to sterilise the meatus of the urethra, but the first portion of the urine passed was discarded in order to reduce the amount of extraneous contamination; on occasions catheter specimens were taken, but where a routine bi-weekly examination is made on

chronic carriers over a period of almost two and a half years this is obviously impossible. The urine was first of all centrifugalised and two or three large loopfuls from the sediment plated on to a Mac-Conkey agar plate.

When isolated non-lactose fermenting colonies were obtained after 24 hours incubation at 37°C. subcultures were made on agar so as to obtain pure growths for subsequent tests. Scheer (1918) suggested plating from the supernatant fluid of centrifugalised specimens of urine on the basis of his experimental results in the separation of B. typhosus from mixtures of organisms. After rapidly centrifuging a mixture of B. coli and B. typhosus for twenty minutes he isolated B. typhosus from the supernatant fluid in almost pure culture. B. coli was isolated from the bottom of the tubes and only with difficulty from the top, although prior to centrifuging it was present in that situation in greater numbers. He believed that this is due to the increased motility of the typhoid bacilli which have thus a greater resistance than the coliform organisms to centrifugation and that even though carried to the foot of the tube they regain the upper reaches sooner on account of their motility. This method, he states, applies also to B. paratyphosus A, and may be employed for stools as well. No extended observations were made

to test Scheer's hypothesis owing to the relative infrequency of urines positive for enteric organisms in the present series, but an extended trial might be made by plating both from the supernatant fluid and from the sediment of centrifugalised urines.

As a routine measure, when no growth of non-lactose fermenters appeared on the MacConkey agar plates in 24 hours, the urine, which had been allowed to stand at room temperature in the interval, was re-plated on to the same plate, using 5 to 10 loopfuls and incubated for a further 24 hours. By this means the specific organism was occasionally isolated on the second day when a negative result was obtained in 24 hours. Evidently the urine itself had inhibited to a certain extent the growth of other bacteria but allowed the enteric organisms to grow. This fact was particularly noted in the case of the B. paratyphosus B carrier (M.D.) on 2.1.23 when after 24 hours incubation there was only a profuse coliform growth. After standing at room temperature for the twenty-four hours and replating the urine on to MacConkey agar only one coliform colony was present and 5 colonies of B. paratyphosus B. had appeared.

In order to recover typhoid bacilli from the small proportion of urines that do not contain them in large numbers Morishima and Teague (1917) recommend

the following methods:-

To isolate typhoid bacilli from urine not collected under aseptic precautions, streak two or three large loops of the urine over the surface of an Endo-agar plate; add to the urine approximately one half of its volume of nutrient broth and incubate the mixture overnight. If the direct inoculated plate is negative on the following morning prepare dilutions of the incubated mixture of urine and broth (1: 1,000,000; 1:10,000; 1:100; and 1 loop of the original) and inoculate them on an eosin-brilliant-green agar plate. Other special media that inhibit the growth of most strains of B. coli while allowing the development of B. typhosus, could probably be substituted for the eosin-brilliant-green agar, if one is not familiar with the latter medium. If the typhoid bacilli are present in sufficiently large numbers to yield colonies on the plate inoculated on the first day, the incubated urine-broth mixture, is, of course, discarded. Morishima and Teague claim that this method offers the advantage of subjecting a large amount of the urine to examination with very little manipulation or loss of time. By its use it is believed that considerably higher percentages of positive results would be obtained in the routine examination of urines for typhoid bacilli than by the methods usually

employed. Owing to the liability of specimens of urine to contamination Browning suggests that Brilliant-green may be added to the mixture on the basis of Benian's experiments. Garbat (1922) suggested that 15 to 20 ccs. of a 24 hours specimen of urine should be cultured in 100 ccs. of broth and then plated on to a solid differential medium, preferably Endo-agar.

Observations on the present series of chronic typhoid carriers.

None of the eight chronic bacillus excreters under observation proved to be true urinary carriers, though in all but one (Mrs. B.E.) the specific organisms were occasionally isolated from the urine. In three others (J.M., M.M., and H.T.) B. typhosus was isolated on only one occasion, in the first two and on three in the latter in 104, 49, and 175 examinations respectively. Table XVIII p. 327 shews the results of the examination of the urines of the whole series of carriers.

Analysis of these cases in detail.

Mrs. G. B. typhosus was continually present in the urine from 23.11.21 to 12.12.21 and after that period disappeared, none being isolated after the latter date in 22 examinations from 14.12.21 to 13.3.22. Throughout the faeces were always positive for B. typhosus. From the nature of the appearance of the

TABLE XVIII.

Table to show results of examination of urine in chronic carriers.

Case.	Number of Examinations.	Result of Examinations.		Remarks.
		Positive.	Negative.	
Mrs. G.	35.	10.	25.	
J.M.	104.	1.	103.	
Mrs. M.M.	50.	1.	49.	
Mrs. B.E.	17.	0.	17.	
Mrs. R.	3.	2.	1.	
Miss K.O.	149	17	132)	
Miss M.D.	178.	41.	137)	Prior to operation.
Miss H.T.	110.	3.	107)	
Miss K.O.	75	1*.	74	(*Positive on 2nd day
" M.D.	86.	0.	86	after operation.)
" H.T.	65.	0.	65.	Subsequent to oper-
				ation.

bacilli in the urine, their persistence for a period, and final disappearance, it would suggest a temporary bacilluria associated with a bacteremia rather than a contamination of the urine from the faeces.

J.M. As the urine showed the presence of typhoid bacilli only once in this case on 23.4.23 (one colony) during 104 examinations made between 16.5.22 and 7.6.23 one may conclude that this resulted from a contamination of the specimen. About this period the patient, who suffered from dementia, was distinctly dirty in his habits and difficult to control.

Miss M.M. A similar contamination appears to have occurred in this case on 2.2.23, this being the only positive result obtained in 50 examinations from 9.10.22 to 31.5.23.

In both J.M's and M.M's cases the faeces were positive for B. typhosus on the same day as the urine was positive.

Mrs. B.E. At no period did the urine show show B. paratyphosus B. present in any of the 17 examinations made from 9.12.22 to 10.5.23.

Mrs. R. Only three examinations were made (31.5.23, 4.6.23, and 7.6.23). Both urine and faeces were negative for B. typhosus on 31.5.23, but positive on 4.6.23, On 7.6.23 the urine alone revealed the presence of B. typhosus.

Miss K.O. In this case the positive urine examinations always coincided with positive faecal examinations except on one occasion (22.12.21) when the urine MacConkey agar plate showed two colonies of B. typhosus and the faeces plates none. This result was never subsequently repeated. The 17. positive urine findings occurred at long and irregular intervals. The largest number of consecutive positive results occurred between 10.1.22 and 19.1.22 when the urine was positive four times. While impossible to exclude the possibility of contamination of the urine by the faeces it is not improbable that in this case a bacteremia may occasionally have occurred, leading to the excretion of bacilli in the urine. The rarity of the positive results is noteworthy.

Miss H.T. On three occasions B. typhosus was isolated from the urine out of 110 examinations from 14.4.22 till the operation of cholecystectomy on 4.5.23. Subsequently 65 negative results were obtained (7.5.23 to 7.2.24). The findings of typhoid bacilli on these occasions would thus appear to be a result of contamination.

Miss M.D. The[41], positive results for B. paratyphosus B in the urine occurred at variable intervals and on no occasion was the urine positive when the faeces was negative. The largest number of consecutive

positives was 4 (12.12.21, 14.12.21, 16.12.21 and 20.12.21). The findings of this case are similar to that of K.O., namely that the many of the positive examinations may be due to some contamination although it is still possible that a temporary bacilluria may have at times taken place. As compared with the excretion in the faeces, the excretion in the urine was scanty and infrequent.

Thus from the above detailed analysis it is seen that none of the carriers could be designated a true urinary barrier.

To illustrate the value of replating the urine on to the same plate of MacConkey agar in spite of the fact that no growth may have occurred after 24 hours' incubation the results shown in the following table illustrate the usefulness of this measure.

Date of Examination.	Case.	Number of colonies after immediate plating.	Number of colonies after replating.
10.8.22.	M.D.	Sterile.	1 B. paratyphosus B.
6.11.22.	M.D.	Sterile.	1 " " "
20.11.22.	M.D.	Sterile.	5 " " "
2.1.23.	M.D.	Coliform growth. Non-lactose fermenters.	1 Coliform. 5 B. paratyphosus B.
5.3.23.	M.D.	Sterile.	4 B. paratyphosus B.
21.12.22.	K.O.	1(B.typhosus).	5 B. typhosus.

BIBLIOGRAPHY.

Adrian (1908): Strasb. med. Zeitung, 1908, H.6. Ref. Centr. f. Bakt., Abt 1, Ref. Bd. XLIII, p.172.

Blumer (1895): John Hopkins Hosp. Rep., 1895. V. p. 327.

Browning (1918): Applied Bacteriology, London, 1918, p.15 and p. 136.

Brownlee and Chapman (1906): Glasgow Med. Journ. 1906, LXVI, p. 634.

Buchan (1908): Liverpool Med. Chir, Journ. 1908, p. 144.

Cole (1901): Bull. John Hopkins Hosp., 1901, XII, p. 203.

Connell (1909): Am. Journ. Med. Sc., Phila., 1909, CXXXVII, p.637.

Cummins, Fawcus and Kennedy (1910): Journ. R.A.M.C. 1910, XV, p. 351.

Dittmar (1922): Proceed. Roy. Soc. Med., 1922, XVI, pp. 1-10.

Flexner (1896): J. Path. and Bact., 1896, III, p.202.

Garbat (1922): Monographs of Rockefeller Institut. for Med. Res., No 16, May 1922.

Greaves (1907): B.M.J., 1907, II, p.75.

Gwyn (1899): Bull. John Hopkins Hosp., Balt., 1899, X, p. 109.

Haibe (1921): Commun. Roy. Soc. Med. Belg., ref. J. Am. Med. Assn. 1921, 76, p.136.

Hall, Walker and Roberts (1911): J. Path. and Bact., 1911, XV, p.120.

Hébert and Bloch (1922): Ann. de l'Inst. Past., 1922, 36, p. 157.

Henes (1920): J. Am. Med. Assn., 1920, 75, p. 1771.

Horton-Smith (1900): B.M.J., 1900, I, p. 827, and Lancet, 1900, I, p. 910.

Houston (1899): B,M.J., 1899, 1, p.78.

Irwin and Houston (1909): Lancet, 1909, I, p.311.

Kach (1920): Zeitschrift. f. Klin. med., Berl., 1920, 89, p. 87.

Konjajeff (1899): Abstract in Centralb. f. Bakt. u. Parasitenk., Jena, 1899, VI, p. 672.

Ledingham and Arkwright (1912): The carrier Problem in Infectious Diseases, London, 1912.

Liebetrau (1906): Arb. a.d. Kaiser. Gesundheitsamte, 1906, XXIV, p. 341.

McCall (quoted Patrick 1914).

Meyer and Ahreiner (1909): Mitt. a.d. Grenzgeb. d. Med. u. Chir. 1909, XIX, p. 486.

Morishima and Teague (1917): J. Infect. Diseases, 1917, XXI, p. 145.

Murstad (1921): Med. Rev. 1921, 38, p. 215, Ref. J.A.M.A., 1922, 79, p. 561.

Napier and Buchanan (1906): Glasgow Med. Journ., Dec. 1906.

Nichols, Simmons and Stimmel (1919): Journ. Am. Assn. 1919, 73, p. 680.

Niepratschk (1909): Zeitsch. f. Hyg., 1909, LXIV., p. 454.

Osler (1913): Modern Medicine, London, 1913, p. 142.

Patrick (1914): J. Patho and Bact., 1914, XVIII, p.355.

Petruschky (1898): Centralbl, f. Bakt, u. Parasitenk., Jena, 1898, Abt. I, orig., XXIII, p. 577.

Pick (1910): Munch. Med. Woch., 1910, p. 1523.

Richardson (1903): Boston Med., and Surg. Journ., 1903, CXIVIII., p. 203.

Rousing (1898): Infektiose Krankheiten der Harnorgane, Berl. 1898.

Scheer (1918): Med. Klin., 1918, p. 758.

Schuder (1901): Deutsch. med. Woch., 1901, p. 762.

Sick and Deist (1923): Deutsche. Arch. Klin. Med., 1923, 143, p. 149.

Stokes and Clarke (1916): J. R.A.M.C., 1916, XXVI, pp. 461 - 470.

Tanon and Dumont (1917): J. Am. Med. Assn., 1917, 68, p. 1999.

Tonney, Caldwell and Griffen (1916): J. Infect. Dis., 1916, 18, p. 239.

Ustvedt (1910): Norsk. Mag. f. Laegevid, No. 4. Ref. Deutsch. Med Woch, 1910, p. 776.

Young (1900): John Hopkins Rep., 1900, VIII, p. 401.

****

CHAPTER X.

VARIATION IN BACTERIA OF THE ENTERIC GROUP IN RELATION TO AGGLUTINATION BOTH BY SALTS AND BY SPECIFIC SERUM WITH OBSERVATIONS ON THE PRESENCE OF ROUGH VARIANTS.

Throughout the whole course of examinations on a carrier of B. paratyphosus B (M.D.) two distinct varieties of colony have been found, often co-existing together. They have been recovered from the faeces by direct plating on MacConkey's medium and also by the brilliant green enrichment method; they were also grown from the urine and from the bile obtained at operation for cholecystgastrostomy. The one variety had the smooth rounded character of ordinary B. paratyphosus B, while the other appeared to be the rough variant type described by Arkwright (1921) and Schutze (1921). Von Lingelsheim (1913) described a somewhat similar variation in the case of typhoid bacilli, though the colonies tended to be dry and wrinkled

with consequent difficulty in obtaining stable suspensions. The characters of the "rough variants" described by Arkwright were found by him at first in 8 out of 9 B. dysenteriae (Shiga) strains and later in B. dysenterae (Flexner), B. typhosus, B. paratyphosus B, B. enteritidis (Gaertner) and other members of the coli-typhoid group. From these cultures he obtained Smooth (S) and Rough (R) colonies which had different cultural characters in broth and on agar but the same sugar reactions. The rough colonies are large, have a granular surface with irregular crenated margins and give rise to stouter and longer bacilli than the smooth variety. The "S" form makes a good stable emulsion in salt solution and in broth cultures causes uniform turbidity. The "R" form agglutinates spontaneously in 0.85 per cent. saline and in broth cultures forms a deposit leaving the liquid clear. Arkwright noted that many cultures which agglutinate spontaneously when emulsified with 0.85 per cent. saline form stable emulsions in weaker salt solution, e.g. 0.42 per cent., 0.21 per cent., or 0.1 per cent., and stated that accordingly weaker salt solution must be used for agglutination experiments with "R" colonies. The "S" form, when agglutinated by a specific anti-serum for the species of organism under consideration but not necessarily derived from the particular strain in question, forms large clumps and the deposit is readily shaken up into a turbid suspension. The two forms differ

decidedly in their agglutinating, antigenic and absorbing properties with specific sera in the case of B. dysenteriae (Shiga) but not so markedly with B. typhosus. Schutze confirmed Arkwright's findings with a substrain from a case of infection with B. paratyphosus B (Schottmüller) obtained from Fletcher, which was a self-agglutinator, but found that variations in colony character and stability in saline do not necessarily go hand in hand; the variant giving the roughest colony need not be the most unstable in saline and vice-versa, and the degree of serological character varies independently of the amount of roughness; again there exists a serological cosmopolitanism among rough cultures, e.g. rough variants of B. Gaertner, B. paratyphosus A and typhoid strains will sometimes agglutinate to titre limit with anti-sera generated by immunising rabbits with rough cultures of the B. paratyphosus B group, while the smooth prototypes from which these have derived remain quite unaffected by similar anti-sera. A "Rough" hog cholera strain on sub-culture over a number of years only gave rise to rough colonies. The affinity of type strains (in the form of their rough variants) may be detected by preparing from it an agglutinating serum and by completely absorbing the specific agglutinins from the serum. The diagnosis of "rough" strains by their

growth inhibitions has been pointed out by Eijkman (1904) and McLeod and Govenlock (1921) by direct inoculation of one strain upon another. The procedure is as follows:- an organism is grown on gelatine at 37°C. for 24 hours, cooled to solidification and the sloped surface inoculated with a loopful of broth culture; after one or two days at 22°C, if the inoculated bacillus is not inhibited, a roughening shows along the tract of the needle contrasting with the smooth surface of the gelatine and gradually develops into a definite line of growth.

These rough colonies do not appear to correspond with the capsulate mucoid forms of B. paratyphosus B of Fletcher (1920) or of Thøjtta and Eide (1920) though they bear some resemblance to them. Fletcher found large white mucilaginous colonies to which he gave the name "mucoids" in the stools of a chronic carrier of B. paratyphosus B. These gave the ordinary sugar reactions but did not agglutinate with B. paratyphosus B anti-serum but absorbed agglutinins from it; eight months later one of the strains agglutinated slowly and imperfectly in low dilutions. The "mucoids" were able on subculture in peptone water to give rise to both ordinary forms and mucoid forms. The ordinary forms derived thus differed from typical paratyphoid bacilli in that their colonies on solid media

were as a rule more granular and more long "involution" types were present than normal. The author describes the "mucoid" colonies as being twice the diameter of typical B. paratyphosus B colonies, dome-shaped, circular in outline, with a regular margin and having a mucoid appearance. In transmitted light they are translucent, appearing like frosted glass. On agar slopes growth is thick and shiny like mucus, but this sliminess disappears except near the top of the tube after a few days; when left several weeks on subculture only non-mucoids and a few ghostly colonies mingled with them, which have a ground glass appearance, are obtained, On subculturing several times the latter give rise to luxuriant "mucoids" once more. Fletcher has never been able to produce mucoids from ordinary B. paratyphosus B such as Revis (1910) obtained. In films numerous small coccoid forms are got from the "mucoids" which have capsules while ordinary type paratyphoid colonies produced from mucoids show long involution forms. Thjøtta and Eide's organism gave practically the same characteristics as Fletcher's. It was immobile or very slightly motile, but proved on serological tests to be a true, though atypical strain of B. paratyphosus B, but agglutinated much more slowly with ordinary paratyphoid B antisera. The patient's serum agglutinated both the normal and atypical strains and after absorption the agglutination for

both was abolished. Rabbit antisera derived from the mucoid type agglutinated two other normal cultures and after absorption by the typical strains the titre of agglutination was lowered practically to the same degree for all three strains. The tests, however, showed the atypical strain to be much slower in its agglutinating reaction since in all the tests agglutination only reached its maximum in 48 hours with it, while 24 hours were sufficient for the two normal strains. Individual bacilli did not appear to be capsulated but 3 or 4 were covered with a common cover of mucus. In their case typical paratyphoid colonies likewise only produced typical colonies while the mucoid types at first produced both varieties but on repeated subculture the mucoid type gave only atypical, i.e. mucoid forms. They claim that their organism establishes a real transmutation of bacteria.

Walker (1922) described the experimental derivation of a mucoid form of B. paratyphosus B by growth in 25 per cent. specific immune serum broth. These colonies were apparently similar to the mucoid types described by Fletcher. On subculture it reverted to the ordinary form of B. paratyphosus B.

Description of rough variants isolated from a B. paratyphosus B carrier (M.D.).

The "rough"colonies gave the normal sugar and biological reactions for B. paratyphosus B and were isolated

from the faeces, urine and bile. Gram-stained films revealed the organism to be a short bacillus which showed no appreciable difference in length to that of the bacillus derived from the normal round B. paratyphosus B colonies isolated at the same time (Fig.8 x 1000). They were actively motile.

Lactose	Glucose	Mannite	Dulcite	Maltose	Saccharose
0.	+	+.	+2	+	0.

Inosite	Litmus Milk.	Indol	Gelatin.
0.	Alk.	0.	Slight gas. No liquefaction.

0 = No change.

\+ = Acid and gas formation.

+2 = Acid and gas formation on the second day.

On MacConkey's bile-salt lactose neutral-red agar the rough colonies are larger than the round varieties; they have irregular margins and a woolly striated or radiate appearance on the surface (Fig.9 x 1000). After keeping at 37°C. and room temperature for two days there is definite heaping-up of the margins. By transmitted light they have a somewhat frosted appearance. Occasionally one half of the colony was round and smooth and the other half of the rough irregular type (5.3.23). On ordinary agar medium the growth had a similar rough appearance; at first it was dry

Microphotographs of the Smooth (ordinary) and Rough Variant varieties of B. paratyphosus B isolated from chronic bacillus excreter M.D.

Fig.8 x 1000

Smooth Colony.

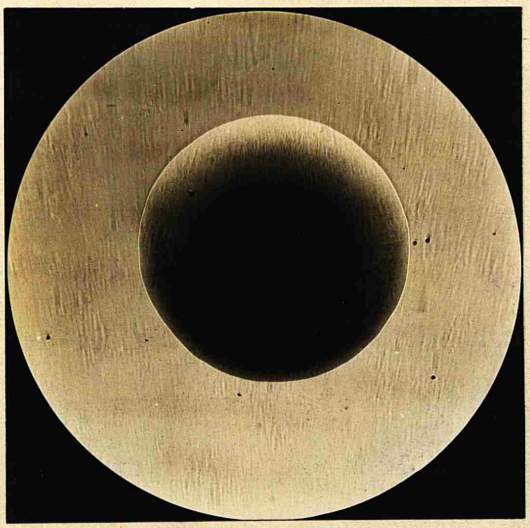
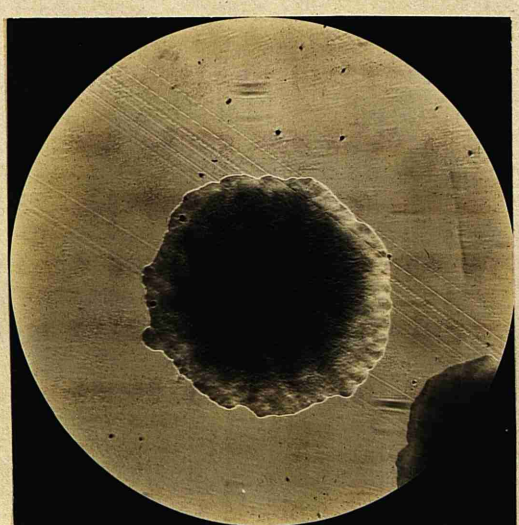


Fig.9 x 1000

Rough Colony.



and wrinkled; but in a few days tended to have a slimy and mucoid character. Fourteen subcultures from 13.7.22 to 7.3.23 of organisms derived from a primary rough colony did not produce any alteration in the character of the growth. This was repeated by other similar colonies on many occasions. The "rough" colonies were tested by plating frequently to see if they would produce round varieties. On every occasion they reproduced entirely the rough prototype. Likewise round colonies were never observed to reproduce rough varieties. In peptone water no differences in growth characteristics were seen except that a distinctly heavier deposit was present in 48 hours at 37°C. at the foot of the tube containing the "rough" cultures. There was no obvious difference in rapidity of growth.

In order to show spontaneous agglutination of the rough colonies saline suspensions of agar cultures were prepared with 0.85 per cent. and 0.21 per cent. NaCl solution as Arkwright suggested. Sometimes 0.42 per cent. saline caused spontaneous agglutination to disappear, but it was always present with 0.85 per cent. and agglutination to titre with serum from another strain, with no evidence of any "rough" element, occurred.

The rough cultures showed no appreciable difference in their resistance to brilliant-green as compared with

the smooth colonies (see Table XIX, p. 344).

The technique of the resistance experiments was as follows:-

1 c.c. of sterile neutral peptone water was placed into each tube - 8 in all - the first 7 also containing brilliant - green and the last one serving as a control. Into each of the first 4 were added .01, .025, .05, .1 c.cs. of 1:10,000 brilliant-green in distilled water and into the next three .025, .05, .1 ccs. of 1:1000 brilliant-green solution, giving the following final dilutions of the dye:- 1:1,000,000; 1:400,000; 1:200,000; 1:100,000; 1:40,000; 1:20,000, 1:10,000. Into each tube was next put 0.1 c.c. of a very dilute saline suspension from a 24 hour agar slope. The naked eye appearances of the tubes were compared with the control tube and agar plates were stroked in 24 and 48 hours.

"R" cultures inoculated intravenously in increasing doses into rabbits produced a high titre (1:25,000) agglutinating antiserum which agglutinated equally well

TABLE XIX.

Table to show Resistance to Brilliant-green of both varieties of B. paratyphosus B derived from carrier M.D.

Strength of Brilliant-green		1/1,000,000	1/400,000	1/200,000	1/100,000	1/40,000	1/20,000	1/10,000	Control.
Rough	Naked Eye-24 hours	+	+	Sl. Inh.	v.m.h.	v.m.h.	v.m.h.	v.m.h.	+
Smooth	Plate -	+	+	+	+	v.sl.inh.	v.sl.inh.	v.sl.inh.	+
Rough	Naked Eye-48 hours	+	+	+	+	+	+	+	+
Smooth	Plate -	+	+	+	+	+	+	+	+

slight inhibition.

\+ = full or heavy growth.
v.sl. inh. = very slight inhibition.
sl. inh. = slight inhibition.
v. m. inh. = very marked inhibition.

---

smooth B. paratyphosus B colonies as well as the rough varieties derived from the same patient as the culture employed to generate the antiserum. Another strain (S type) was agglutinated similarly to the same titre.

Variation in agglutinability of various strain of typhoid and paratyphoid bacilli.

Inagglutinability.

Immediately after isolation some cultures were found to be more or less insusceptible to agglutination with the corresponding stock antisera. In every instance repeated sub-culture on agar or in broth (four or five transfers) was sufficient to restore this power. It is therefore unsafe to base a final conclusion on deficient agglutinability until the organism has been frequently subcultured. Inagglutinability is said also to depend on certain substances, such as malachite-green, which may have been added to the medium on or in which the organism has been grown: the test is therefore better carried out with a subculture from such an original plate.

No difference in agglutinability was noted by the author in cultures of B. typhosus or B. paratyphosusB isolated via the brilliant-green fluid enrichment medium of Browning from those isolated by direct plating on MacConkey agar from the stools of enteric carriers investigated by him. In rare instances inagglutinability

has been found to persist for a long period and may be accompanied by lack of motility. Numerous observers have recorded the isolation of typhoid bacilli, which were agglutinated only slightly or not at all (Horton Smith 1900), Remy (1901), Sacquepée (1901) Nicolle and Trenel (1902), Prantschoff and Porges (1906), Fornet and Rochaix (1913), Gay and Claypole (1913), McIntosh and McQueen (1914), Arkwright (1914), Bull and Pritchett (1916), Blankenhorn, Ecker and King (1923) and Yu (1924). These bacilli have been isolated from acute and carrier cases and have fulfilled all the tests for typhoid bacilli, and in certain cases it has been found that immunization of animals against feebly agglutinable strains (Arkwright, McIntosh and McQueen, Blankenhorn, Ecker and King and Yu) resulted in the production of a serum which agglutinated laboratory strains. According to Paltauf (1914), not infrequently agglutination is lessened with the patient's serum as well as with an artificial anti-typhoid serum. If, then, an organism which resembles a member of the enteric group fails to agglutinate with suitable sera, it should not be rejected until it has been subjected to repeated sub-culture in broth. Fabry (1920) states that cultivation in an alkaline peptone bouillon containing small amounts of phenol (0.1 c.c. of 5 per cent. phenol to 5 c.c. broth) renders such strains more susceptible to

agglutination. In obstinate cases the true nature of an inagglutinable strain may be revealed either by immunizing an animal with it, when the serum yielded may be found to agglutinate typical strains, or by means of the absorption test.

Description of the findings in the case of an "inagglutinable" bacillus isolated from a chronic faecal carrier by Watt (1923).

Subsequent to the reported cure of a chronic B. typhosus excreter by a detoxicated B. typhosus vaccine, an organism, which gave typical biological reactions of B. typhosus but did not agglutinate with any of the stock antisera, was isolated on more than one occasion from the case. This organism was examined by Dr.J.W. Tocher who described it as follows: Gram negative, feebly motile, giving all the sugar reactions of B. typhosus; did not agglutinate with any of the stock sera; emulsions of the culture, injected intravenously into a rabbit failed to produce agglutinins towards the organism. The growth in broth was inclined to form filaments.

Another report of the organism stated that an emulsion of the bacillus was not agglutinated by any of the "Standards" sera, and that so far it was not agglutinated by inoculated animal serum. Inoculation with 2 c.cs. of a 24 hour's broth culture failed to

kill a guinea-pig, so that the organism is evidently non-virulent. This latter observation is of no value since there is no criterion which would yield satisfactory information as regards virulence in the human subject.

A culture was received from Dr. Watt on 25.3.23 and subjected by the author to extensive investigation.

Biological reactions.

Gram.	Motility	Lactose	Glucose	Mannite
Negative	+ (not very active).	0	A	A

Dulcite	Maltose	Saccharose	Litmus Milk	Indol
0	A	0	sl.acid. alk.	0

Gelatin.
No gas. No liquefaction.

A = acid formation.

sl.acid = slight acid formation.

alk = alkaline.

0 = No change in reaction.

Agglutinability.

When first tested, a saline suspension of a 24 hours agar culture, heated at 56°C. for ½ hours, of the organism, agglutinated to ⅛ th. (1:1600) of the titre limit (1:12,800) in 2 hours at 56°C. (and standing

overnight at room temperature)with antityphoid serum obtained by injecting a rabbit with a known strain of B. typhosus (R.L.L.).

After subculturing on agar at daily intervals on three occasions the culture was once more similarly tested. Five individual colonies all agglutinated to the titre of two different rabbit anti-typhoid sera (R.L.L. and Court.). The same result was obtained on 27.8.23, some five months later. After the mixture of serum and organisms had stood for 2 hours at 56°C it shewed much less agglutination than did a control series with a stock culture of B. typhosus (R.L.L.);- (1:800 of serum agglutinated the Watt organism to the same extent as 1:6,400 did in the case of the stock culture) - even after 24 hours the flocculi were coarser with the latter.

Agglutinin Absorption.

(a) A stock antityphoid serum was only partially deprived of its agglutinins by absorption with the Watt culture (titre of untreated serum for stock B. typhosus = 1:12,800, titre of treated serum = 1:6,400: the method of the experiment was practically that given in Browning's Applied Bacteriology p.28). Three agar slopes inoculated with Watt culture, incubated at 37°C for 24 hours were each emulsified in 1 c.c. of saline. The mixture was killed by heating at 56°C. for 1 hour and treated with 3 c.cs stock rabbit antityphoid serum

(titre 1:12,800) in a 1:50 dilution at 37°C for 2 hours and then centrifuged. The supernatant fluid was used and tested with a similarly killed and prepared emulsion of a known stock B. typhosus culture (R.L.L.).

The same stock serum on similar treatment with two known strains of B. typhosus, separately, in each instance was practically deprived of agglutinins for the Watt culture and the other two strains of B. typhosus (titre of treated serum in each case = 1:200).

(b) An antiserum to the Watt bacillus was obtained from a rabbit by intravenous inoculation of a killed saline suspension of a 24 hours culture, which agglutinated the Watt culture more slowly than it agglutinated two stock strains of B. typhosus (R.L.L. and Court): (titre in each case after 24 hours = 1:25,600). This serum was absorbed with the two stock typhoid cultures as well as with the homologous culture. After treatment with the stock cultures separately the serum failed to agglutinate the Watt culture in a dilution of 1:200, but after absorption with the Watt culture it still agglutinated the latter slightly in a dilution of 1:400. This confirmed the weakness in binding power of the Watt organism previously noted.

These results permit of only one conclusion, namely, that the organism is B. typhosus. The dubiety as to

its character probably arose from its manifesting deficient agglutinability shortly after isolation.

Unfortunately the true nature of the organism was not discovered until a cure by means of detoxicated vaccine had been reported. As the carrier was an exceedingly intermittent excreter of B. typhosus the importance of continued examinations of the faeces over a period of one to two years before a cure is reported cannot be sufficiently stressed.

It is possible that the vaccination may have had some effect upon the specific organisms in causing a deficiency in their agglutinating power. No such effect, however, was noticed in organisms isolated from any carriers of the author's series subsequent to vaccine treatment.

Dissussion of variations in agglutinability.

Assuming an antiserum of high titre, the agglutinability of B. typhosus is primarily dependent upon the nutrient medium. Hohn (1922) states that agglutinability is not a characteristic, and primarily present property of the typhoid bacillus but that it is acquired from the culture medium. Inagglutinability depends on the inability of a particular strain to produce from available nutrients the materials that promote agglutinability. To promote maximium agglutination of typhoid strains he recommends 1 per cent. galactose agar. Arkwright's (1914) experiments on agglutination with watery extracts

of B. typhosus would appear to support this view. Agglutinability is not affected by growth on 10 per cent. rabbit blood agar (Bull and Pritchett 1916) though Gay and Claypole (1913) claim this is the case and also Eisenberg (1913) by growth in blood bouillon.

Spontaneous agglutination.

Certain strains of bacteria, particularly those of the rough variant variety, whether in broth culture or saline suspension, are apt to undergo agglutination of their own accord. This has been already mentioned in the case of the rough variant colony of B. paratyphosus B. isolated from the carrier (M.D.). As suggested by Arkwright, for this reason, a control observation in which the specific serum is replaced by normal saline, or better by normal serum, should always be introduced. This source of fallacy is usually betrayed, in a series of tubes in which gradually increasing serum dilutions are tested, by the absence of any gradation of the agglutination as the limit of titre is approached. The difficulty is usually overcome by diminishing the percentage of sodium chloride. Verzar (1917) obtained a culture of B. typhosus of this type from the urine of convalescents. He also noted the transformation of a typical B. typhosus into a variety which shewed spontaneous agglutination. Twenty subcultures and passage through a guinea-pig

did not cause the property to disappear but heating to 60°C as suggested by Porges and Prantschoff apparently caused it to disappear. The addition of 0.03 to 0.20 per cent. of formalin added to agar or broth cultures is claimed by Ishi (1922) to prevent spontaneous agglutination. He noted that spontaneous agglutinating colonies had a heavier growth, rougher surface, were irregularly round with a serrated border, and were of the rough variant type. In most cases the organisms were longer and relatively more motile, Kabeshima (1918) reported a spontaneously agglutinating strain of B. paratyphosus A isolated from the blood.

****

REFERENCES.

Arkwright (1914): Journ Hyg., 1914, XIV, p. 261.

Arkwright (1921): Journ. of Path. and Bact., 1921, XXIV, p. 36.

Bergstrand (1923): J. of Bact., 1923, VIII, p. 173.

Blankenhorn Ecker and King (1923): J. Infect. Dis., 1923, 32, p. 95.

Bull and Pritchett (1916): Journ. Expt. Med., 1916, 24, p.35.

Eijkmann (1904): Centralbl. f. Bakt., 1904, I, orig., XXXVII, p. 436.

Eisenberg (1913): Ergeb. der Immunitats. Exper. Ther. Bakt. u. Hyg, 1913, 73.

Fabry (1920): Compte rend. Soc. de biol., 1920, 83, p. 201.

Fletcher (1910): Lancet, 1918, July 27th.

Fletcher (1920): Journ. R.A.M.C., 1920, 34, p.219.

Fornet and Rochaix (1913): Compte. rend. Soc. de biol. 1913, LXXIV, p. 797.

Gay and Claypole (1913): Arch. Int. Med., 1913, 12, p. 614.

Hohn (1922): Münch. med. Woch., 1922, 69, pp. 7 -10.

Ishi (1922): J. of Bact., 1922, VII, pp. 39 - 71.

Kabéshima (1918): Compte rend. Soc. de. biol., 1918, 81, p. 744.

McIntosh and McQueen (1914): Journ. Hyg. 1914, 13, p. 409.

McLeod and Govenlock (1921): Lancet, 1921, I, p.900.

Nicolle and Trenel (1902): Ann. de l'Instit. Pasteur, XVI, p. 562.

Paltauf (1912): In Kolle and Wassermann Handbuch d. path. Mikroorgan, II, p. 502.

Prantschoff and Porges (1906): Centralbl. f. Bakt., I. Abt. Orig., XLI, pp. 466, 546.

Remy (1901): Ann. de l'Instit. Pasteur, XV, p. 249.

Revis (1910): Centralbl. f. Bakt., 1910, XXXVI.

Sacquépée (1901): Ann. de l'Instit. Pasteur, XV, p. 249.

Schutze (1921): Journ. Hyg., 1921, XX, p.330.

Smith, Horton, (1900): Lancet, I, p.821.

Thøtta and Eide (1920): J. of Bact., 1920, 5, p.501.

Verzar (1917): Centralbl. f. Bakt. I, Abt, orig, LXXX, p. 161.

Von Lingelsheim (1913): Centralbl. f. Bakt., 1913, I, orig., LXVIII, p. 577.

Walker (1922): Journ. Hyg., 1922, 21, p. 87.

Watt (1923): Lancet., 1923, I, No 5191, p. 378.

Yu (1924): Zeitsch. f. Immunitatsf., 1924, 41, p.114.

****

CHAPTER XI.

THE ISOLATION OF B. TYPHOSUS FROM ABNORMAL SITES FOLLOWING AN ATTACK OF TYPHOID FEVER.

Since typhoid fever is a generalised blood infection it follows that the organisms may lodge and multiply in various situations in the body, the site being predisposed to by some previous injury or disease. As is well known typhoid bacilli have a special predilliction for multiplication in the gall-bladder and in the majority of true chronic intestinal carriers can be isolated from the bile. This phenomenon is discussed in another section (see p. 137). A similar condition apparently exists in the kidneys. This aspect is fully dealt with under the section on urinary carriers (see p. 306).

Numerous cases have been recorded in which typhoid bacilli have been isolated from other sites, both during the acute attack, during convalescence and many years after the original infection. The

specific organisms have been isolated from the blood of carriers as long as 14 years after the acute attack; thus Ebeling (1914) described the case of a woman whose temperature and pulse were normal but whose serum agglutinated B. typhosus in a dilution of 1:200. In addition to the blood the urine and faeces were also positive for B. typhosus. Similarly Sieliger and Lüdke reported carriers of B. typhosus in the blood who suffered from no ill effects.

Many cases have been recorded of suppurating lesions following attacks of enteric fever in connection with bones. Bureau and Marchand had a patient who developed, after typhoid fever, a fistula in Scarpa's triangle, pointing under Poupart's ligament, the pus of which gave B. typhosus in pure culture. This fistula closed and broke down six times in 2½ years and was finally cured following 16 injections of an autogenous vaccine. In Wohlgemuth's case a typhoidal osteo-myelitis developed 21 years after the primary infection.

Napier and Buchannan (1906) reported a case where pyelitis with chronic nephritis was due to typhoid bacilli alone, and these were isolated in pure growth from abscesses in connection with the ribs and other bones many years after the attack. Weil (1917) confirmed Bureau and Marchand's findings

and emphasized the value of vaccine therapy in that type of case. He stated that one patient was cured in three weeks by vaccine therapy of a typhoidal suppurative bone process that had dragged on for two years during which time it had been subjected to seven futile operations. The cure, effected by the vaccine, was complete in one week in another case after eleven months of the infection and three operations. Improvement amounting to a cure was obtained in two weeks in processes of six and twelve months' standing in other cases. It failed only in one case which commenced healing after removal of the sequestrum. Stephan (1916) and Eschbach (1917) both described cases of suppurative osteitis due to B. paratyphosus B.

Typhoid bacilli have been isolated from the bone marrow and spleen at post-mortems on chronic carriers by Knauer (1921). An interesting case is described by Blankenhorn, Ecker and King (1923) of atypical typhoid fever with a slowly agglutinable typhoid bacillus in a periosteal lesion.

Among cases where the typhoid bacilli have been isolated from other sites Drüner reported one of strumitis occurring 26 years after the original attack of enteric fever and Laubenheimer (1911) found typhoid bacilli in ovarian cysts. Bennett (1917) had

two patients who suffered from otitis-media following upon an acute typhoid attack with excretion of typhoid bacilli from the ears. These became aural typhoid carriers.

In 1921 Haibe observed a case of appendicitis eight months after typhoid fever. B. typhosus was isolated in pure culture from the appendix. He considered it to be true appendicitis of typhoid character but this has not been irrefutably proved, as it was not certain that the patient did not harbour the bacilli in the gall-bladder.

The author observed a case of perichondritis in a man, (M.M.) aged 48 years, who suffered from an acute attack of typhoid fever towards the end of November 1922. In February 1923 he first noticed a small swelling over the front of the chest which grew larger and became somewhat tender. Physical examination revealed a rounded swelling over the sternal end of the 4th rib and cartilage on the left side. The skin on the surface was slightly inflamed. The swelling was tender to touch, slightly movable but non-fluctulant.

Dr. Scoular Buchanan, Western Infirmary, Glasgow (to whom I am indebted for the details regarding operation) made an incision over the swelling on 11.5.23. and found a small soft focus in the centre

of a mass of material of cartilaginous hardness. The soft material was brownish, resembling old blood, and mucoid in consistency, but no definite connection could be detected between the lesion and bone or cartilage. The part was scraped and touched with pure carbolic acid. The material from the swelling was subjected to bacteriological and histological examination. A MacConkey agar plate gave a pure growth of non-lactose-fermenting-colonies, which gave the typical morphological, fermentative and agglutinative reactions of B. typhosus.

The histological appearance of the tissue removed at operation showed it to be composed of fibro-adipose tissue, with numerous areas of inflammatory change. In many instances the inflammatory cellular infiltration was perivascular in distribution. The constituents of the exudate were plasma cells, large mononucleated cells, lymphocytes, and swollen connective tissue cells. There were also a smaller number of polymorphonuclear leucocytes present. It was also noted that more especially where the inflammatory change is to be seen at the junction of the fibrous with the adipose tissue, eosinophile leucocytes were conspicuous among the cellular elements.

The urine and faeces were subsequently examined

on 12 different occasions, bi-weekly examinations being made from 17.5.23 till 23.7.23, with a view to determining whether he was a urinary or intestinal excreter as well. On no occasion, however, was B. typhosus isolated, though occasionally both the urine and faeces contained other non-lactose fermenting bacilli.

The Widal reaction was carried out on 14.6.23 and the serum gave standard agglutination in a dilution of 1:500, 100 standard agglutination units being present per cubic centimetre of patient's blood. On the same day a cutaneous test for hypersensitiveness was performed according to the technique described by McKendrick (1923), slightly more than 0.06 c.c., of the standard emulsions being injected intra-dermally, but with a completely negative result. The patient was, thus, never proved to be a true chronic intestinal typhoid carrier as B. typhosus was found only at the site of the lesion. When last seen by the author towards the end of July 1923, the patient's wound had practically healed but since then Dr. Scoular Buchanan reports that breaking down has occurred in November and December 1923.

Isolation of B. typhosus from the throat in acute typhoid fever and in chronic carriers.

According to Forster and his co-workers in Strasburg sore throat is not an uncommon initial

symptom in typhoid fever, the bacilli being supposed to gain entrance to the circulation through the tonsils. Eggebrecht (1916) found typhoid bacilli present in the mouths of 4.2% of patients in an asylum with endemic typhoid fever. Many of the cases suffered from catarrh of the mouth and the upper part of the respiratory tract. Vincent and Murilet (1916) confirm the previous worker's results and state that the existence of tonsillitis or sore throat is seen in 40% of the cases. They conclude that although the bacillus has been isolated in the tonsillar exudate (Gallois) these lesions should be regarded as a result of a secondary growth. With a view to testing whether acute cases and chronic typhoid carriers harbour the bacilli in the throat, swabs were taken in the usual manner from the tonsils and inoculated directly on to MacConkey agar plates.

Aucte cases.

The examinations were carried out through the various stages of the disease from the beginning of the second week onwards. The faeces were examined by the author, however, on the same day in only one case, but B. typhosus was isolated in the faeces of all on other occasions by the Resident Staff at Belvidere Fever Hospital, Glasgow.

Table of Throat Examination in Acute Cases of Typhoid Fever.

Case.	Result of Examinations of Swabs from Throat.		Remarks.
	Positive.	Negative.	
I.L.	0	5	
C. McD.	0	5	
D. McD.	0	3	
E. McC.	0	1	Faeces positive for B. typhosus on the same day. ~~on one occasion.~~

Chronic Carriers. Examinations of the faeces were made on the same days as the throat examinations.

Table of Throat Examinations on Chronic Enteric Carriers.

Case.	Result of examinations of swabs from Throat.		Faeces Examinations.	
	Positive.	Negative.	Positive.	Negative.
K.O.	0	5	4	1
M.D.	0	5	5	0
H.T.	0	5	2	3
B.E.	0	1	0	1
M.M.	0	1	0	1
J.M.	0	1	1	0

It is notewhrthy that none of the acute cases or chronic carriers complained of any symptoms relating to the throat.

Thus in no case was B. typhosus or B. paratyphosus B isolated from the throat in four acute cases or from six chronic carriers, in spite of the fact that the faeces in many of the cases were positive for the specific organisms on the same day as the throat examinations were made.

****

BIBLIOGRAPHY.

Arnd (1923): Schweiz. Med. Woch. 1923, 53 Jahrb, p. 423.

Bennett (1917): J. Amer. Med. Assn., 1917, 68, p.33.

Blankenhorn, Ecker and King (1923): J. Infect. Dis., 1923, 32, p. 95.

Bureau and Marchand (quoted Arnd):

Drüner (quoted Arnd):

Ebeling (1914): Berlin Klin. Woch., 1914, p. 689.

Eggebrecht (1916): quoted Arnd.

Eschbach (1917): Bull. Soc. méd. Hôp., Par., 1917, p. 1089.

Forster and Kayser (1905): Münch. med. Woch, 1905, p. 4173, and Forster ibid 1908 p.1.

Haibe (1921): Commun. Roy. Soc. Belg. quoted J. A.M.A. 1921, 77, p.136.

Knauer (1921): Centralbl. f. Bakt. I. orin., 1920, LXXXV, p. 237.

Laubenheimer (1911) quoted Arnd:

McKendrick (1923): J. Path. and Bact. 1923, XXVI, p. 535.

Napier and Buchanan (1906): Glasgow Med. Journ. 1906. Dec.

Sieläger and Lüdke (quoted Arnd):

Stephan (1916): Deutsche. Med. Woch., 1916, XLII, p. 1473.

Vincent and Murilet: Fievres typhoide et paratyphoides, Paris, 1916, (also London 1917).

Weil (1917): Bull. de L'Acedemie de Méd., Par., 1917, LXXVII, p.108.

Wohlgemuth (quoted Arnd):

****

CHAPTER XII.

THE TRANSFORMATION OF THE INTESTINAL FLORA WITH SPECIAL REFERENCE TO THE IMPLANTATION OF B. ACIDOPHILUS

While it is a well established fact that diet exercises a profound influence in determining the predominance of one or other type of bacteria in the alimentary canal, the attack on the problem of transforming and simplifying the ordinary mixed intestinal flora through diet in conjunction with the oral administration of bacteria or by the latter procedure alone is still in its early stages. Rettger and Cheplin's (1921) treatise is a storehouse of information on this subject and gives an extensive historical review. The following resume is by no means exhaustive but supplements it by adding some of the opinions of later workers.

With regard to B. bifidus, first described by Tissier (1899-1900), the predominance of this organism

in the faecal flora of breast fed infants has been ascribed to the lactose in human milk (see Sittler 1908). In the change from breast to bottle-feeding B. bifidus is in a large measure replaced by B. acidophilus, as has been claimed by Logan (1914) and others. B. acidophilus was added to the list of known intestinal bacteria by the classical researches of Moro (1900), but as Mereshowsky (1905-1906) and Distaso (1911) showed it would be more descriptive to call them acid resistant rather than acidophile. The term "aciduric" has been suggested by Kendall (1910).

Herter and Kendall (1909) were the first who correlated definitely specific types of intestinal flora with the chemical composition of the ingested food. These facts were, for the most part, established through feeding experiments in monkeys and cats. To provide a high calory carbohydrate diet they fed these animals with cow's milk and lactose, and the faeces in three or four days came to resemble very closely those of the normal human nursling, in which B. bifidus and B. acidophilus are said to be typically dominant. On the other hand, prolonged feeding of a proteid diet containing no carbohydrate (eggs to monkeys and meat to cats), caused the presence of large numbers of proteolytic bacteria in the faeces and entirely suppressed purely fermentative types. With

proteid diet putrefactive products, such as indol, phenols, sulphuretted hydrogen and ammonia were then also strongly in evidence in the faeces.

EFFECT OF FEEDING WITH ACIDURIC AND LACTIC BACTERIA ALONE.

The acclimatization of B. bulgaricus in the intestine after oral administration has been claimed by Metchnikoff and his followers Cohendy (1906 a and b), Belonowsky (1907) and Leva (1908), but this has not been substantiated by either investigators. Luerrsen and Kühn (1908) failed to implant it in the intestine in men by the continued use of Yoghurt. Herter and Kendall (1908), Distaso and Schiller (1914) and Rahe (1915) had similar results, though Distaso in 1913 - 1914 observed in persons with an ileum fistula B. bulgaricus present in the faeces in large numbers twenty-four hours after feeding with milk soured with this organism. Hull and Rettger (1917) repeatedly failed in their attempts to implant B. bulgaricus by feeding patients with large numbers of this organism either alone or in association with the glycobacter peptolyticus, an active starch-digesting organism which was originally isolated by Metchknikoff from the dog's intestine, and which he regarded as an important aid in bringing about the acclimatisation of the Bulgarian bacillus. The

glycobacter peptolyticus is claimed by Wolman (1912) to attack the starches and make them available for digestion. Rettger and Cheplin's (1921) results confirm those of the previous workers. They state that B. bulgaricus, which is not an intestinal organism normal to the rat, is unable under any circumstances to establish itself in the alimentary canal of the albine rat. The experiments of these authors on man by feeding whith B. acidophilus whey-broth and milk show that implantation invariably occurs while this is not the case with B. bulgaricus. Milk infected with B. bulgaricus had the effect of causing a partial transformation of the flora into an acidophilic one, but the flora reverted in a few days in every case after stoppage of feeding to the ordinary mixed type. Claims made by Raubitschek (1912) for the implantation of other organisms in the intestine have not been substantiated by Hull and Rettger (1917), whose attempts to acclimatise foreign strains of B. coli and B. proteus vulgaris in the intestine of the white rat failed in spite of preliminary immunisation at the same time. Their results accord with those of Seifert (1911). Raubitscheck was able to implant B. prodigiosus in rabbits and dogs after preliminary immunisation by a vaccine of the same organism.

Weiss (1904) demonstrated the presence of large numbers of B. acidophilus in the intestine of man after the administration of milk, but Rettger and Cheplin (1921) only found it present in small numbers in man when taking a quart of milk a day. Hull and Rettger (1917) and others have pointed out that B. acidophilus is a normal inhabitant in the intestine of man, but is present only in small numbers when an ordinary mixed diet is being taken. According to Torrey (1915), Hull and Rettger (1917), and Rettger and Cheplin (1921) the implantation of B. acidophilus invariably occurs in man as a result of feeding with cultures in whey-broth and milk, and with lactose and dextrin alone or in conjunction with the broth or milk. Rettger and Cheplin state that by the daily administration of B. acidophilus suspensions to albino rats implantation occurs to the extent of 90 to 99% of the total flora, but that on ceasing to administer the suspensions the character of the flora gradually reverts to the normal or usual mixed type.
These workers recommend the oral administration to human subjects of 300 c.c. of whey-broth culture which has been incubated for forty-eight hours at 37°C., to be taken in one dose usually before the noon luncheon; or still better 500 c.c. to a litre of milk soured by B. acidophilus to be taken in three equal

quantities between meals. The method of preparation of this milk is described in detail later (see p.385). The transformation of the flora reaches its maximum in both these cases in five or six days. They state that the use of pure milk cultures of B. acidophilus has great advantages over all the other methods of effecting the transformation of the intestinal flora into purely an acidophilic one, and emphasize the special significance of the very marked and almost immediate transforming power on the intestinal bacteria which such milk cultures possess. Other advantages they mention are that the milk can be prepared by trained specialists with comparatively little expense and with a high degree of certainty of obtaining a uniform product which is decidedly wholesome and palatable.

Gompertz and Vorhaus (1922) recapitulate the methods of administration and add that rectal implantation may be tried. They state that milk cultures must be taken by mouth in quantities from a pint to a quart daily (600 c.c. to 2400 c.c.), but that of broth cultures much smaller amounts (one or two tablespoonfuls thrice daily i.e. 30 c.c. to 60 c.c.) suffice and produce more rapid implantation.

ACCESSORY FACTORS IN TRANSFORMATION OF THE INTESTINAL FLORA.

Diet is the principal factor involved and, as has

been demonstrated by Herter and Kendall (1909), the chemical character of the ingested food is mainly responsible, a high calory carbohydrate diet being particularly suitable. Hull and Rettger (1914) demonstrated in experiments on white rats, that not all carbohydrates exercise an equal transforming effect. Lactose, whether present in milk or added to a meat diet, alone of the sugars caused the development of a purely fermentative flora dominated by B. acidophilus. Distaso and Schiller (1914) confirmed the latter result and showed that dextrin likewise exercised a marked transforming power. Torrey (1915) reported that a diet containing 250 to 300 grammes of carbohydrate, made up of milk and bread, was sufficient to induce a B. acidophilus type of flora in the faeces of man, and in a later article (1919) that lactose and dextrin are the best carbohydrates for transforming the flora in both dogs and man. Hull and Rettger (1917) in their experiments on white rats and man state that diet is the most important factor in determining the aciduric character of the intestinal flora. Lactose, milk and mixed grains (wheat, oats, etc.) are specific articles of diet which exert such an influence. Lactose, when fed in sufficient quantities (2 - 3 grammes daily) brings about a complete transformation of the flora of white rats within two or three days; milk requires a longer time and

does not bring about a complete change. Milk and lactose together form the most practical and effective diet for man. Bread does not foster the development of the aciduric bacteria, as it contains cooked starch which is digested so quickly that no available sugar remains in the intestine long enough to be attacked and utilised by this group of bacteria. Meat or other high protein diet increases the indol producing bacteria and other organisms of the so-called "putrefactive" type. These workers also found in man that eight pounds of milk sugar taken over eleven days produced a marked aciduric flora, and emphasize the value of laxatives in conjunction, so that by hurrying the lactose through the intestine a sufficient amount reaches the lower parts, where the aciduric organisms multiply most abundantly under suitable conditions. Without the use of the laxative or some other agent which saves the lactose from rapid absorption in the small intestine, in which most marked bacterial changes, putrefactive or otherwise, take place, little encouragement is given to the aciduric types. Their results are analogous to those of Tonney, Caldwell, and Griffen (1916), Morris, Porter and Meyer (1919), Rettger and Cheplin (1921) and Bass (1923). All these investigators found that transformation of the intestinal flora by the ingestion of 300-400 grammes of lactose, in addition to the ordinary diet,

took place, so that B. acidophilus could be isolated from the faeces after four to eight days. Several days after the lactose was stopped the faecal flora reverted to the ordinary mixed type.

Dragstedt, Cannon and Dragstedt (1922) found that a meat diet produced a proteolytic flora and that there was a disappearance of Gram-positive aciduric organisms from closed intestinal loops in dogs, probably due to the absence of utilisable carbohydrate and to the alkaline reaction of the medium. Intestinal stasis or complete obstruction leads to the development of a proteolytic intestinal flora irrespective of the character of the diet.

Bassler and Lutz (1922) state that several teaspoonfuls of lactose per day stimulate the growth of B. acidophilus in the intestinal canal of human beings and this costs less than B. acidophilus milk while it gives the same results by rendering theintestine acid and hindering putrefactive processes. On the other hand Rettger and Cheplin (1921) and Bass (1923) both agree that the quantity of lactose necessary to cause complete aciduric transformation of the intestinal flora is too excessive for general use and recommend instead milk soured by B. acidophilus in conjunction with the ordinary diet or with a small quantity of lactose added to it.

Possible reasons for failure to effect transformation.

The use of an insufficient quantity of carbohydrate, milk or broth infected by B. acidophilus, may be the cause of failure to transform the intestinal flora into one containing a preponderance of these organisms. The nature of the carbohydrate is also of importance as of these only lactose and dextrin are capable of causing transformation . Bass (1923) points out that striking therapeutic results have been claimed from the administration of broth cultures of B. acidophilus in teaspoonful doses, only a small fraction of the amount of culture that others have found necessary to change noticeably the intestinal flora. 300 grammes of lactose; 300 c.c. whey broth infected with B. acidophilus; 500 - 1000c.c. milk infected with B. acidophilus are recognised by the majority of workers (Rettger and Cheplin (1921), Bass (1923) etc.), as the quantities necessary to bring about transformation of the intestinal flora to one in which B. acidophilus is the predominating organism. The milk or broth should be freshly prepared daily as the number of viable organisms diminish rapidly on keeping a few days. Keeping B. acidophilus whole milk at 20°C. is satisfactory for three or four days, after which the numbers of viable organisms decrease rather rapidly and the acidity

increases to the point of impalatability.

The preparation of the milk must also be carried out with great care. The effects of badly prepared milk are discussed later (see p. 390).

Kopeloff and Cheney (1922) think that the failure to obtain a greater ascendancy of B. acidophilus in the intestinal flora may be due to the fact that the pabulum has been whole milk and not skimmed-milk, as employed by Rettger and Cheplin (1921). They found that the flora becomes changed on treatment with B. acidophilus whole milk andlactose, but the relative percentage of Gram positive rods rarely exceeds 70 per cent.

Since various commercial preparations of B. acidophilus have been placed on the market in tablet, capsule and liquid form, it is of interest to report the researches of Bass (1923) on these preparations. Of the tablets, none examined had as many as 1000 viable bacteria of any kind per tablet, thus 1000 million tablets would be necessary to contain as many bacilli as are contained in 1000 c.c. or the usual daily dose of milk infected by B. acidophilus, found by most observers as necessary to transform the flora. Bacteria were more numerous in the commercial liquids examined but here again 7 or 8 gallons would be necessary to get as many as in 1000 c.c. of the

acidophilus milk culture. Bass's finding is that only fresh cultures produced according to the proper bacteriologic methods should be used.

Hydrogen-ion concentration.

This factor has already been considered to some extent (p. 206).

Rettger and Cheplin (1921) have concluded that the process of elimination of an ordinary mixed flora and the establishment of B. acidophilus apparently does not depend on any changes of the H - ion concentration within the intestine but depends on diet as the acidity of the faeces where the flora had been changed to one consisting of ninety per cent B. acidophilus was not increased. On the other hand Cannon and McNease (1923) declare that these authors assume that the acidity of the faeces and of the higher reaches of the large intestine are alike, a result with which their findings disagree. They found that the simplification of the intestinal flora varies directly with the hydrogen - ion concentration Ph 7.0 being characteristic of a gas producing proteolytic type, whereas an increasing acidity is characterized by a diminution of proteolytic types and their replacement by aciduric types dominated mainly by B. acidophilus. The determination of actual acidity of faeces is of slight

value in interpreting the intestinal contents higher up. Kendall's (1921) interpretation of the factors involved in the transformation of the intestinal flora are that an available carbohydrate is acted on by both proteolytic and fermentative types of organisms and as a result an acidity develops which is distinctly unfavourable for the former group. Robinson (1922) believes the physiological factor is the predominating one in influencing faecal reaction, no result being noticeable as a result of the introduction of acidophilic bacteria into the intestine, the intestine apparently exerting a regulating influence, which prevents the development of acidity by micro-organisms. Kopeloff and Beermann (1923) conclude that the establishment of B. acidophilus in theintestine is not a physical phenomenon and not strictly a chemical phenomenon but appears to be essentially a bacteriological one, since in the former case patients receiving pasteurized B. acidophilus milk were not relieved of constipation, nor were those receiving sterile milk, while those taking milk containing living B. acidophilus were benefited.

Effect of Purgatives and absorption.

Hull and Rettger (1917) were the first to emphasize the importance of employing a laxative in association with the taking of lactose in aiding the transformation of the intestinal flora into an

aciduric one. The laxative acts by hurrying the lactose through the intestine so that a sufficient amount reaches the lower parts, where the aciduric organisms multiply.

Similarly Robinson (1922) has pointed out that laxatives raise the acidity of the colon by hastening the passage of the acid contents of the small intestine and produce an acid stool such as is met with in diarrhoea. McClindon, Shedlov and Karpman (1918) showed that the progressive decrease in acidity of the contents of the bowel from above downwards was probably due to the progressively greater absorption of CO_2, thus the effect of laxatives would be to tend to prevent absorption or neutralisation. Rettger and Cheplin (1921) state that the diarrhoeal stool is acid provided the stimulus which produces increased peristalsis does not produce increased secretion. Lactose causes the production of an almost pure acidophilic flora and produces acidity in vitro, but in vivo this acidity is controlled by the regulating factors of the colon.

Effect on Nature of Stool

The character of the faeces after the transformation of the intestinal flora into an

acidophilic one is of interest. Torrey (1918) noted that hard formed stools were the exception in patients treated with B. acidophilus milk or lactose, the stools being mainly soft and moist; Gompertz and Vorhaus (1922) found the stools became colourless, soft and partially formed and of a light colour.

Application in treatment of various disease conditions in man.

Hirschler (1886), Winternitz (1892) and Schmitz (1894) demonstrated that lactose could inhibit intestinal putrefaction when fed by mouth. Poehl (1887) noted that sour milk had a similar property. This has subsequently been confirmed by many other workers. Tissier and Martelly (1902) considered that the chief agent in effecting inhibition of putrefying bacteria was probably the lactic acid produced by the lactic acid bacilli.

Effect on Treatment of Typhoid Fever and of Typhoid Carriers.

Liefmann (1909) suggested milk soured by Yoghurt as a means of eliminating typhoid bacilli from the faeces of carriers and claimed to have obtained a cure by that means for the condition. Fawcus (1909) Thomson and Ledingham (1910), Cummins, Fawcus and Kennedy (1910) and Nichols (1919) employed Yoghurt

or B. bulgaricus milk without any beneficial results. Zweig (1910) tested Lactobacillin in two carriers and supports Liefmann's view. His results are not reliable as only six examinations were made during the whole period.

Mayer (1910) claimed by the continued use of sweet milk to have reduced the number of typhoid organisms in the faeces in typhoid carriers. Litchfield (1914) and Barker (1914) fed typhoid patients with fermented milk reinforced with lactose with excellent results, and Torrey (1915) reported that typhoid bacilli were isolated less frequently from the stools of typhoid patients on a high calory carbohydrate diet than from those of another series in which the feeding was less liberal, while Haibe (1921) recommended a strict dietetic regimen also for chronic carriers with milk as a basis.

Gomportz and Vorhaus (1922) make the suggestion that B. acidophilus may be given as a prophylactic against ascending infection of the gall-bladder, and ducts by B. coli in the case of typhoid fever etc., as they found that B. acidophilus inhibits and replaces B. coli and lessens the risk of autointoxication.

Therapeutic value in relieving chronic constipation and diarrhoea.

B. acidophilus broth and milk has been claimed by numerous authors to be of great therapeutic value

in relieving chronic constipation and diarrhoea; Rettger and Cheplin (1921) report its value in two of their cases; Gompertz and Vorhaus (1922) found it relieved both these conditions but they had fifteen per cent. of failures; Bass (1923) and Kopeloff and Cheney (1922) employed it with success; Kopeloff (1923) found that relief from chronic constipation has persisted for six months after the ingestion of B. acidophilus has been discontinued, stating that viable B. acidophilus in appreciable numbers have been recovered from the faeces of patients months after stoppage of the ingestion of B. acidophilus milk. Bassler and Lutz (1922) emphasize the very limited value of B. acidophilus milk in intestinal disorders. Cheplin, Post and Wiseman (1922) from investigations into the therapeutic effects of B. acidophilus milk in toxic intestinal conditions, show its value when implanted in the alimentary canal as the dominant organism by the oral administration of the milk culture in proper amounts. The bacillus, while it does not produce gases or toxins, suppresses B. coli and inhibits such toxigenic microbes as the enterococci, B. welchii, proteus and putrificus. Experimental evidence in nine cases established its beneficial influence upon constipation, diarrhoea and mucous colitis, toxic symptoms being relieved with the

regulation of the bowels and disappearance of mucus. The acidophilus milk was prepared by the method of Rettger and Cheplin, living twenty-four to thirty -six hour cultures being administered daily in addition to the ordinary diet, one litre of milk culture with fifty to one hundred grammes of lactose being ingested in three equal doses two to three hours after meals. It is essential that a minimum averaging fifty billions of viable organisms should be given daily for at least six weeks, and it must be borne in mind that the ingestion of relatively few bacilli will not lead to implantation and bodily improvement, and administration in tablet form is useless. While it is not claimed as suitable for every gastro-intestinal disturbance, careful acidophilization of the enteric tract by means of B. acidophilus milk is recommended as a valuable means of therapy in toxic intestinal conditions.

An editorial review on B. acidophilus and intestinal putrefaction in the Journal of the American Medical Association (1922,Vol. 1. p. 186) states that with the disappearance of putrefaction as a result of implanting B. acidophilus the somewhat hypothetic toxic products might also be reasonably expected to be lacking but this is not so, as indican still remains high, and also phenol excretion in the

urine is increased even when liberal amounts of milk, lactose and cultures of B. acidophilus are added to the diet, although the concentration of these micro-organisms in the faeces is high. Hence it may be that the large amount of tryptophane yielding protein in milk may serve as a possible indican precursor. These facts suggest that the favourable clinical results obtained are not primarily dependent on decreased production of the antecedents of indican.

Original Observations.

Preparation of Bacillus Acidophilus Milk for Human Consumption.

Much time and effort have been given in recent years to the production of sour milk and sour milk products. Milk soured with B. bulgaricus and lactic acid bacilli powders or tablets have been widely sold throughout the world more especially in the United States of America and France. Apart from the benefits alleged to result from the transformation of the intestinal flora, these preparations appear to possess undoubted therapeutic value. Bass (1923), however, warns against the use of commercial tablets, powders and liquids and quotes striking figures of the huge numbers and quantities necessary to give the same effect as freshly prepared sour milks.

In any case soured milk constitutes a valuable food and serves as a substitute for ordinary milk, which may not be tolerated by certain persons.

B. acidophilus milk resembles milk soured by B. bulgaricus. Coagulation of the casein takes place in both due to acid production and no gas is produced. Both are acid to taste and quite palatable. However, acidophilus milk never attains as high a degree of acidity as does bulgaricus milk even in old cultures; in fact there is little or no danger of its becoming too sour if the time of incubation is within reasonable range.

The following method was employed in the preparation of B. acidophilus milk during the twelve months' administration to the typhoid carriers under the author's care and is almost identical to that described by Rettger and Cheplin (1921). The details of preparation were kindly supplied by Dr. G. Paterson, Belvidere Fever Hospital, Glasgow, who had the clinical charge of the typhoid bacillus excreters.

In summer the same day's milk after the cream has been roughly removed is used, while in winter the previous day's milk is used, to enable it to be partially skimmed. The milk is sterilised by autoclaving at a temperature of 125°C. for forty-five minutes. (The best results are got by this pressure and duration

of heating as regards subsequent palatability and appearance of the soured milk, which is of a creamy but never sticky or stringy consistency. The sterilised milk is thin and has a brownish tint, resembling rich cream, but is of no greater consistency than ordinary milk. When the milk is sterilised for longer than forty-five minutes at twelve pounds pressure it becomes burnt and unpalatable in flavour and in the course of souring the casein separates out into a clot of rubber-like consistence and whey forms when it is taken out of the incubator. If the pressure is lower the milk appears quite normal on coming out of the autoclave but after inoculation with B. acidophilus and incubation clot and whey are formed. Thus when the pressure is too great or too little the resulting milk is poor, but if the pressure is lower and the milk is autoclaved for a longer time the final result may be quite good. The milk is next allowed to cool (either rapidly or simply by allowing to stand at room temperature), and is then inoculated with eight to ten cubic centimetres of the previous day's B. acidophilus milk to the pint and incubated at 37° C. for eighteen to twenty-four hours. It is important that the inoculum should be a milk culture not more than seventy-two hours old. The strain used should have been repeatedly subcultured; recently isolated

strains of B. acidophilus are slow and ineffective, thus it is necessary to employ strains which have been grown in milk at 37°C. for at least two or three weeks, preferably after daily transplantation during that period. The amount of the inoculum for each transplantation should be large and transfers should be made with sterile glass pipettes. These pipettes consist of sections of glass tubing drawn out at one end, but the opening must be large enough to admit coagulated milk. Platinum loops are useless for making transfers.

As soon as the milk has coagulated at 37°C. it is placed in the refrigerator and may be used for several days. The acidophilus milk has now a semi-pultaceous consistency and is as palatable as ordinary sour milk. There are no gross clots nor does whey separate and it retains its brownish tint andhas a definite aroma and flavour which add materially to its palatability and which are more or less absent in the case of B. bulgaricus milk. The strain mainly used in the investigation was one of Rettger's obtained from the National Standard Type Collection. A strain isolated from a dog's intestine by the author was also employed for a period. There was no notable difference in the products obtained with these two strains.

Smears were made daily from the milk and stained by Gram's method and in addition, subcultures in whey

Microphotographs of B. acidophilus (Moro) in Milk stained by Gram's Method.

Fig.10 x 1000

2 days old.

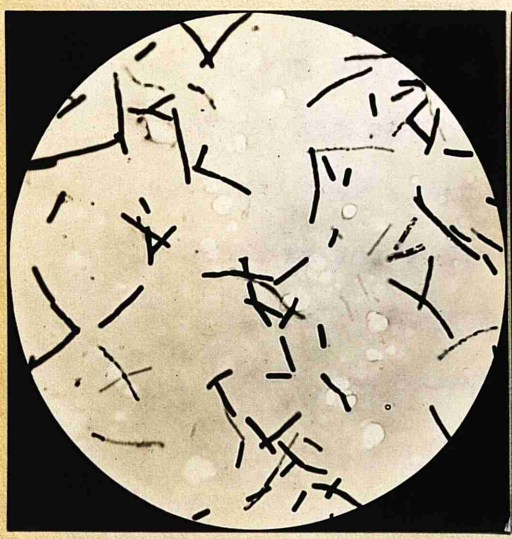
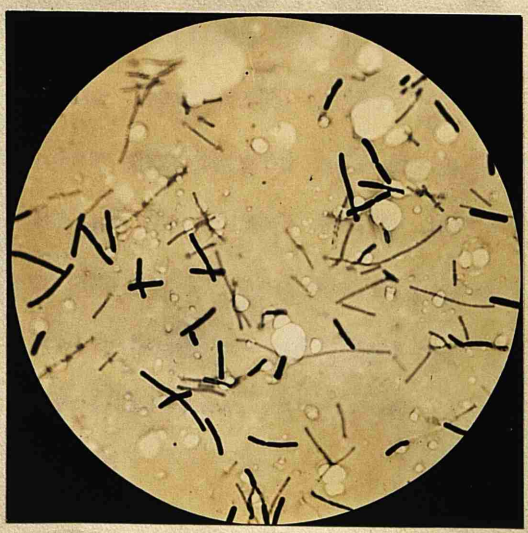


Fig. 11 x 1000

2 days old.

Fig.12 x 1000

5 days old.



agar, whey broth and liver glucose agar were made. The smear shows mainly long stout or fine, Gram positive rods of pleomorphous character, characteristically curled towards their ends, but in addition Gram negative forms with or without Gram-positive beading and true branching forms are present. (Figs. 10 & 11 x 1000)

When the conditions are suitable growth is abundant and the organism remains alive and continues to multiply for a considerable time. When the conditions of sterilisation are unfavourable the number of long Gram-positive forms becomes markedly lessened in the first day of storage in the ice-chest or at room temperature, and diminish rapidly in the next two days. The branching forms disappear and many more long Gram negative forms are seen and the total number of organisms present is greatly diminished (Fig. 12x1000). Thus there appears to be parallelism between the physical condition of soured milk and activity of B. acidophilus growth. However, on re-transplantation to suitable medium the organisms return once more to the normal state. Some forms of B. acidophilus occur as short Gram-positive bacilli with a tendency to chain formation, especially on isolation from rats' or dogs' faeces (Figs. 13 and 14 x 1000).

Microphotographs of B. Acidophilus (Moro).

Types I and II.

Fig.13 x 1000.

Fig.14 x 1000.

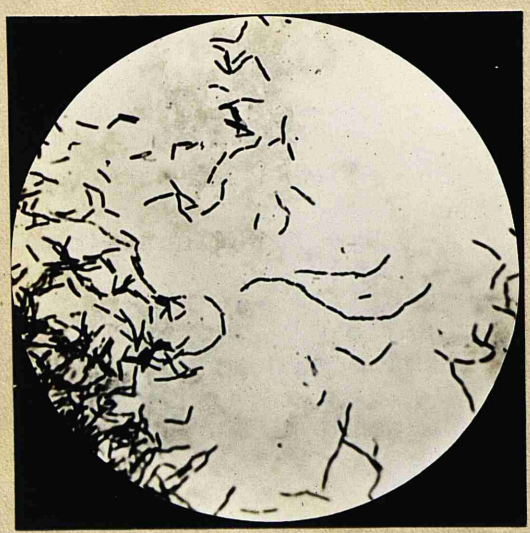
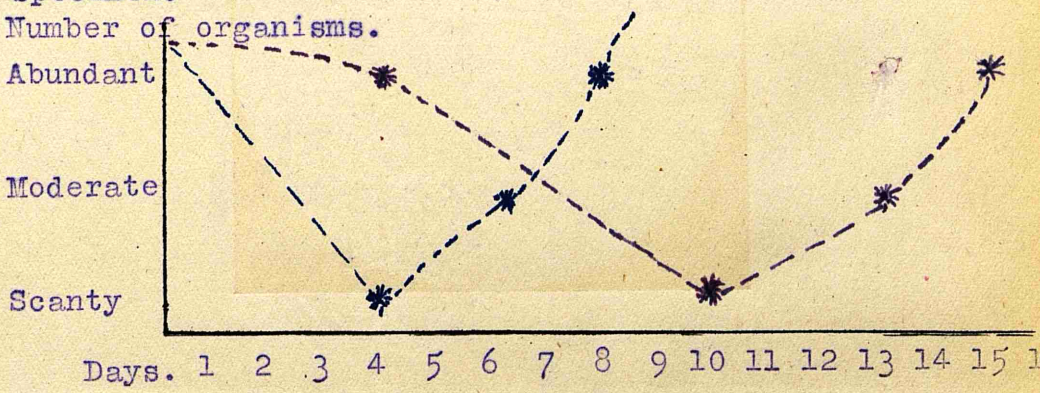


Chart to show the effect of adverse and favourable conditions of preparation of milk on growth and numbers of B. acidophilus present in milk cultures as shown by Gram-stained smears and whey agar cultures.

The results show the relative abundance of organisms when films are made under constant conditions as regards amount used to make smear and area of specimen.



Good Milk ---(red)

Poor Milk -.-(blue)

* Transplantation into fresh specimen of autoclaved milk with incubation at 37°C for 24 hours, and then allowed to stand at room temperature for twenty-four hours.

A similar result is got by simply allowing a suitably autoclaved specimen of milk after rich inoculation with B. acidophilus to stand for many days at room temperature. Here again approximately three transplantations are necessary to restore the milk to its normal condition.

Experiments were carried out to find which

temperature of incubation gave the best coagulation and the results obtained were similar to those of Rettger and Cheplin (1921) in that 37°C. is best, though satisfactory results are got with a temperature of 30°C. With lower temperatures than this, namely room temperature (20°C) coagulation is slow, there being little change visible in the appearance of the milk in the first three or four days.

Occasionally in summer the milk was slightly sour to start with i.e. before autoclaving, and after sterilisation appeared normal, but after inoculation with B. acidophilus and incubation for twenty-four hours clot and whey separated to some extent. In two days the growth of the organisms had definitely fallen off, but not to the same extent and much more slowly than with milk prepared under adverse conditions of temperature and pressure, where clot and whey formation are present and the numbers of organisms are scanty. These organisms were likewise capable of growing abundantly and producing specimens of soured milk, whose physical characters were satisfactory on a few transplantations into fresh sterile milk.

The quantity given to each "Carrier" was approximately two and a half pints per day, a third of this amount being drunk thrice daily between meals.

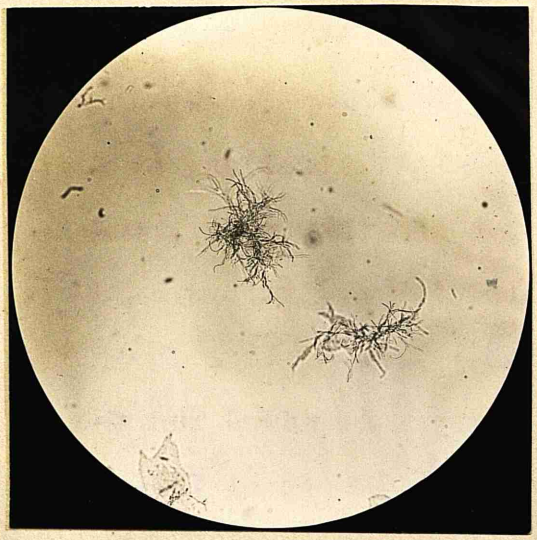
Methods employed in the routine examination of the faeces.

The carriers faeces were emulsified for purposes of preservation in thirty per cent. glycerine in saline (Teague and Clurman, 1916) or in saline alone, in the proportion of one part of faeces to approximately four of the diluent. The glycerine did not appear to have any harmful effect on the isolation and growth of B. acidophilus from the faeces.

2% lactose agar and 4% lactose agar proved quite efficient in the isolation of the aciduric organisms from dogs' and rats' faeces, but did not give quite so good results in the case of human faeces, where whey agar or whey broth gave the best results. The reaction of the lactose agar employed should be acid, Broth which has been rendered acid by 1 per cent. acetic acid is claimed by Cruickshank and Berry (1924) to give excellent results, owing to only the Gram positive organisms multiplying in this medium. Whey agar was prepared according to Rettger and Chep - lin's method (difficulties may be at first encountered however, in obtaining clear whey). The skimmed milk is heated to 80°C or 90°C., and five cubic centi- metres removed to a test tube; while the test sample is still hot 10 per cent. solution of hydrochloric acid is added drop by drop until complete coagulation

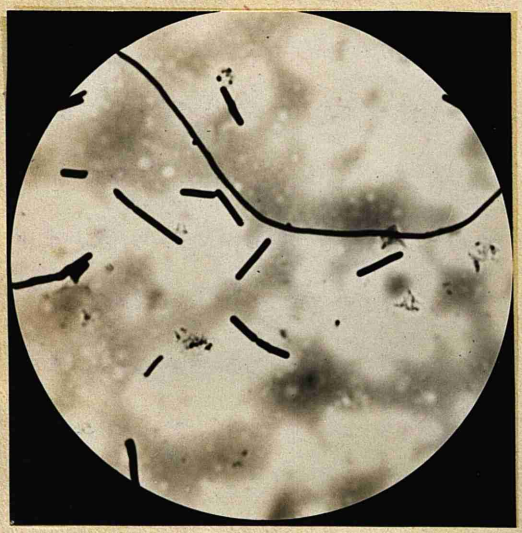
Microphotograph of B. Acidophilus colony from Whey Agar.

Fig.15 x 60.



Microphotograph of B. bulgaricus in milk.

Fig.16 x 1000.



takes place. This test is pepeated two or three times. The calculated amount of acid required to coagulate the milk in bulk is then added and thoroughly mixed with the milk, the temperature of which is still 80° to 90°, and the casein allowed to settle. This is followed by filtration, at first through absorbent cotton and then paper, and neutralisation of the filtrate with sodium hydroxide to yield a Ph value of 6.8 - 7.0. Before further filtering, 0.5 per cent peptone is added and the medium is autoclaved for fifteen minutes at fifteen pounds extra pressure. The precipitate is separated by filtering through paper, and the clear filtrate is employed for preparing whey broth or whey agar. To convert into whey agar, 1.2 per cent of standard shredded or powdered agar is added to the whey broth. A comparison was made experimentally between the three media, viz. 2 and 4% lactose agar and whey agar, using strains from milk cultures and from the faeces of carriers fed with them and it was found that the B. acidophilus colonies on whey agar were more abundant, larger and presented a more typical appearance. On the latter medium B. acidophilus colonies appear in plates as fine feathery branching colonies mainly in the deeper parts of its substance (Fig. 15x 60).

One or two platinum loopfuls or more of the faeces

emulsion are seeded into the whey agar tubes, made liquid and cooled to 40°C, mixed and poured into a Petri capsule and incubated for twenty-four or forty-eight hours. Likely colonies are picked off and inoculated into whey broth where they give a clear supernanant fluid after incubation, and appear as whitish threads at the bottom of the tube. Transplantation back into whey agar or milk is carried out until a pure culture is obtained.

This method yielded pure growths of B. acidophilus with comparative ease in the case of dogs and white rats, but at times isolation proved exceedingly difficult or even impossible in the case of human faeces where the organisms, although abundant in stained films, appear to be for the most part dead.

Later on liver-glucose-agar, as described by Torrey (1917), was employed and found to give exceedingly satisfactory results in human cases, the B. acidophilus colonies appearing as flat dingy colonies with serrated edges, but the results were no better than with whey agar. Torrey found during the course of his study of Gram-positive aciduric bacilli of the intestinal tract that solid media prepared with an infusion of ox liver instead of muscle tissue as a base was particularly favourable. In brief, the preparation of this medium is as follows: Cut 500

grammes of ox-liver in small pieces and add 100 c.c. of distilled water, boil for two hours in a double boiler, filter through flannel and cotton, and to the filtrate add 10 grammes of peptone and 20 grammes of agar. Heat the flask in the Arnold's steriliser for one hour, adjust to the reaction desired and clear with eggs if necessary; to the clear filtrate add 10 grammes of glucose and 1 gramme of di-potassium phosphate.

No blood is added to the medium on which B. acidophilus will develop with a range of reaction from neutral to phenophthaleih to +5 acid. A reaction of +3 acid appears to be most favourable, but for faecal work +4 acid is used in order to inhibit the development of streptococci and most strains of B. coli. The type of colony of B. acidophilus most frequently encountered forms a small fluffy deep colony resembling a fleck of cotton (~~Fig. 15~~). Certain other strains form globular deep colonies with a serrated border. Acid-tolerating colon bacilli give rise to lenticular deep colonies or sharp pointed triangular ones. These were seen in specimens of faeces from "Carriers" fed with milk soured by B. acidophilus. Anaerobic plates are not necessary, as with this medium the development of the B. acidophilus colony is quite satisfactory under aerobic conditions.

In cultivation of the bacteria of the type of B. acidophilus incubation for two or three days is desirable, though this is not always necessary.

The B. acidophilus colonies are easily recognised with the aid of the lower power lens of the microscope and their relative number as compared with all other colonies can be determined.

The two types of colony of B. acidophilus designated by Rettger and Horton (1914) as x and y were seen quite frequently. The former have a decidedly fuzzy appearance and are indistinguishable from the colonies of B. bulgaricus (cf Fig. 15), while the y type is small and round or spindle-shaped and is only partly fringed and at times appears almost perfectly smooth. The latter colonies may be designated as crab-like. The two appear to be interchangeable.

For B. bifidus, Torrey's medium is titrated to +1 to phenolphthalein and to each 10 c.c. about 1 c.c. of sterile defibrinated rabbit blood is added, just before the plate is poured. For its optimum development B. bifidus requires a certain degree of anaerobiosis, but it is not an obligate anaerobe, and will grow fairly well on this medium.

B. bifidus colonies are raised, more or less globular, and buff to reddish-brown in colour. They can readily be distinguished from the flat dingy

Microphotographs of Smears from faeces of Carriers fed on B. acidophilus milk.

Fig.17 x 1000.

Fig. 18 x 1000.

colonies with serrated edges of B. acidophilus, and a quantitative estimation made of the viable organisms.

In one per cent. maltose broth, after incubation at 37°C. for forty-eight hours, B. acidophilus produces acid, but B. bulgaricus does not. This was personally confirmed in the case of the strain obtained from dog faeces. In certain cases, as has been seen, neither microscopic nor cultural appearances enable B. acidophilus and B. bulgaricus (Fig. 16 x 1000) to be distinguished with certainty, According to Rahe's (1918) scheme of classification of the aciduric bacilli all strains of B. acidophilus ferment maltose while none of the Bulgarician strains do so. B. acidophilus is said to be differentiated from B. acidophil-aerogenes, described by Torrey and Rahe (1915), by the fact that all varieties of the latter organism form gas in maltose.

Veillon tubes were not employed by the author though they are recommended by Rettger and Cheplin (1921).

Direct microscopical examination of faecal suspensions were made as well as cultural examination, films being stained by Gram's method using carbol-fuchsin (1 - 10) as the counterstain. Such preparations are of considerable value in that they show the relative predominance of different types of bacteria, and

emphasize in particular the conspicuous, long B. acidophilus-like rods when present in any numbers. (Figs. 17 and 18 x 1000). This method has, of course, the drawback that is does not give a true picture of the viable organisms present in the intestinal tract, as dead organisms may form a considerable proportion of those seen in films, Nevertheless, in spite of the recognised limitations of this method, it has proved to be indispensable in the present investigations. Stained faecal films showed occasionally, the presence of long B. acidophilus-like organisms although cultures were negative. The question arises as to whether replacement of the coliform bacilli in the intestinal flora by B. acidophilus occurs or whether feeding with milk soured by this organism only causes the addition of Gram-positive bacilli to the flora. As far as one can see a true replacement occurs (see p. 398).

A note was also kept of the reaction of the faeces to litmus after the method described by Goéffon.

Experimental feeding with B. acidophilus milk to human Typhoid bacillus carriers: the effect on the intestinal flora.

With daily quantities of two and a half pints of freshly prepared B. acidophilus milk in addition to a mixed diet containing approximately 250 to 300

grammes of carbohydrate, the intestinal flora of three typhoid and one paratyphoid carriers was so transformed within four days that the predominant organism was of the nature of B. acidophilus, long Gram-positive rods preponderating in Gram-stained films from the faeces (cf. Figs. 17 and 18), and typical feathery B. acidophilus colonies being always isolated in the manner already described. On MacConkey agar plates the number of colonies of B. coli were markedly reduced. This appearance persisted so long as feeding with B. acidophilus milk was continued. After discontinuance of milk-feeding the ordinary mixed flora re-appeared in four or five days, while after the first forty-eight hours no growth of B. acidophilus was obtained in whey agar, or whey broth. Even after feeding with B. acidophilus had been discontinued for 5 days, however there were still present small numbers of B. acidophilus-like bacteria in the Gram-stained films (see Tables XX and XXI. p. 400 - 1.)

Commercial lactose was given in the dry state in quantities rising from 4 drachms to 6 ounces per day, in three equal doses with meals, along with the amounts of B. acidophilus milk above mentioned, to two of the carriers, and was continued for periods up to about a month.

After feeding with B. acidophilus milk was

TABLE XX.

TO ILLUSTRATE THE TIME OF DISAPPEARANCE AND REAPPEARANCE OF B. ACIDOPHILUS AS THE PRE* DOMINATING ORGANISM IN HUMAN FAECES ON FEEDING WITH MILK SOURED BY B. ACIDOPHILUS.

Case 1. (M.D.). Female, aged 39 years.

Date.	Quantity of Milk.	Character of Faeces	Direct Gram Film of faeces.	Cultures in 4% Lactose Whey Agar.	Agar.
3/8/22.	2½ pints daily	Semi-formed	80% B. acidophilus.	+	+
7/8/22.	"	"	75% B. acidophilus.	+	+
8/8/22.	Milk stopped	"	75% B. acidophilus (approximately)	+	+
9/8/22.	"	"	25-30% B. acidoph.	-	-
10/8/22.	"	"	10% B. acidophilus (approximately)	-	-
11/8/22)	"	"	Only a few B. acid-	-	-
14/8/23)	"	"	ophilus-like bacilli similar to what one w would expect in ordinary mixed flora.		

Date.	Quantity of Milk.	Character of Faeces.	Direct Gram Film of Faeces.	Cultures in 4% Lactose Whey Agar.	Agar.
15/8/22.	Milk restarted.	Semi-formed.			
17/8/22.	"	"	60% B. acidophilus.	+	+
18/8/22.	"	"	70-80% "	+	+
19/8/22.	"	"	80% "	Not done.	Not done
20/8/22.	"	"	80-90% "	Not done.	Not done
24/8/22.	"	"	80% "		
28/8/22.	Milk stopped.	Semi-formed.	80-90% B. acidoph.	+	+
29/8/22.	"	"	40-50% "	-	+
30/8/22.	"	"	20-30% "	-	-
31/8/22.	"	"	10% approximately	-	-
1/9/22.	"	"	10% approximately	Not done.	Not done
4/9/22.	"	"	Less than 5% B. acidophilus & like ordinary mixed flora.	Not done.	Not done

TABLE XXI.

Repetition of Experiment as in TableXX with Case 11, K.O. Female aged 29 years.

Date.	Quantity of Milk given.	Gram Films.	Whey Agar Culture.
26/6/22.	Two and a half pints daily.	70% B. acidophilus	+
28/6/22.	"	70% B. acidophilus	+
	Stopped on29/6/22.		
3/7/22.	None.	Less than 5% B. acidophilus.	-
6/7/22.	None.	Less than 5% B. acidophilus.	-
24/7/22.	None.	Less than 5% B. acidophilus.	Not done.
26/7/22.	Two and a Half pints daily.	Less than 5% B. acidophilus.	-
27/7/22.	"	30-40% B. acidophilus.	-
31/7/22.	"	60-70% B. acidophilus.	+
3/8/22.	"	80-90% B. acidophilus.	+
7/8/22.	"	75% B. acidophilus	+

discontinued lactose was still given in order to determine whether B. acidophilus would in consequence persist in large numbers in the intestine.

Although the quantities of lactose are less than those recommended by Hull and Rettger (1917), who got a partial failure with one of their human carriers, quite a satisfactory persistence of B. acidophilus occurred in one case (K.O.): with the other (M.D.) no marked effect resulted, (see Tables XXII&XXIII), although a certain effect due to the lactose was distinctly evident (compare Tables XX and XXIII). These results were repeated on several occasions.

Lactose feeding alone without the administration of milk soured by B. acidophilus successfully transformed the intestinal flora in one carrier (K.O.), but not to the same extent as was obtained with the milk-feeding. Thus with lactose alone approximately 60 per cent. of the organisms present in stained films were of B. acidophilus type, which was also isolated in whey agar cultures from the faeces. In a second carrier (M.D.) lactose feeding was not successful in a marked degree. The use of a laxative or purgative in conjunction with lactose feeding appeared to aid the transformation somewhat, but at no time in either case was the preponderance of aciduric organisms so complete as during the administration of

TABLE XXIJ.

TO SHOW THE EFFECT OF LACTOSE ADMINISTRATION ON THE INTESTINAL FLORA IN CONJUNCTION WITH THE GIVING OF B. ACIDOPHILUS MILK.

Case II (K.O.). Female aged 29 years.

Date.	Quantity of Milk.	Quantity of Lactose.	Gram Film.	Whey Agar.	Remarks.
26/12/22	2½ pints daily	4 ozs. per day.	90% B. acidophilus	+ (Ratio 1:2 other colonies.).	
8/1/23.	"	"	80-90% B. "	+	
9/1/23.	None.	"	60% B. acidoph.	+	It will be noted that
10/1/23.	"	"	30% B. acidoph.	-	the numbers
4/2/23.	"	"	20-30% B. "	-	of B. acid-
12/2/23.	"	"	30% B. acidoph.	Not done.	ophilus fall
13/2/23.	"	6 ozs. per day	30% B. acidoph.	Not done.	from the or-
15/2/23.	"	"	30% B. "	Not done.	iginal high
16/2/23.	"	"	50% "	+	level to a
17/2/23.	"	"	60% "	+	relatively
18/2/23.	"	"	60% "	+	low number
19/2/23. to 26/2/23.	"	"	60% "	+	and rise again on the increased dose of lactose being given.

T A B L E XXIII

REPETITION OF EXPERIMENT AS IN TABLE XXII, BUT WITH CASE I (M.D.), FEMALE AGED 3½ years.

Date.	Milk.	Lactose.	Gram Film.	Whey Agar.	Remarks.
4/12/22.	2½ pints daily	4 ozs. per day	90% B. acidophilus	+	
5/12/22.	Milk stopped.	"	60% B. acidophilus	+	
6/12/22.	None.	6 ozs. per day	20-30% B. acidoph.	-	
7/12/22.	"	"	10% B. acidophilus	-	
8/12/22.	"	"	10% B. acidophilus	-	
9/12/22.	"	"	5-10% B. acidoph.	-	
11/12/22.	"	"	less than 5% B. acidophilus.	Not done.	
12/12/22.	"	"	" " "	"	
13/12/22.	"	"	" " "	"	
14/12/22.	"	"	" " "	"	
15/12/22.	"	"	5%-10% B. acidoph.	"	

Date.	Milk.	Lactose.	Gram Films.	Whey Agar.	Remarks
11/1/23.	2½ pints daily.	6 ozs. per day.	90% B. acidophilus.	+	
15/1/23.	"	"	80% B. acidophilus.	+	
16/1/23.	Milk stopped	"	40-50% B. acidoph.	-	
17/1/23.	None.	"	20-30% B. acidoph.	-	
18/1/23.	"	"	10-20% B. acidoph.	-	
19/1/23.	"	"	5-10% B. acidoph.	-	
22/1/23.	"	"	Less than 5% B. acid.	-	
25/1/23.	"	"	30% B. acidophilus.	-	
29/1/23.	"	"	20-30% B. acidoph.	Not done	Purgative given (Calomel gr. IV. previous evening, 24/1/23 - 1/2/23. inclusive)
1/2/23.	"	None.	20-30% B. acidoph.	"	
5/2/23.	"	"	5-10% B. acidoph.	"	

of B. acidophilus milk (see Table XXIII).

The effect of high calory carbohydrate diet on transformation of the intestinal flora.

Alteration of diet to correspond with that recommended by Torrey (1915) was tried alone without feeding with milk soured by B. acidophilus; this diet contained 250 to 300 grammes of carbohydrate obtained by the taking of milk, potatoes, arrowroot and bread, but, without the feeding of the milk soured by B. acidophilus, no very marked alteration of the flora to an aciduric one, was noted, though some increase in the numbers of acidophilic organisms took place.

Attempt to implant B. bulgaricus.

The author received a culture of B. bulgaricus from the Agricultural College, Glasgow, which had been used in cheese-making but for some unaccountable reason had fallen off in its fermentative properties. The organism appeared to be quite typical otherwise, so it was proposed to pass it through a human being and re-isolate and see if any increase in its fermentative powers had resulted. Half a litre of sterile milk, infected with the organism and prepared in the usual way was given to three different persons, but in no case was it possible to isolate the organisms from the faeces in spite of calomel being given the previous evening. Again, another culture of B. bulgaricus obtained from the National Collection of

Type Cultures was employed similarly without success. This result is similar to that obtained by Rettger and Cheplin (1921) and many other workers.

Feeding with special types of B. coli.

Enteric carriers were fed with milk infected with B. coli of the lactis aerogenes variety and by that means the flora was temporarily transformed into one almost entirely dominated by this type of organism. The B. lactis aerogenes strain employed in these experiments was isolated from the faeces of the B. paratyphosus B. carrier (M.D.). It proved to be a non-motile, moderately short, Gram-negative bacillus, in which no capsule was demonstrated. The bacillus lactis aerogenes was originally described by Escherich, in connection with his work on the bacteriology of the intestine in children as an organism differing from the ordinary milk-souring bacteria by its producing gas from milk in the absence of air. Although it is a free gas-producer, this property is not specific for it, and within recent years it has attracted attention chiefly from its being apparently closely allied to B. pneumoniae of Friedlander, Like the latter, this organism is stated when injected into animals to appear in a capsulated form. The strain employed in these experiments fermented lactose, glucose, mannite,

maltose, inosite and saccharose with the production of acid and gas, but not dulcite. It did not produce indol, but gave acid and clot in the first day in litmus milk, and a positive Voges and Proskauer's reaction.

The milk was sterilized by autoclaving at five pounds extra pressure for twenty minutes, inoculated from a twenty-four hours' agar culture and incubated for 18 to 24 hours at 37°C. The milk had a distinct tendency to form clot and whey, but was not too unpalatable when freshly prepared. Increasing doses from one drachm thrice daily to a dose of four fluid ounces thrice daily was not in itself sufficient to cause cultures of the faecal flora to present to any considerable extent the large, opaque, spreading-margined or cream-like coli, though occasionally some of these were present as shown by MacConkey-agar plates, although ~~given~~ the full dose was given for a fortnight before the administration of the vaccine. A vaccine was given to the paratyphoid carrier (M.D.) of this strain of lactis aerogenes, commencing with five millions and increasing in ten doses (given every second day), (rising by approximately doubling the dose,) up to a thousand millions, and the milk again administered so that four fluid ounces were being taken thrice daily. The intestinal flora now became almost entirely replaced by the organism to the

exclusion of other varieties of B. coli. This continued so long as the milk was being given, but within four days after the cessation of administration these heavy cream-like colonies began to disappear from the faeces until in three weeks the flora had once more regained its former appearance. The numbers of B. paratyphosus B. colonies in the faeces during the period of feeding and vaccine administration were greatly diminished but likewise increased in number on stoppage, For further details see under Methods of treatment which aim at altering the intestinal flora (p.224-8).

Thus it was possible by feeding by mouth to implant this organism for a period and to transform the intestinal flora thereby, a result which would appear to agree with that of Raubitschek (1912) and disagree with Seifert (1911) and Hull and Rettger (1917). This result would appear to corroborate the statement that preliminary immunisation enables the foreign organism to attain a foothold.

Effect of acidophilus milk and lactose feeding etc. on reaction and consistency of faeces.

The reaction of the faeces of two of the patients (M.D. and K.O.) throughout the B. acidophilus milk and lactose feeding was alkaline or slightly alkaline to litmus. In the case of the third carrier (H.T

the reaction was much more variable, being alkaline, neutral and acid on various occasions, but no relationship could be established during the acid periods with the taking of B. acidophilus milk or the consistency of the faeces. When milk infected with B. lactis aerogenes in large quantities was being given to M.D., the faeces tended to become slightly acid to litmus and remained so with only slight variations to neutral and slight alkalinity. A similar result was got with milk soured by B. coli (inosite-fermenting type) in doses up to one and a half fluid ounces daily (p.215&227) the flora, however, was by no means entirely altered to that variety of B. coli. In all three carriers while fed with B. acidophilus milk the faeces remained more or less semi-pultaceous and light yellow in colour, being soft-formed or semi-fluid and moist. This was very evident in comparison with the faeces of many other carriers of the author's series and of the same carriers under other regimes, whose stools were frequently hard-formed, thus rendering the isolation of B. typhosus and B. paratyphosus B. much more difficult, if not impossible.

Clinical Results.

As regards the value of B. acidophilus milk from a clinical stand-point in curing or alleviating

chronic constipation or diarrhoea, no accurate record was kept of the times when purgatives or laxatives were necessary for the female patients under treatment and observation. They all, however, stated that they found that they did not require any laxatives as a general rule while taking the milk, and that previous to the onset of this form of treatment aperients were used fairly regularly to keep their bowels open. On no occasion did they suffer from diarrhoea. The effect of replacement of the usual intestinal flora by B. acidophilus or special types of B. coli on the excretion of bacilli of the enteric group, was negligible.

The occurrence of B. acidophilus in the intestine of young dogs.

Professor Noel Paton, University of Glasgow, asked the author to carry out bacteriological examinations of dogs' faeces in connection with his experimental work on Rickets. Ten animals in all were examined. The oldest of the series was nineteen weeks old and the youngest fourteen weeks. The intestines of the dogs were sent for examination immediately they were killed and the material was taken from the middle of the rectum except in one case; cultures were made from faeces where present; in some cases there was no gross faecal matter and

scrapings from the bowel were used. In every case stained films were made direct from the intestinal contents, cultures were also made - aerobic (on MacConkey's medium) and anaerobic (minced meat broth), as well as cultures on 2% lactose agar for the detection of B. acidophilus types. Representative colonies of coliform organisms were further examined for sugar reactions etc. In general, the flora throughout the series showed no great variation, B. acidophilus being present in all, but was notably abundant in one, from which it was recovered quite easily in pure culture. The diet throughout the series was separated dried milk ("Cow and Gate" Brand) and dried bread. The last four dogs in the series had only the dried bread and lean beef and no very marked alteration from aciduric to proteolytic bacterial types was observed in this flora.

Effect of diet on the intestinal flora of rats.

At a later date a bacteriological examination was carried out for Drs. Murray and Craig (Physiology Department, University of Glasgow) on rats' faeces. The rats, which were nearly full grown, were fed for three days previous to commencement of experiments on a diet composed of porridge and milk. The diet in series A. was then changed to an entirely meat diet, while series B. were given bread and potatoes,

Method of Examinations. The site from which the material was taken was the ileum, about one inch from the ileo-caecal valve; in every instance stained films were made direct from the intestinal contents; the faeces were emulsified in 10 c.c. physiological saline; a standard 4 millimetre platinum loopful of this suspension was used for inoculation; for further dilutions, three to five loopfuls of the first tube were inoculated into fresh tubes; cultures were made - aerobic (on MacConkey's medium, in litmus milk and gelatin) and anaerobic (minced meat broth) as well as glucose broth, whey agar and 2% lactose agar; representative colonies of coliform organisms and non-lactose fermenters were further examined for sugar reactions etc., and various forms of acidophilic organisms were isolated by subculturing from the various media (Whey, and 2% lactose agar) into glucose broth and replating in either whey or liver glucose agar, and tested in 1% Maltose Broth .

Results of Examinations in detail. (Table XXIV)

In AI the flora was partially proteolytic, as shown by the digestion of the milk-casein and liquefaction of the gelatin. Coliform bacilli, scanty aciduric organisms, and Gram-positive bacilli of

Specimens examined.

TABLE XXIV.

Series.	Number.	Date of Examination.	Type of faeces.	Diet.
A.	I.	16/5/23.	Dark coloured and soft-formed, of somewhat gelatinous character.	Meat.
	II.	16/5/23.	Ditto.	Ditto.
B.	I.	18/5/23.	Yellowish brown and soft-formed, of somewhat gelatinous character.	Bread and potatoes.
	II.	16/5/23.	Ditto.	Ditto.

anaerobic types were present as well, but the flora in the main was proteolytic. In addition, one non-lactose fermenting colony, which gave the sugar reactions of Morgan's No. 1. bacillus was isolated. The coliform bacilli, as shown by culture on MacConkey's medium, were abundant and when tested by fermentation reactions etc. were found to be of common varieties. (Where there were distinct difference in the appearance of colonies, several were always tested from the same animal).

AII. The flora while partially proteolytic showed the presence of a greater proportion of B. acidophilus types than did A I. Coliform bacilli and

anaerobes were abundant and presented no striking variation in character or numbers as compared with A I.

B I. Aciduric types predominated though proteolytic organisms were present also. As before, the coliform bacilli and anaerobes were of the common varieties. However, a few of the coliform bacilli were either inosite-fermenters or late lactose-fermenters.

B II. The flora was mainly coliform and aciduric and showed only scanty preteolytic elements. The anaerobes, as shown by the late liquefaction of the gelatin, belonged probably to the saccharolytic group; the coliform bacilli were of the same common varieties as met with in theother animals, a few late lactose-fermenters being present.

Conclusions and Summary of Results.

In general, although the intestinal flora of both series showed distinct differences in character these were only a matter of degree, as both proteolytic and aciduric elements were present in all. The intestinal flora in ~~the A~~ A series was more proteolytic and less aciduric, while in the B. series the opposite was the case. The above findings agree with those of Cannon and McNease (1923) and others in this field of work. According to Torrey (1919)

the nature of the feed would account for the difference. In A. series there was a meat diet which is stated to favour the production of proteolytic organisms, and in B. carbohydrate diet, which is stated to induce an aciduric flora; but the carbohydrate given here was potatoes and bread, the latter of which contains cooked starch, and according to Hull and Rettger (1917) is soon absorbed in the upper parts of the digestive tract, and thus little or no available sugar remains in the intestine long enough to be attacked and utilized by the aciduric group of bacteria. A point worthyof note is that the type of B. acidophilus found to be present in large numbers in this series of rats was of the Y type described by Rettger and Horton (1914).

Note on experimental results of Co-workers.

Dr. Craig's experiments showed that a marked increase in urinary indican and phenolic substances, coincided with the meat diet. These results are in conformance with the findings that the intestinal flora in the A series was more proteolytic and less aciduric, while in B. series the opposite was the case, although both proteolytic and aciduric elements were present in all. The latter point is supported by the third examination of urine which showed a slight indican output even in B. series, after three weeks

on a carbohydrate diet. This result agrees with that of Hertzer and Kendall (1909) who found that the intestinal flora of kittens and monkeys underwent a distinct change when the diet was changed from meat and eggs to milk and glucose, an acidophilic instead of a strongly proteolytic faeces being got. At the same time a marked decrease occurred in the intestine of indol, skatol, phenol and bound sulphuretted hydrogen and of the indican and aromatic oxy-acids in the urines. They conclude that the change was probably brought about by milk. Dr. Murray in his examination of the thyroids of the rats found the most striking difference between the two series was the large amount of colloid material in the vesicles in A series, compared with the colloid content of B. series, where the gland vesicles were almost empty and the walls collapsed.

Summary.

1. The history is given of investigations on the replacement of the common coliform intestinal flora in man by Gram-positive, aerobic, lactose - fermenting bacilli.
2. The characters and method of isolation of B. acidophilus are discussed.
3. By means of ingestion in sufficient quantities, two and a half pints a day, of recent cultures of B. acidophilus in milk it is possible in the

human subject to transform an ordinary mixed intestinal flora into an almost entirely acidophilic one. (80 -90 per cent. of the organisms present in Gram-stained films). On stoppage of feeding with B. acidophilus the original type of intestinal flora is seon re-established. But they may persist in some cases as a result of continued ingestion of lactose.

4. Similarly on feeding with milk, soured by B. lactis aerogenes this organism replaces the ordinary faecal flora provided that preliminary vaccination with the organism is practised along with feeding with cultures in confirmation of Raubitschek..

5. The transformation effected by means of a high calory carbohydrate diet by itself is not so complete as that brought about by B. acidophilus-feeding in man, but is quite successful in the case of rats.

6. Ingestion of lactose in large quantities can transform the flora, but the results obtained are not as complete with B. acidophilus milk and are somewhat variable, a failure being met with in one of the patients tested.

7. Laxatives are of value in conjunction with lactose, apparently by hurrying on the contents of the small intestine, so that the lactose is not absorbed and is available in the lower reaches of the bowel.

8. Failure resulted in repeated attempts to implant B. bulgaricus in the intestine of man.

9. The reaction of the faeces has no relationship to the administration of B. acidophilus milk or lactose, but appears to be influenced by milk infected with B. lactis aerogenes, being rendered acid thereby.

10. The character of the faeces is soft, moist, odourless and of a light yellow colour, during the taking of milk soured with B. acidophilus.

11. B. acidophilus milk seems to have a therapeutic value in relieving chronic constipation.

12. Administration of B. acidophilus and the other organisms tested has no effect upon the excretion of specific organisms in enteric carriers, although B. coli is suppressed to a great extent by feeding with B. acidophilus milk.

******

BIBLIOGRAPHY.

Barker (1914): J. Am. Med. Assn., 1914, 63, pp. 929 - 931.

Bass (1923): Southern Med. Journal Birmingham, Ala, 29, p.1; ref. J.A.M.A. 1923, 80, p. 508.

Bassler and Lutz (1922): J. Am. Med. Assn., 1922, 79, p. 607.

Belonowsky (1907): Annales de L'Institut. Pasteur, 1907, 21, pp. 991 - 1004.

Cannon and McNease (1923): J. Infectious Diseases, 1923, 32, p. 176.

Cheplin, Post, and Wiseman (1923): Boston Med. and Surg. Journ., Sept. 20th 1923, p. 405.

Cohendy (1906 a): Compte rend. Soc. de biol., 1906, 60, pp. 602 - 4.

Cohendy (1906 b): Compte rend. Soc. de biol., 1906, 60, pp. 364 - 386.

Cruickshank and Berry (1924): B.M.J. Nov. 22nd. 1924. p. 944.

Cummins, Fawcus and Kennedy (1910): J. R.A.M.C., 1010, 14, p. 351.

Distaso (1911): Centralbl. f. Bakt. u. Parasit., 1911, 59, p. 648.

Distaso (1913 - 1914): Centralbl. f. Bakt., Abt. I, Ref. 60, p. 255.

Distaso and Schiller (1914): Compte rend. Soc. de Biol., 1914, 86, p. 243.

Dragstedt, Cannon and Dragstedt (1922): J. Infect. Diseases, 1922, 31, p. 207.

Fawcus (1909): J. R.A.M.C., 1909, 12, p. 147.

Goéffon: Manuel de Coprologie Clinique.

Gompertz and Vorhaus (1922): J. Am. Med. Assn., 1922, 80, pp. 90 - 92.

Haibe (1921): Communications to Roy. Soc. of Med. Belgium. Ref. J. Am. Assn., 1921, 76. p. 136.

Herter and Kendall (1909): J. Biol. Chem., 1909, VII, p. 203 - 236.

Hirschler (1886): Zeit. f. Physiol. Chem., 1886, 10, pp. 306 - 317.

Hull and Rettger (1914): Centralbl. f. Bakt., Abt. 1. orig. 1914, 75, pp. 219 - 229.

Hull and Rettger (1917): J. of Bacteriology, 1917, 2, p. 47.

Kendall (1910): Journ. Med. Res., 1910, 22, p. 153; Ibid. 1911, 25, p. 117.

Kendall (1921): Text-book of Bacteriology, 1921, p. 625.

Kopeloff (1923): J. Amer. Med. Association, 1923, 80, p. 602.

Kopeloff and Beermann (1923): Abstracts of Bacteriology, 1923, VII, p. 28. (No. 66).

Ko;eloff and Beermann (1924): Arch. Int. Med., 1924, 33. p. 55.

Kopeloff and Cheney (1922): J. Amer. Med. Ass., 1922, 79, p. 609.

Leva (1908): Berlin Klin. Woch., 1908, 45, p. 922.

Liefmann (1909): Münch. Med. Woch. 1909, 56, pp. 527 - 551.

Litchfield (1914): J. Am. Med. Assn. 1914, 63, pp. 935 - 936.

Logan (1914): Journal of Path. and Bact., 1914, 18, pp. 527 - 551.

Luerrsen and Kühn (1908): Centralbl.f. Bakt., Abt. II, 1908, 20, pp. 234 - 248.

Mayer (1910): Centralbl. f. Bakt. abt. I, Orig. 1910, 53, pp. 236 - 279.

Mereshowsky (1905): Centralbl. f. Bakt. u. Parasit., 1905, 39, pp. 39, 380, 584, 696, ibid. 1906, 40, p. 118.

Moro (1900): Jahrb. f. Kinderh., 1900, 52, pp. 38 - 55.

Morris, Porter and Meyer (1919): J. Infectious Diseases, 1919, 25, p. 349.

McClendon, Shedlov and Karpman (1918): J. Biol. Chem., 1918, 34, p.1.

Nichols (1919): J. Am. Med. Assn. 1919, 73, p. 680.

Pochl (1887): Jahreber., u.d. Fortschr. der Thier. Chem., 1887, 17, p. 277.

Rahe (1915): J. Infect. Diseases,1915, 16, pp. 210 - 221.

Rahe (1918): Journal of Bacteriology, 1918, 3, p. 407.

Raubitschek (1912): Virchow's Arch., 1912, 209, pp. 209 - 220.

Rettger and Cheplin (1921): Treatise on the Transformation of the Intestinal Flora with special reference to the implantation of B. acidophilus, New Haven 1921.

Rettger and Horton (1914): Centralbl. f. Bakt., Abt. 1., orign 1914, 75, pp. 362 - 372.

Robinson C.S. (1922): J. of Biol. Chem., Balt., 1922, 52, p. 445.

Schmitz (1894): Zeit. f. Physiol. Chem., 1894, 19, pp. 401 - 410.

Seifert (1911): Deutsche Med. Wochenchr., 1911, 37, pp. 1064 - 7.

Sittler (1908): Centralbl. für Bakt., abt. I., 1908, 47, p. 14.

Teague and Clurman (1916): J. Infectious Diseases, 1916, 18, p. 652.

Tissier (1899): Compt. rend. Soc. Biol., 1899, 51, p. 943.

Tissier (1900): Recherches sur la flora intestinale normale et pathologique du nourrison, Thése, Paris, pp. 85 - 96.

Tissier and Martelly (1902): Ann. de l'Inst. Pasteur, 1902, 16, pp. 865 - 873.

Tonney, Caldwell and Griffen (1916): J. Infectious Diseases, 1916, 18, p. 239.

Torrey (1915): J. Infectious Diseases, 1915, 16, p. 72 - 108.

Torrey and Rahe (1915): J. Infectious Diseases, 1915, 17, p. 437.

Torrey (1917): J. Bacteriology, 1917, 2. p. 435.

Torrey (1919): J. Med. Research, 1919, 30, p. 415.

Thomson and Ledingham (1910): Loc. Govt. Board, Report of Med. Off. 1908-09. Appendix B.

Weiss (1904): Centralbl. f. Bakt. Abt. i. orig, 1904, 36, pp. 13 - 28.

Winternitz (1892): Zeit. f. Physiol. Chem., 1892, 16, pp. 460 - 487.

Wolman (1912): Annal. de l'instit. Pasteur, 1912, 26, pp. 610 - 624.

Zweig. (1910): Deutsch. Med. Woch., 1910, p. 1803.

****

CHAPTER XIII.

Economic Aspect and Administrative Treatment of the Typhoid Carrier Problem.

As carriers provide the source of most of the sporadic outbreaks of enteric fever at the present time the necessity for their control, when discovered, becomes of paramount importance.

As is well-known anti-typhoid inoculation with a vaccine of dead organisms of B. typhosus, B. paratyphosus A and B has proved a most successful preventive measure of the acute disease, and should be even more widely practised than is now done, especially in the case of those coming in constant contact with enteric fever patients, since among them typhoid infections are well known to run a very fatal course. The statistics of the incidence of the disease both from numbers infected and case mortality of the troops in the Great War shew the immense value of anti-typhoid vaccination as a preventive measure

especially when compared with the figures of the South African War.

Preventive inoculation, however, does not appear to have eradicated the carrier as the 5 carriers discovered by McCarthy and Simmons (1924) in 84 enteric convalescents had been previously vaccinated. Their findings confirmed the results of Hébert and Bloch (1919) though the latter found that the organisms were more abundant in the faeces and urine of the unvaccinated. Garbat (1922) believes that vaccination has a somewhat beneficial aspect in prevention. Meyer (1921) and his collaborators who have made extensive animal experiments in spite of the previous use of a vaccine were still able to render rabbits carriers. The blood serum of vaccinated rabbits had no bactericidal effect on typhoid bacilli. As vaccination proved incapable as in McCarthy and Simmons' cases of preventing the occurrence of infection it does not seem surprising that it also failed to prevent the development of the carrier state. Whether preventive inoculation will lessen or prevent the occurrence of bacillus excreters among those who do not become clinically affected with enteric fever is still a question which deserves attention.

Since it is impossible to cure the human chronic bacillus excreter by any means, except surgical, and

many might refuse operation, the question of prophylaxis comes to be of prime importance, if the spread of enteric fever is to be checked. In the first place, chronic carriers, as well as cases of latent infection must be sought for among the apparently healthy population, and, secondly, patients suffering from enteric fever must be examined during convalescence for the presence of typhoid bacilli in their faeces and urine. Further, the examinations must be frequently repeated over a considerable period of time after convalescence before a negative result can be accepted, since the chronic excretion of typhoid bacilli may not begin, until a considerable time after convalescence has been established (6 weeks), the faeces having been free of typhoid bacilli at earlier stages.

Again, there may be long periodic intermissions of excretion as Prigge (1910) pointed out, and as is shown by the negative phases of the carriers investigated by the author. Five of whom (K.O., J.M., M.D., M.M., and Mrs. G.) proved to be almost continuous excreters with short periods lasting two or three weeks - when no bacilli were isolated in bi-weekly examinations, while in other two (H.T., and B.E.) the excretion was very interrupted, negative phases lasting up to four months occurring. Cholagogues proved

ineffectual in inducing the re-excretion during these periods.

Only too frequently are patients discharged from hospital without a series of examinations being made. A rational procedure (Browning and Gilmour 1910) is to examine both faeces and urine at intervals of 6 days on 5 occasions during convalescence, and again to make a similar series of examinations after an interval of two months. I think again after periods of 6 and 12 months would render the escape of carriers improbable. If the results are all negative, then and only then, may it be safely assumed that the subject is free from typhoid bacilli. Garbat suggested two consecutive negative duodenal cultures as being sufficient evidence of cure.

If any good is to be gained from the above procedure carriers must be legally restrained ffom the persuit of occupations involving the handling of foodstuffs ready for consumption, and special care be taken in regard to the disposal of the infective excretions.

America is far in advance of this country in that respect, numerous States e.g. Minnesota, New York etc. having special regulations for the control of typhoid carriers. The New York State Regulations (1922) lay down that carriers must not handle food

either cooked or uncooked; motions must be disinfected with chlorinated lime if not passed directly into a water closet; carriers should not use toilet if possible away from home, but if they do the hands must be well washed afterwards; clothing must be disinfected before sending to the laundry; every person in the house of a carrier must be immunized against typhoid fever; movements and whereabouts of carriers to be notified to the Health Department; Stools to be examined from time to time. Recently in Baltimore there was a test case of considerable interest in which the Public Health Authorities won the decision, namely that they could isolate and quarantine a woman who had allowed her excretions to be bacteriologically examined and found to contain typhoid bacilli. The woman in question was a chronic bacillus excreter. (J.AM.A. 1922, 79, No.2., p. 1331).

In Illinois the Industrial Board gave compensation (2000 dollars) to the relatives of 14 employees of the Elgin National Watch Coy., who died as a result of a typhoid epidemic due to the contamination of drinking water furnished by the employers. This is an important finding as it tends to establish the principle that typhoid fever is a disease, under some conditions, consequent on occupation. (J.AM.A. 1917, 68, p. 383).

On 1st. March 1921 the Scottish Board of Health

introduced regulations regarding the control of Carriers-Public Health, (Infectious Disease Carriers) Regulations (Scotland)1921, made in terms of Section 78 of the Public Health (Scotland) Act 1897. These give the Local Authority power to deal with a person adjudged to be a carrier of an infectious disease in the same manner as if he actually suffered from the disease. Before a person is to be deemed a carrier he must be certified as such by a Medical Officer of Health, and also by another registered medical practitioner, such certification to have effect for a period not exceeding three months. Further examinations may be made at any time after the date of a certificate, and the carrier may demand to be re-examined during the currency of a certificate on giving the Medical Officer of Health not less than 48 hours' notice in writing. A further certificate on re-examination may be given, but if no certificate is necessary these regulations cease to apply. Provision is made for an appeal to the Board by a person certified to be a carrier. Under Articles XII and XIV of the Public Health (Pneumonia, Malaria, Dysentery, etc.), Regulations (Scotland), 1919, power was given to Local Authorities to prevent persons suspected of being carriers of dysentery and enteric fever from being employed in or concerned with the preparation or handling of food or drink for

human consumption. These Articles are now revoked, the powers in them being replaced by the New Regulations of 1st. March 1921.

The Board have also under their consideration the question of special treatment which it may be possible to afford in the case of different classes of carriers, with a view to removing the danger, or so modifying it as to render restraint of the carrier unnecessary. Circular D.B. No III. 1921.

Dittmar (1922) in commenting on the Scottish 1921 Regulations states that while the need for supervision in the public interest of the disease carrier is recognized, the liberty of the carrier has been guarded in the regulations. This is no doubt necessary, but in the case of enteric carriers who form the most difficult class to deal with administratively, the time during which a certificate is valid seems to be too short. It is known that carriers of enteric infection may be so for years and thus he suggests that after the lapse of the first certificate in enteric carriers the period for re-certification should be yearly. But the most important disability of the carrier, most of whom are women, is their inability to earn a living as housekeepers, cooks and dairyworkers. If they must change their means of livelihood in the public interest, the change Dittmar

thinks should fall on the public purse. Each case of course would have to be carefully adjudged on its merits. If a public health authority could certify on the results of careful investigation into all the circumstances of the individual, that he or she could not earn a living at another form of occupation, then, and only then, would a disability pension be payable by the State to the individual so circumstanced and would be liable to revocation should the carrier on investigation prove to have lost his power of infectivity. It should cease at the age of 70 years when he would become entitled to an old age pension. For the payment by the State of a disability pension Parliamentary sanction would of course have to be obtained.

No similar Regulations are in force in England and the only Regulations in that Country dealing with "carriers" are the Public Health (Pneumonia, Malaria, Dysentery etc.) Regulations, England, 1919, (Articles XII and XIV). These are similar to the Regulations in force in Scotland up till 1921.

In the case of enteric carriers isolation or quarantine would seem to be rather an extreme and too drastic a measure, especially where they refused operation and had to be detained for life, and again on the grounds of expense alone, ~~especially in America where there are so many carriers.~~

The best one can hope for is some legal form of restraint and control as outlined in the New York Public Health Regulations, and an education of the afflicted persons in personal hygiene. Mayer (1910) stated that by the exercise of similar measures in a certain district in Germany the yearly number of enteric cases has been steadily reduced from 484 in 1904 to 70 in 1908, whereas in other districts of equal size and density of population where such provisions have been omitted there has been no such reduction.

The enteric carrier in Mental Asylums constitutes a grave menace to all the inmates and attendants and there are frequently recurring epidemics. To combat this Dittmar has suggested the setting up a Central Institution for dealing with all Asylum carriers. This seems to be the most feasible way of dealing with the problem.

It was suggested as a ~~result~~ corollary of the good results which had followed the operative treatment in the three chronic bacillus excreters of the author's series that the insane carriers might be subjected to the same mode of treatment. However the scheme fell through as the Legal Authority of the Scottish Asylums Board of Control held that carriers were not ill and any operative measures were not for their benefit but solely for the good of the community. Hence if

they were allowed to be operated on the Board of Control would become liable to a penalty should the carriers regain their sanity, and bring an action against the Authority concerned.

Comment is hardly necessary upon this decision. But it must be strongly emphasized that the carrier is not a healthy person and the cure of the condition involves undoubted benefit to the person concerned, since, for example, the danger of gall-stones and the sequels will be reduced if not observed by curing the gall-bladder conditions.

****

*

B I B L I O G R A P H Y.

Browning and Gilmour (1910): Glasgow Med. Journ., August, 1910.

Dittmar (1922): Proc. Roy. Soc. Med. 1922, Vol. XVI., pp. 1 - 10.

Garbat (1922): Monos. Rockefeller Instit., No. 16., May 10th 1922.

Hébert and Bloch (1919): Ann. de l'Inst. Past., 1919, 36, pp. 157 - 208.

McCarthy and Simmons (1924): J. Lab. and Clin. Med., 1924, IX, pp. 513 - 18.

Mayer (1910): Centralbl. f. Bakt., 1910, 53, p. 234.

Meyer (1921): J. Infect. Dis., 1921, 28, 0. 381.

Prigge (1910): Klin. Jahrb, 1919, 22, p. 245.

******