THE PATHOGENESIS OF THE ANAEROBIC CORYNEBACTERIUM DIPHTHERIAE, ANAEROBIC DIPHTHEROID, AND ANAEROBIC LEPTOTHRIX INFECTIONS IN RELATION TO THE PSYCHOSES, NEUROSES, AND NEUROTOXIC STATES, COMPARED WITH 260 CASES OF SYMPTOMATIC PHYSICAL DISORDER.

(Illustrated by 17 Tables)

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1. INTRODUCTION.

The subject of this thesis is the outcome of what might be termed a "hereditary interest" in the complex problem of the relationship of chronic infections to the psychoses and allied mental disorders. Circumstances enabled me, while still a student, to undertake very humbly the continuation of my father's researches in the Scottish Asylums' Laboratory at a point where his illness and death might have proved the closing of a long and arduous chapter. That this would have been so is, as far as I know, borne out by the fact that up to the present no work directly bearing on his later bacteriological studies has been published. The researches I have undertaken during the past six years have been an attempt to elucidate more clearly what exactly are the bacteriological factors at work, and, further, in what manner they attack the economy generally, and with what result. In my endeavour to verify and extend Ford Robertson's views I have been singularly fortunate.

After nearly two years in the Laboratory of the Scottish Asylums' I had the opportunity as Honorary Bacteriologist and Pathologist to the Southport Infirmary and assistant to Dr E. Cronin Lowe, (consulting pathologist and director of the Southport Infirmary laboratory) of studying a large number of private and hospital patients during the course of two years work. In addition to undertaking bacteriological research by anaerobic methods, it fell to me to organise a pathological clinique, the object of which was to

aid the physician in the diagnosis and treatment of obscure physical disorders by the method of a "diagnostic survey". The scheme evolved necessitated a systematic study of each patient by haematological and biochemical tests including functional efficiency tests such as the glucose tolerance and fractional test meal. Each case was also studied with a view to discovering if possible some underlying bacteriological infective condition which might have a bearing upon the state of the patient. In the course of undertaking a large number of patients by this method of approach, some fifteen mental and borderland cases came under review. In many instances I was much struck by their similarity to control patients in the underlying physical disturbance found, and, while in this respect there was something in common, it was striking to note in some of the borderland, and certainly in those who were definitely mental, the absence of clinical symptomatology, in spite of the fact that they showed more gross functional physical disorder on the average than did the non-mental cases. In gastric disorders for example non-mental patients, almost without exception, gave a clear clinical history referrible to that organ. In two mental patients however no such evidence past or present was ascertainable, although both were found to be suffering from severe gastritis and disordered secretory function. Exceptional as these two cases may appear to be, this experience, which has been by no means confined to the gastric mechanism, has been enormously

amplified by the work I have carried out since then. In the study of the bacteriological flora, especially of the intestine, there were bacterial elements common to the majority of the fifteen cases which, on the other hand, were relatively infrequently met with in ordinary hospital patients. This fact further impressed itself upon me when it had to be realised that these additional infective factors were in every respect similar to those so commonly seen in the course of routine bacteriological work at the Scottish Asylums' Laboratory. This evidence I believed at the time formed a valuable link which, if opportunity offered, might result in a chain of facts establishing the importance of this group of bacteria.

Early in November, 1927, the New Reception Hospital,(Wantage House), of St Andrews, Northampton, was opened, and I was offered the opportunity of organising the very work which had been begun amongst cases of mental disorder at the Southport Infirmary. It is not often one is fortunate enough to start from the foundation a scheme of research with a limited number of patients whose whole care clinically and scientifically comes within one's own province. Further, the facilities afforded in each department are those of the best equipped modern general hospital in miniature, a fact which has very materially contributed, not only to the extension of research, but to the correlation as far as my knowledge has permitted, of the wide range of facts and observations that have been collected in the course of nearly three years.

My experience of the value both scientific and therapeutic of the diagnostic survey method of research led me to adopt it in an extended form on all cases admitted to Wantage House. The following is a brief outline of the scheme of research which up to the present has been undertaken on 155 patients, 137 of whom presented definite mental disorders.

(1) Systematic physical examination including blood pressure, central nervous system, teeth, etc.

(2) Examination of upper respiratory passages for evidence of focal infection.

(3) Laboratory tests.
   a. Cytology of the blood.
   b. Biochemical examination of the blood. Non-protein nitrogen, CO_2, calcium, Van-den-Bergh, and phosphates.
   c. Gastric analysis by fractional method.
   d. Glucose tolerance.
   e. Urine, 24 hrs. sample. Biochemical qualitative and quantitative examinations and cytological.
   f. Intestinal content. Chemical and microscopical.
   g. Cerebro-spinal fluid. Cytological, biochemical qualitative and quantitative in about ¼ of the cases.

(4) Bacteriological examinations of the main foci throughout the alimentary canal in all cases by the anaerobic methods given in this thesis. Pelvic organs and accessory sinuses in some.

(5) Radiography. Teeth and accessory sinuses in all cases, and gastro-intestinal tract in some.

(6) The study of the psychological aspects past and present of each case, especially in relation to heredity, environment, and past physical disorders.

The above tests have been repeated as circumstances demanded, and, with the exception of the gastric analysis and glucose tolerance, are carried out again

case it has been possible,under ideal circumstances of observation and control, to study and where possible correlate the mental symptoms with the underlying physical disorders. The main outlines of this scheme and the circumstances that led up to its inception have been mentioned so that those whose position it is to criticise and judge should know that the basis of this thesis has been a wide one and that the views expressed therein have been given without conscious bias and in the hope that others may be stimulated to carry out similar researches.

I have to thank Dr D.F.Rambaut, Medical Superintendent, for his permission to write this thesis.

II. HISTORICAL REVIEW.

Researches bearing upon the Bacteriology, Toxicology, and Haematology of the Psychoses and Allied Disorders.

1875 - 1929.

While in no way professing to be complete, I have compiled this review in order that investigators in this field of research may not only be reminded of what early as well as more recent pioneers have accomplished, but also in the hope that those less versed in the technicalities of research, and who may justifiably feel somewhat bewildered at the complexities of the problems confronting them, will be perhaps stimulated to contribute their share in the advancement of the diagnosis and treatment of mental disorders.

As early as 1875 Savage, the English alienist, reported the recovery of cases of mental disorder following the extraction of infected teeth. Between 1889 and 1897 several Italian workers, D'Abundo (1), Piccinino (2), (3), Grimaldi (4), Montesano and Montessori (5) published observations on the presence of micro-organisms in the cerebro-spinal fluid and brain of general paralytics.

In 1901 Lewis Bruce and Ford Robertson (6), working independently, studied the clinical and bacteriological aspects of general paralysis of the insane, and came to the conclusion that it was a disease directly due to poisoning by the toxins of bacteria whose point of attack is through the gastric and intestinal mucous membranes. Another investigator,

Chalmers Watson, (7) working in the Laboratory of the Scottish Asylums', advanced very similar views regarding the pathogenesis of tabes.

In the following two years Ford Robertson, D. McRae and J. Jeffrey (8) carried out extensive bacteriological researches into general paralysis. In this work evidence was adduced on cultural grounds that an organism resembling the Klebs-Loffler bacillus could be isolated post-mortem from the tonsils, bronchial secretions and alimentary canal. This was done in 17 cases out of 20. In 4 of these this organism was obtained from the brain. In 6 cases of other forms of insanity 2 were found to have a similar organism isolated, in 1 from the ileum, and in the other from the tonsil. During life the same bacillus was obtained from the tonsils, pharynx, carious teeth and saliva. At the same time Ford Robertson (9) produced histological evidence of the presence of a similar organism in the respiratory or alimentary tract in all of 20 cases examined. Concurrent with these investigations Shennan, in collaboration with Ford Robertson, (10) conducted two series of animal experiments demonstrating that very striking morbid phenomena could be produced by feeding rats with living cultures of a threading type of diphtheroid isolated from the bronchus in a case of general paralysis. This organism was non-pathogenic to guinea-pigs. The tentative conclusion arrived at was that general paralysis of the insane is the result of chronic toxic infection

especially dependent upon the abundant growth of a Klebs-Loffler bacillus of modified virulence which gives the disease its special character.

Later, in 1903, Ford Robertson (11) in a summary of his work and that of his collaborators stated that "the view that general paralysis of the insane is essentially syphilitic in origin, although probably held by the majority of neurologists at the present day, has never been proved. That syphilis strongly favoured the development of general paralysis of the insane is however beyond question; the doubt is merely as to its manner of action". Four main arguments against the syphilitic hypothesis were put forward, and the theory of chronic toxic infection by a micro-organism of a distinctive character, advanced. Evidence based on clinical, haematological, histological, bacteriological and experimental work was given to support this view.

In 1905 Ford Robertson and McRae (12) published further evidence of the presence of diphtheroid bacilli in the genito-urinary tract in cases of general paralysis and tabes dorsalis.

A year later Ford Robertson(13) in the Morrison Lectures for 1906 reviewed these investigations, adding much recent work on the subject. Further evidence of the presence of diphtheroid organisms in the brain, cerebro-spinal fluid and blood of general paralytics had been obtained, and on account of its special characteristics the name of bacillus

paralyticans had been given. Information on the increased phagocytic and lysogenic action of the blood upon this bacillus had been obtained and compared to that of control cases. The closely allied condition of tabes was brought into line on the evidence of experimental and bacteriological work. Twelve months later Ford Robertson and McRae (14) in pursuing their bacteriological researches, ascertained more definitely the cultural and morphological characteristics of the bacillus paralyticans, its fermentation reactions to litmus broth sugars being tested. Numerous strains were isolated, six of which proved non-virulent to guinea-pigs. Some however were lethal to mice and others produced morbid symptoms.

In 1909 Ford Robertson (15) summarises the results of a large number of experiments carried out in the course of six years work. In the early experiments four rats were fed with cultures of a threading diphtheroid bacillus from the bronchus of a general paralytic. All four animals died after manifesting paretic symptoms and morbid vascular and cerebral tissue changes. They showed extensive invasion by the thread form of bacillus identical with that isolated from the patient. Dr Lewis Bruce, in undertaking the production of an immune serum in a goat, inoculated the animal subcutaneously with cultures of the same bacillus. The animal was known to lick the spots at which the injection was made. After a time it developed severe alimentary disturbance, became tottering in gait, and, about six months from the time

when the last injection was given, had a seizure closely resembling the congestive attack of a general paralytic, and died a few days later. A culture was made from the oesophagus after death and a growth of the diphtheroid bacillus was readily obtained. The brain was found to have morbid changes resembling those found in general paralysis. Later, sixty rats formed a series of experiments, being fed with six strains of diphtheroid bacilli derived from cases of general paralysis and tabes. The pathogenic effect of the six strains varied, only a few of the species causing paretic manifestations. Others again proved markedly toxic, symptoms developing rapidly (in one within seven weeks). Three out of twelve showed paresis with signs of dementia. The others however became progressively emaciated and lethargic, and finally died. All showed well-marked chronic degenerative changes of the cord and less advanced changes in the cerebral cortex. In another series of experiments four rabbits were fed with cultures of the bacillus paralyticans brevis. The results recorded show the selective action of this organism in producing well-marked nervous symptoms, accompanied by morbid changes in the spinal cord and, in some, the brain. The absence of results from hypodermic injections is remarked upon.

Three years later the same investigator (16) produced further experimental evidence. Infection of the genito-urinary tract in sixteen rabbits with the

bacillus paralyticans resulted in ten developing paresis and ataxia; some also showed signs of dementia and two succumbed to their illness. Further, it was proved that the disease had the character of a contagious venereal one. Three male rabbits that were not infected but which nevertheless showed well-marked paresis and ataxia of the hind limbs, had merely been placed beside females, the genital tract of which had been infected with the bacillus paralyticans. One animal, a male, developed signs of dementia after some weeks, paresis and ataxia being relatively slight. It became thin, and died in two years three months after the commencement of the experiment. Microscopical changes were not typical of general paralysis but the cortical and spinal nerve cells showed very severe and widespread chronic changes. It was mentioned that the morbid changes as well as the features presented during life resembled to some extent those of dementia praecox. A second rabbit, a female, given intra-vaginal injections of the bacillus, became ataxic and paretic nineteen months later, and developed distinct signs of dementia. The animal became completely paralysed in its hind quarters and was shortly afterwards killed. The pelvic organs were intensely congested, the lumbar vertebrae inflamed and softened, and microscopical examination of the lumbar cord showed dense infiltration of the pia with lymphocytes and plasma cells, with marked hypertrophy and

proliferation of the neuroglia, and peri-arteritis of the vessels. In this animal the morbid changes in the cord resembled those that occur in the brain of dementia paralytics.

I have given these experiments in some detail because, in spite of the striking results produced, only transient interest was taken in them, owing no doubt to the view held at the time that para-syphilis was the last word to be said regarding etiology. At the present time, owing to extended knowledge of the role of syphilis in general paralysis and allied disorders, it is being gradually recognised that serious blanks as yet remain in the chain of evidence supporting the pure syphilitic etiology of the diseases in question. I would here make a plea which I partly base on the work in this thesis, that the whole possibilities of Ford Robertson's researches, which up to the present have never been refuted, should once more be explored. The results I believe would justify the elaborate nature of such investigations, and would perhaps lead to a fuller understanding of the complexities of this problem which is as yet by no means fully understood.

Contemporary with the foregoing experiments and having a bearing upon the results of the last method of transmitting the infection, the same investigator in a paper "The Infective Foci in General Paralysis and Tabes Dorsalis" (17) produced evidence in two cases that general paralysis is a venereal disease

not only in respect of its etiological relationship to previous syphilis, but also in respect to the source of bacterial infection, which experimental evidence has shown to be capable of producing many of the essential phenomena of the disease. The source of this infection would appear to be commonly a bacillary endometritis. The uterine nidus forming a suitable cultural medium which raises the virulence of the organism, the source of infection may be primarily attributed to either sex. It is suggested that the serious relapses which are common in patients returning home are, in some instances at least, due to re-infection. Two "carriers", both wives of general paralytics and both suffering from severe endometritis, were shown to be carrying infection by the bacillus paralyticans which proved lethal to six mice tested in the usual way. In the first case, the bacillus proved identical with that previously isolated from the urethra and nose of the husband. A vaccine prepared from the bacillus isolated from the husband produced in the wife reactions in the form of a severe and characteristic attack of endometric pain. Further, histological evidence of invasion by this bacillus of the cranial lymphatic system is given, the paths being by way of the cribriform plate and the foramen rotundum.

In 1907 Ford Robertson and McRae (18) recorded observations on the treatment of general paralysis and tabes by vaccines and anti-sera. 34 cases of general

paralysis and 2 cases of tabes dorsalis were subjected to anti-serum treatment, sheep being immunised with virulent diphtheroids of the bacillus paralyticans, longus and brevis type. General reactive effects were marked, both in the production of subjective and objective symptoms, and moderate pyrexia occurred usually lasting 24 hrs. Out of 12 cases thus treated under the authors' own immediate supervision for a period of over three months, all showed at first remarkable degrees of improvement. 2 were able to leave hospital, 3, after temporary improvement, later became steadily worse in spite of continued treatment. Control cases, all mental patients, showed no reactive response, likewise control sera proved unable to produce reactions in general paralytics. One of the conclusions formed was that a polyvalent anti-bacterial serum would be likely to be more efficacious than the mono- or bi-valent serum used.

In 1909 the same investigator in collaboration with Dods Brown (19) added to the evidence already obtained as to the presence of the bacillus paralyticans in the cerebro-spinal fluid in cases of general paralysis. 20 cases of general paralysis and 10 cases of other forms of insanity were studied by cultural methods, and the examination of the centrifugal deposit. In all cases the fluid was obtained by lumbar puncture. The cultures and centrifuge deposit proved negative to the bacillus paralyticans, and the presence of cells

containing this organism in the 10 control cases. Out of the 20 cases of general paralysis however, bacilli could be distinguished in 12, lying free or in the cells, and in 4 positive results to the diphtheroid bacillus were obtained on culture: 3 were in a congestive attack and 1 in a very advanced stage of the disease. All the bacilli were feeble growers and none showed any marked virulence to mice. The authors refer to O'Brian's (20) work as confirming their own observations. Out of 62 cases of general paralysis he isolated bacillus paralyticans in 70%, whilst in all 30 cases of other forms of insanity the cultural results were negative.

In 1913 Barton White (21) in his bacteriological investigations of the urine in general paralysis found various species of bacteria to be more numerous than in other forms of mental disorder. The action of hexamine as an antiseptic was observed in this series of cases.

In the same year Harvey Baird (22) recorded the findings from the bacteriological examination of the urethra in 21 paralytics and 13 non-paralytics. In only 3 of the former were the results negative, organisms, especially diphtheroids, being more abundant in the paralytics than in the others.

In 1919 Cotton (23) and his collaborators in America published their observations, which had begun in 1916, on "The Role of Focal Infection in the Psychosis". Five years later their exhaustive researches were

dealt with in his book "The Defective Delinquent and Insane". Again, in 1923, he reviewed his elaborate investigations in a paper given in this country, in which he outlined the methods employed in the eradication of septic foci throughout the alimentary canal and pelvic organs. The relation of chronic sepsis to the so-called functional psychosis was discussed, and the results obtained by detoxication given in statistics covering 1412 successfully treated cases. In spite of the striking evidence brought forward by Cotton and his co-workers, bacteriological and allied research with a few exceptions has gone forward relatively slowly. One reason probably underlying this is the remarkable progress made in the fields of biochemical research. More recently however, and especially in the past five years, co-ordinated clinical and bacteriological researches have been undertaken.

In 1919 Ford Robertson (24) in a paper "The Infective Factors in some types of Neurasthenia" reviewed 66 cases in which extensive examination for infective foci had been made, especially of the intestinal and genito-urinary tracts. He referred to the importance of anaerobic methods if factors of primary importance are not to be missed, and the value of the methods of focal reaction and therapeutic immunisation as furnishing trustworthy evidence of the causal relationship or otherwise of a particular organism isolated. His statistics showed that 49 cases had

diphtheroiduria and 25 similar infections of the intestine. While submitting that mental or physical trauma or strain are important causal factors, he asserted that, if after a period of rest the symptoms due to such causes do not subside, they must have a toxic basis. The same investigator (25) in 1921 in a section of his book "Therapeutic Immunisation" summarises the role of the diphtheroid group as pathogenic factors in the production of nervous, and mental disorders, and in many common diseases hitherto unrelated to bacterial toxaemia.

A year later (26) he reviewed the results of extensive bacteriological investigations by anaerobic methods in 32 cases of dementia praecox. A number of cases illustrating the causal relationship of streptococcal, pneumococcal, anaerobic diphtheroid, and "streptothrix" infections, to physical disorders and borderland mental states, were cited and correlated with those found in the dementia praecox group. The successful results of immunisation in a number of dementia praecox cases were reported, and in concluding the writer asserted that these chronic bacterial infections are the most important of several factors that determine the mental disorder.

In 1922 Hunter (27) whose paper in 1900 marked the first publication in the field of chronic sepsis in relation to medicine, referred to the nervous and mental disorders of severe anaemias in relation to their infective lesions and blood changes, and later

in 1927 contributed further researches in cases of mental disorder.

A year later Chalmers Watson (28) in a paper covering some years of research recorded his observations upon the role played by auto-intoxication or auto-infection in mental disorders.

In 1923 Goodall (29) in his presidential address gave a survey of researches bearing upon the toxicology, haematology, and bacteriology of the psychosis from 1911 - 1923. He referred to his work with Barton White in 1913 (30) on the bacteriological investigation of the stool of newly admitted cases of mental disorder. From the results they concluded that neither the Gram positive or coliform flora were abnormal in the preponderance of cases. Agglutination against B. Coli gave negative results for cases of recent and acute insanity. These findings were later confirmed and extended by Scholberg and himself to obligatory anaerobes, B. putrificus, and B. enteriditis sporogenes, the latter giving no indication of the development of agglutinism or increase of opsonic index. He referred to an observation recorded by Scholberg and himself on the presence of pus cells in the fasting stomach of mental patients, 60 out of 68 having this condition. This, he observed, is unusual amongst infirmary patients apart from lesions of the gastric mucosa. Experiments on the toxicity of the blood, cerebro-spinal fluid, and urine in cases of insanity were recorded by himself, Scholberg, and

Cameron, rabbits and fowls being injected with these materials under varying techniques. The results, although not conclusive, showed that to rabbits the blood stroma from cases of general paralysis and dementia praecox is in some way toxic, the serum less so, while the cerebro-spinal fluid failed to be lethal but nevertheless produced morbid changes in some of the animals. The interesting experimental work of Meyer, (31), Cuneo (32). and Loewe (33) was cited as deserving further study.

In the same year Graves (34) recorded some observations on the chronic septic foci in the teeth of borderland and mental patients, citing numerous instances from case histories where dental sepsis alone seemed to be causal to severe emotional disturbances. This work marked the beginning of the extensive researches now carried out by Graves and Pickworth (35) on sinusitis in relation to the causation of mental disorders. Pickworth, (36) in more recent work, has accumulated striking pathological, histological, and bacteriological evidence of the grossly septic conditions that may exist in the accessory sinuses, especially the sphenoids, in chronic and acute cases of insanity. The disturbances of the closely associated pituitary gland in these cases is evident, and have been enumerated and correlated with the more common subjective and objective symptoms found in mental disorder. The more clinical aspects of these investigations appeared in two recent papers

by Graves (37) in which case histories recording treatment and progress were given in great detail and form an interesting study.

In 1927 Goodall in his Maudsley Lecture dealt in retrospect with the progress of morbid histology and its relation to present day researches. In his summary on the subject of toxaemia he referred to his radiological investigations with Knox and Stanford (39) into the evidence of disordered intestinal function in mental disease, and to those recorded by Chalmers Watson and Hendry (40), the latter remarking that such study shows evidence of the relationship between the psychosis and altered function of the vegetative nervous system. The recent post-mortem and histological investigations of Mazzanti (41) in 12 cases of confusional insanity gave evidence of macroscopic lesions of the intestinal wall in 8, while in 3 microscopical examination showed intense congestion, haemorrhage and degenerative changes. Pardo, the Italian observer, recorded the increase of putrefactive anaerobes in the intestine in acute cases of mental disorder. Goodall also referred to the interesting researches of Loewe who found that the dried insoluble adialysate of urine from the catatonic type of dementia praecox, dementia paralytica (after seizures) and delirium tremens was highly toxic. From cases of epilepsy after seizures he produced seizures similar to epileptic ones. The urine from normal persons was found to be non-toxic.

F.H.Stewart, (42) in the course of several years work, has carried out elaborate investigations relating to the coliform group in mental patients. He has demonstrated that B. paracoli and B. coli mutabile are at least four times as common in the insane than in the general community, and in greater numbers in at least one half of the former during the acute phase of illness. He suggested that this may be due to the action of some other intestinal organism. The production of phenol from tyrosine has been studied, and the organisms with this capacity have been found to be B, Morgani, M. phenologenes, Berthelot, and a paracolon. With the first mentioned organism patients were found to be reactive to inoculation, and, the bacillus having been eradicated from the bowel, improved bodily health resulted. In another paper the physiological and pathological action of tyramine was discussed, its action being sympathomimetic and similar to adrenaline but one-twentieth less potent. Sixty-five patients and 28 controls have been studied for the phenol producing power of their coliform flora, the media being bullion broth containing 0.1% tyrosine. The former showed a wide departure from the normal in half the cases. The question however as to whether the rise in phenol percentage is causal or merely symptomatic in the onset and development of illness, remains as yet unsolved. This work was later extended to ascertain whether functional mental disorder is, as an effect or cause, associated with an

increased absorbtion and elimination of the following toxic substances, phenol, tyramine, indole, scatole, indolethylamine, and histamine. These are poisonous or semi-poisonous derivatives of three important amino-acids, tyrosine, tryptophane, and histadine. Special cultural broth (Koessler and Hanke) had been used for the study of the action of the coliforms in producing the toxic derivatives mentioned. Quantitative analysis of the bacterial decomposition of this broth was described, and the technique given for parallel estimation of the urine. The results of this research are interesting and suggestive but insufficient cases precluded any deductions being made.

Brief reference to some of my own researches (43) must be included here as they have a bearing upon the present work. One hundred and fourteen cases of mental disorder have been studied in relation to the bacteriological findings in the mouth and stomach and for the efficiency of gastric function as it affects the condition of the lower alimentary canal. Disorders of the gastric mechanism as ascertained by the fractional test meal were discussed in the 114 cases; only 46% of these came within the normal range, hyperchlorhydria accounting for 30%. The special cultural methods of determining the bactericidal power of the resting juice were given, and the results showed that free HCl is essential if conditions of sterility are to be maintained and the onset of gastritis prevented. Part

of the bacteriological analysis had been directed towards tracing the descent to the intestine of streptococci usually only found in the teeth, tonsils, or pharynx. Six cases were given to show how it is possible to find in the intestine streptococci such as those of the pyogenes and anginosis group having the peroxidase reaction on chocolate agar. Correlation of the intestinal bacteriological findings with the gastric groups showed a reversal of what one might logically suppose to be the case, viz. that the achlorhydric and achylic groups were found to have the smallest incidence of peroxidase types, only 42.9%, whereas the hyperchlorhydrics had 66.7% and always with greater numbers of colonies. Amongst the conclusions arrived at was that the degree of gastric infection did not increase the expectation of invasion of the intestine, and further, that owing to the highly developed bactericidal barrier in the hyperchlorhydric group, infection of the intestine must usually occur via the blood stream or lymphatics. The high incidence of anaerobic species of bacteria, especially the diphtheroid-leptothrix group, in over 200 bacteriological examinations was referred to.

In a paper "Some Cases of Mental Disorder; a Patho-Clinical Study" (44) I gave a detailed survey of four cases out of 120 suffering from mental disorder which had been studied under the scheme of a diagnostic survey by carefully correlated laboratory, clinical and radiological investigations in an endeavour to

ascertain not only the underlying pathology of each, but whether or not rational treatment based on the findings had any influence on the course of their illness and subsequent recovery. The salient features of each case were reviewed and the probable reasons discussed for the success or failure of treatment. The evidence obtained emphasised the fact of how complex if not elusive are the problems of the physical disorders underlying insanity. Diagnosis and treatment by clinical methods alone had, in 3 out of the 4 cases, failed to bring about improvement or recovery. This was due to the fact that none gave definite clinical indications of the severity of the pathological and toxic processes at work. The importance of anaerobic methods in the study of the special types of infection so prevalent in insanity was stressed, and the fact that many of the anaerobic species of diphtheroids found were morphologically identical with the Klebs-Loffler bacillus and were without doubt neurotoxic in action was emphasised.

III. CLASSIFICATION OF CASE GROUPS AND FOCI OF INFECTION STUDIED IN EACH, WITH TABLE OF CASES ON WHOM THE DIAGNOSTIC SURVEY HAS BEEN DONE.

In the introduction I have outlined the source of the material on which I have based this research. The following table epitomises the cases, which for convenience have been placed in four groups.

TABLE 1 SHOWING CLASSIFICATION OF CASES.

Groups.	Classification of Mental State.	No. of Cases.
1.	Definite mental disorder including recent, acute, quiescent, and chronic cases.	145
2.	Borderland cases including psycho-neurosis, neurasthenia, and allied states.	22
3.	Neurotoxic cases all showing in some form or other the milder manifestations of psychic disturbance with or without physical symptoms.	22
4.	Control cases suffering from either well-defined or obscure physical disease.	261
	TOTAL	450

GROUP 1. The majority of the cases included in this group are admissions to Wantage House and have been under observation and treatment for varying periods from some months to well over a year. Nine are cases dealt with as out-patients, being resident

in the main hospital. Table 2 gives the subdivision of this group which has been based on psychological symptomatology and is as accurate as classification by this method will allow with the exception of the delusional states in which are included, for convenience, several involutional insanities, one lactation, and one post-puerpueral.

TABLE 2.

CLASSIFICATION OF MENTAL CASES (GROUP 1.)

Diagnosis.	Numbers.
Melancholia.	47
Dementia Praecox.	30
Delusional States.	30
Confusional States including Stupor.	17
Manic-Depressive.	8
Mania.	7
Neurosyphilis and G.P.I.	6
TOTAL	145

GROUP 2. Of the 22 borderland cases 8 have been admitted to Wantage House, the others being investigated at Southport, 2 of the latter being under my personal care for some time. No case, unless showing proper insight into their condition, has been included.

GROUP 3. The cases in this group have been carefully selected as being those to whom the term neurotoxic can apply. The majority are cases leading useful and active lives but from time to time suffering from modified but well-defined psychic disturbances such as inability to concentrate, irritability,

temporary deficiencies in association of ideas, mild depression without environmental cause, periods of sleeplessness and undue mental fatigue. In many these manifestations are associated with either well-defined or somewhat vague physical disorders. The most frequent of these are loss of appetite, constipation, physical fatigue, indefinite visceral or head pains, and usually loss of weight. Observation and treatment of this group has not only yielded ample evidence of underlying physical disorder, but has shown very clearly, in those that have come under my personal supervision, how closely related were the psychic disturbances to the physical. The value of most of the cases belonging to this group is their capacity to respond and to appreciate spontaneously the nature of their condition, which, when relieved, frequently results in the self-understanding of cause and effect, a result which, when correlated with the knowledge of their toxic and infective processes, yields comparative data of very considerable value. Later it will be seen that there are reasonable grounds for assuming that those in this group. through being inherently more stable psychogenically, are able to carry as much if not more potential or active toxic focal infection than those in group 2. If however environmental conditions of psychic or physical trauma or strain undermine the whole economy, the individual is then in a much more serious position. In many this must be so since this very psychic stability and capacity to resist brings the victim either gradually or, more

frequently, precipitately beyond the stage of spontaneous recovery. I believe that most of the toxic or exhaustion psychoses belong to this group but the mental picture is immaterial since it is purely symptomatic. I have dwelt on this hypothesis because I believe it forms an essential link in the correlation between cause and effect in other mental disorders whose etiology may appear at first to be purely psychogenic, but whose physical basis is in reality similar, varying only in the degree of severity.

GROUP 4. The majority of this group are cases examined at Southport laboratory, a number of them being fully investigated by the method of diagnostic survey and therefore run parallel to the cases in the other groups admitted to Wantage House except that, owing to circumstances, it was only possible to undertake anaerobic cultural methods at Southport in the examination of the stool. Five cases were subjected to the complete survey at Wantage House and in these anaerobic methods were applied to all possible foci of infection. It has not been practicable to ascertain a diagnosis in all the Southport cases but most of them came under the category of chronic physical conditions for which advice had been sought. In those cases presenting a bacteriological flora of any severity which approximated to the type found in the other groups, a diagnosis has been ascertained in 44 out of 64 cases. The range of the physical diseases presented is a wide one and covers most of the

recognised entities in pathology. Cases where the term "general physical, or nervous, debility" has been applied, or where the diagnosis could not be obtained, have been retained in the control group unless it was definitely established that psychic disturbances were present. Thus it is possible that some cases having toxic infection similar to those of the other three groups should have been relegated to group 3. With the large number of cases however, the facts to be deduced from these controls can be taken as being a fairly accurate basis for comparison.

The figures given in table 3 represent the bacteriological analysis which has been carried out on specimens subjected to aerobic and anaerobic cultural methods simultaneously for purposes of comparison. As far as possible systematic bacteriological examination has been planned in each case with the object of tracing where possible the spread of aerobic and anaerobic infection from primary to secondary foci. This scheme applies more strictly to groups 1, 2, and 3. In the control cases (group 4) opportunity for doing this was available only in some cases, anaerobic methods not being applied to foci involving the teeth, tonsils and stomach. For the purpose of this research these are necessarily incomplete and have therefore been omitted. In group 1 however, I have added 124 aerobic cultures as the findings from them have a bearing upon the state of the stomach in relation to the aerobic tonsillar and dental streptococcal flora

and certain obligatory anaerobes intermediate between the diphtheroid and leptothrix groups which recent work has shown to exist.

TABLE 3.

TABLE SHOWING NUMBER OF COMBINED AEROBIC AND ANAEROBIC BACTERIOLOGICAL EXAMINATIONS CARRIED OUT IN THE FOUR GROUPS.

Sites of Focal Infection.	Group 1. 145 cases No. of Exams.	Group 2. 22 cases No. of Exams.	Group 3. 22 cases No. of Exams.	Group 4. 261 cases No. of Exams.	TOTAL
Nasal	7	2	2	1	12
Antrum	6				6
Sphenoid	2				2
Tonsil	45	4	5	11	65
Dental	56	9	3	7	75
Sputum	1	1	1	4	7
Stomach R.Juice	124	9	5	5	143
Intestine	249	29	25	264	567
Urine	8	2	2	2	14
Cervix	6	2	2		10
C.S.F.	30	1			31
Ear	2				2
Breast Milk	3				3
Pleural Fluid	1				1
Pus from abcess or ulcer.	3	2		1	6
Totals	544	63	45	295	947
Aerobic Cultures only, Group 1					124
TOTAL EXAMINATIONS					1071

TABLE 4.

TABLE SHOWING THE NUMBER OF CASES IN THE FOUR GROUPS IN WHOM THE DIAGNOSTIC SURVEY HAS BEEN UNDERTAKEN.

	Group 1.	Group 2.	Group 3.	Group 4.	Totals.
Wantage House Cases	128	8	5	5	146
Out-Patients from Main Block	9	0	0	0	9
Southport Infirmary Cases	2	2	0	50 (approx)	54
TOTALS	139	10	5	55	209

IV. BACTERIOLOGICAL TECHNIQUE.

PREPARATION OF MEDIA.

Although the fundamental principals of Ford Robertson's cultural methods as detailed in his book "Therapeutic Immunisation in Asylum and General Practise" have been adopted from the outset in this research, time and experience have however led to the development of anaerobic methods both in the use of solid and fluid media, which fulfil more closely the conditions of strict anaerobiasis so essential for the growth of the diphtheroid and leptothrix group. During the past eight months anaerobic broth and litmus sugar broth methods were started in the laboratory in Wantage House, and their usefulness has enabled me to push forward the research to its present stage. In order to complete the information relating to the technique employed, it is necessary to include a brief outline of the aerobic media used, which, having run parallel with the anaerobic methods, serves as a basis of comparison.

A. AEROBIC MEDIA.

1. MACCONKEY'S NEUTRAL RED BILE-SALT-AGAR. This media in 4" petri dishes has been used for the study of coliform flora.
2. WARREN CROWE'S BLOOD CHOCOLATE AGAR. This media, poured into 4½" petri dishes, has been used for the identification of the streptococcal and allied groups of bacteria. It has been found especially useful in intestinal work as the coliform growth

is relatively slow and gives time (48 hrs.) for the full development and differentiation of the streptococcal flora. Aerobic species of diphtheroids and leptothrix also grow and differentiate well. The following method of preparation has given the firm and smooth surface so essential to this type of media.

About a litre of defribinated bullock's blood is obtained and strained through muslin. 750 cc. is placed in a water-bath at 50°C. 250 cc. of a 3% peptone or trypsin 1% agar, having a reaction of + 18, is melted, and cooled to 50°C. The two are then mixed, 1% of glucose is added, and the mixture is maintained at 50°C whilst plates or tubes are filled in the ordinary way. At least $\frac{1}{4}$" in depth is required in each plate, and, after pouring, the plates are allowed to stand overnight; this allows uniform setting and liberation of the air bubbles. A litre should make thirty plates 4" in diameter. By far the best steriliser is an electric hot air oven provided a small basin of water is put in with the plates to keep the atmosphere moist. A piece of blotting paper is inserted into the cover of the plates and each one is conveyed, without tilting, into the oven. The temperature is raised to 65°C or 70°C, where it should be maintained for an hour or two, and then raised to 85°C or 90°C. The media becomes semi-solid and on no account must the temperature of the plates be allowed to reach 100°C or the surface of the media may be

ruined. The temperature is again raised to 85°C or 90°C on three successive days, making four in all. On inspection the surface should be perfectly smooth and glossy, These plates should keep for two weeks at room temperature. It is of the greatest importance that the media should not be scratched or roughened when inoculating either material or cultures. To prevent this a fine curved piece of glass rod, carefully cleaned before sterilising, must be used - never a platinum loop or wire.

3, HAEMOGLOBIN AGAR. This media is used mainly for subcultural work from the chocolate plates, and for differentiating the haemoglobinitic properties of streptococci. Most anaerobic diphtheroids will grow well and develop the maximum number of granules.

Nutrient Agar.

Lemco	10 grams	(1%)
Peptone	10 "	(" ")
Sodium Chloride	5 "	(½%)
Agar	30 "	(3%)
Distilled Water	1000 cc.	

Place in steamer until dissolved, then standardise to Ph. 8.0, clear and filter as for agar, then add 1% glucose, put up into 250 cc. flasks, and sterilise in steamer ½ hr. for three days. It daily has been found that if the initial Ph. of the media is standardised to 8 this rises consistently to Ph. 7.6 after the three periods of sterilisation. A Ph. of 7.6 has been used during the past two years and would appear to be the optimum acidity for anaerobic work.

To make the tubes, dissolve media in steamer and pour into sterile 5" x $\frac{5}{8}$" test tubes. Place in water-bath at 60°C and, when cooled to this temperature, add to each tube 4 - 5 drops of sterile haemoglobin standardised to 10 grams%. Rotate tube in hand until media is of a uniform colour, slope, and allow to set. The media should be perfectly clear and of a moderately deep sepia brown colour. Incubate for sterility.

4. LEMCO-PEPTONE BROTH. This media forms the basis of the aerobic broth for streptococci (standardised to Ph. 8.6), anaerobic broth and sugar broth (standardised to Ph. 8) for the anaerobic diphtheroid and leptothrix groups.

Lemco	10 grams.	(1%)
Peptone (B.D.H.)	10 "	(" ")
Sodium Chloride	5 "	($\frac{1}{2}$")
Distilled Water	1000 cc.	

Place in steamer to dissolve for $\frac{1}{2}$ hr. and standardise to Ph. 8.6. Return to steamer for 10 mins. to precipitate phosphates, cool, filter, and add 1% lactose. Transfer media to 100 cc. flasks and sterilise in steamer, 20 mins. daily for three days.

To make the tubes, add 10% sterile sheep's serum and 1% sterile standardised haemoglobin. Decant into 4" x $\frac{1}{2}$" sterile test tubes and incubate for sterility. This media has been found to grow most strains of streptococci and pneumococci abundantly. Experiment has shown that although the original Laboratory of the Scottish Asylums' method

of adding 10% serum greatly improved growth when compared to that from nutrient broth alone, the addition of 1% haemoglobin not only further assisted growth, especially in the more delicate strains, but served to differentiate species more clearly by their microscopical characteristics including length of chain. Sugars according to Holman's classification have been used for further identification. Aerobic strains of diphtheroids and some leptothrix species show varied growing powers in this media, but a number form a definite deposit and sometimes cloud in 24 - 48 hrs.

B. ANAEROBIC MEDIA.

1. ANAEROBIC GLUCOSE HAEMOGLOBIN AGAR. This media has been used throughout this research for all primary and subcultural work in the identification of the anaerobic diphtheroid and leptothrix species. The nutrient agar of Ph. 8 is used containing 1% glucose, but beyond this stage the details of preparation are essentially different. For anaerobic work 6" x ¼" extra heavy test tubes must be used or much of the media will be spoilt through breakages. Further, they are plugged with absorbent wool as the ordinary cotton wool will not hold the chemicals employed prior to sealing. The agar is melted as before and 4 - 5 drops of specially prepared sterile haemoglobin added. The tubes are sloped at a slightly greater angle than for the aerobic haemoglobin agar; this allows

room for the wool plug carrying the chemicals. As soon as set the top of the wool plug is cut off and the remainder pushed down within ¼" of the agar. Place on the wool plug as much sublimated pyrogallol as will lie on the spatula recommended below for the purpose and then add 3 - 4 drops of 10% NaOH. More is unnecessary and will saturate the plug with the risk of spoiling the media. Seal at once with best quality bark corks (length 1¼") taken from melted paraffin (melting point 57°C). It is important that the wax is sufficiently hot (i.e. just short of smoking) and that the corks, especially if new, have sufficient time to become thoroughly impregnated with wax as otherwise imperfect sealing will result and the tubes will be spoilt. In practise it is best to retain a complete apparatus for this part of the technique. This should consist of an ordinary Bunsen burner with bye-pass, asbestos cement sheet 24" square, retort stand and 5" ring, two 7" or 8" diameter enamelled iron basins with handles, one or two pairs large size dissecting forceps for handling corks, and one pair of dental tweezers with right-angled bend for removing old wool plugs, one bottle of 10% NaOH with pipette and I R teat, 1 lb. tin of sublimated pyrogallol, one spatula 7" - 8" long with right-angled end having approximately ⅜" square surface; this carries the necessary amount of pyrogallol for each tube.

The tubes are next incubated for sterility. The temperature of 37°C hastens the maturing process which, when complete, should result in a plum colour of the agar. The time required for this change is usually 24 - 36 hrs. and any tubes not showing this differential colour should be discarded. The marked contrast between the fully de-oxygenated tubes and those which are not is one of the many advantages of this media, as at any time in the course of cultural work it is possible to note whether or not growth has occurred under strictly anaerobic conditions. A marked alteration in colour can be observed in tubes imperfectly sealed even if only incubated overnight. Up to a point the media improves with keeping.

2. ANAEROBIC LEMCO-PEPTONE GLUCOSE HAEMOGLOBIN BROTH.

The lemco-peptone broth is used as the basis for this media which is indicated for the further propogation of anaerobic diphtheroids and leptothrix species. Two degrees of Ph. have been used, one at Ph. 8.6, the other at Ph. 8. The Ph. has been found to rise during sterilisation in the same way as for the nutrient agar. 1% glucose is added in place of 1% lactose and the media transferred to 100 cc. flasks prior to sterilisation. Broth containing 3% haemoglobin without serum was used for some time but it has been found that this strength inhibited the growth of some strains and that serum also was required to promote growth.. The following

method is now employed and gives very good results.

To make the tubes, add 1% of specially prepared haemoglobin and 3% sterile serum to one of the flasks and pour into sterile 5" x $\frac{5}{8}$" test tubes, not more than 4 cc. of the mixture in each. Prepare and seal in the same way (with pyrogallol and NaOH) as for anaerobic haemolglobin agar, and incubate for sterility. If the tubes are sealed immediately, they retain a bright cherry colour which in time tends to become a shade lighter. Again failure to seal properly can be readily made out, since if this has occurred the pink colour of the broth changes to a sepia brown in the course of incubation overnight.

The more alkaline broth has been found to suit a few strains of anaerobic leptothrix and diphtheroid, but experience has shown that the slightly more acid reaction Ph. 8 is much more suited to the majority of strains, and has now been adopted for routine use.

3. ANAEROBIC LEMCO-PEPTONE-SERUM-HAEMOGLOBIN LITMUS SUGAR BROTH. This media has been used to test the fermentation reactions of the anaerobic diphtheroid and leptothrix group. Again the basis is the nutrient broth standardised to Ph. 8. The media is transferred to 100 cc. flasks, and 1% litmus and 1% of the sugar required are added. These are sterilised in the steamer for 20 mins. daily for three days.

To make the tubes, add 3% sterile sheep's

serum and 1% prepared standardised haemoglobin and pour into 5" x $\frac{5}{8}$" labelled sterile test tubes, not more than 4 cc. in each. Chemically prepare with pyrogallol and NaOH (2 drops) and then seal immediately as for anaerobic haemoglobin agar. Incubate overnight for sterility.

This final formula for the anaerobic sugars has been in use for the past four months and is the outcome of much experiment, varying strengths and combinations of serum and haemoglobin being tried. The present strength of haemoglobin in no way masks the colour of the litmus, the tubes being a faintly purple blue when freshly made.

Under conditions of complete anaerobiasis an interesting change occurs in the colour of some of the sugars after they have stood for 5 - 6 days at room temperature. All the nine sugars in use are affected in time, but glucose, galactose, and laevulose show the change most readily, and lactose rather later. The affected tubes gradually lose their purplish blue colour and change to the light pink of anaerobic broths. At first this was thought to be due to some change in the sugar under anaerobic conditions and the Ph. was tested but found to be unaltered. Later it was noticed that, if the tubes were left open to the air for a few minutes, the original colour was restored. It would thus appear that this change in colour is only due to a bleaching effect which occurs as the free oxygen is taken up by the action of the

pyrogallol and NaOH, the monosaccharides appearing to be more readily affected than others. A similar bleaching occurs, only more slowly, in Hiss's serum broth under the action of the chemicals, the colour being restored but not quite completely. Why some sugars are more affected than others has not been ascertained, but this lack of colour uniformity does not, however, affect the fermentation reactions. It has been found necessary to give sufficient time before inoculation and after fermentation for complete restoration of colour to take place. This essential point, and others in the determination of fermentations, will be discussed in another section.

4. HISS'S ANAEROBIC SERUM BROTH. This media is being used for fermentation reactions to be run parallel with the previous sugar method. 20 cc. of serum, 1% of the required sugar, and 1% of litmus are added to 80 cc. of nutrient lemco-broth and sterilised for 20 mins. on three successive days.

To make the tubes, 4 cc. is poured into 5" x $\frac{5}{8}$" sterile test tubes and, while hot, these are sealed in the same way as for the anaerobic serum haemoglobin sugars. A few parallel tests with glucose have been carried out and, as with the other media, bleaching occurs during incubation and at room temperature, masking the change of colour due to acid production. Clotting also occurs occasionally. It has been found however

that if fermentation has taken place exposure to air brings up the pink colour very clearly, the process taking longer than for the haemoglobin litmus sugar broth.

THE SPECTROSCOPIC CHARACTERISTICS OF THE HAEMOGLOBIN MEDIA. The great difference in colour in the aerobic and anaerobic tubes strongly suggests that there must be some essential difference in the character of the haemoglobin, one which must be all-important to the survival of strict anaerobes under primary conditions of artificial growth. I am indebted to the Clinical Research Association for a report upon four samples of media which I sent for examination. This was as follows;-

No. 1. Aerobic Haemoglobin Glucose Agar.

1st. Band	648 - 636
2nd. "	592 - 574
Cut off below	550 -

No. 2. Anaerobic Haemoglobin Glucose Agar.

Two Bands	600 - 565
	555 - 525

No. 3. Anaerobic Haemoglobin Glucose Broth.

1st. Band	593 - 560
2nd. "	550 - 526

No. 4. Anaerobic Haemoglobin Glucose Broth exposed to the air.

1st. Band (faint)	648 - 630
2nd. " (well-defined)	590 - 570
3rd. " (" ")	550 - 528

No. 1 Tube contains a mixture of methhaemoglobin and oxyhaemoglobin.

No. 2 " " carboxyhaemoglobin only, although traces of oxyhaemoglobin may be present, this latter being masked by the stronger bands of the carboxyhaemoglobin.

No. 3 " " The same as 2.

No. 4 " " Chiefly oxyhaemoglobin with a little methhaemoglobin.

It would therefore appear that carboxyhaemoglobin, along with inert gases such as nitrogen and CO_2, are essential for the growth of strictly anaerobic species, and this would imply that a minimum degree of oxygen tension is required. The difference between the state of the two haemoglobins in tubes 1 and 4, and 2 and 3, is marked and is only brought about by an essential stage in the treatment of the haemoglobin which is added to the anaerobic tubes. Without this preparation chemical action alone is quite inadequate to remove the heavy charge of fixed and nascent oxygen carried by concentrated haemoglobin.

THE EXTRACTION OF OXYGEN AND GASES FROM THE HAEMOGLOBIN. After the haemoglobin has been standardised to approximately 10 grams % it is transferred to sterile 6" x ¾" wool plugged test tubes, not more than 1½" being allotted to each. This is essential in order to provide room for the marked

ebullition of gas which takes place under the vacuum. Four to five tubes containing haemoglobin are placed in a small tin and covered with the bell-jar of a 6" vacuum plate, care being taken to ensure close fitting of the plate and cover. The Geryk pump is then attached (a $1\frac{1}{2}$" bore single cylinder is sufficient), and exhaustion of the air proceeds. Effervescence of the haemoglobin usually begins after about twenty revolutions of the pump, and great care must then be taken or the whole fluid will "boil over" and saturate the plug. The time required for complete extraction of the gases varies, but as a rule 24 hrs. is sufficient. During the day the quickest method is to pump until the bubbles collect and rise up near the wool plug; if this is done every 10 - 15 mins. extraction may be found to be complete next morning. Once the extraction is finished it is necessary to store under a negative pressure. Another essential part of the technique is to avoid as much as possible contact with the air; thus it is important to remove the tubes from the vacuum only when the agar is sufficiently cool (60°C) and to make the process of adding the drops of haemoglobin as rapid as possible. Lastly, no time must be lost in setting up the tubes chemically and sealing. The time required for the agar to set varies according to room temperature but if placed in a draught usually 5 - 10 mins. suffices. The setting

of the last tube to be handled should be carefully tested from time to time. When solid they should then be placed vertical and the next stage begun.

METHOD OF OBTAINING FRESH SUPPLIES OF STERILE HAEMOGLOBIN AND SERUM. Various methods have been tried from time to time to maintain a supply of fresh sterile haemoglobin. Citrated or oxylated human blood does not haemolyse well and from clotted blood the amount of serum obtained is quite inadequate. Further, the risk of contamination is much greater than from the present source and with the method employed. Commercial haemoglobin has been found to be variable in quality and its growing properties are not quite so good as haemoglobin derived from sheep's blood. By far the most reliable and satisfactory method is to arrange for a small flock of sheep which should be kept for the purpose and bled at regular intervals. This is the ideal, but sheep picked at random from the flock serve the purpose equally well and this practice has obtained at Wantage House. Sheep retained for this purpose for a year in no way suffer, and, provided that they are not bled more ~~often~~ than three to four times, gain weight and condition, thus improving in market value. The preparatory work and output necessary to maintain the supply of serum and haemoglobin may at first appear to be somewhat elaborate and expensive. Once however the small flock of sheep and

their house has been obtained, the amount of time and skill required to carry out the periodical bleeding is very small. It is preferable to have the house, a wooden hut, placed in an isolated position in the corner of a field or park. A small hurdle pen with gate, fixed to the door end of the hut, greatly facilitates selection of the animals with a minimum of disturbance. The animal is lifted on to a specially constructed trestle and strapped securely across the back. The side of the neck is then clipped, lathered and shaved. The head is covered with a clean towel which is secured by two small Spencer Wells forceps to the wool. The skin and surrounding wool is soaked with a solution of 1/50 (about) Lysol or carbolic. The operator, sitting on a low stool, compresses the external jugular vein at its lower end with the thumb of the left hand. As the vein stands out prominently there is rarely any difficulty in inserting the large sterile needle into the upper end. The point of the needle is flamed just prior to use, thus removing any trace of cotton wool that may adhere to it on account of having been kept in a sterile tube with wool at the bottom. Compression is maintained and the blood is allowed to flow into 8" x 1" test tubes, about 50 cc. being collected in each. Twelve tubes are taken from each sheep and it is as well to bleed two sheep at a time, the supply usually lasting the laboratory five to seven weeks according to circumstance. In summer it is important to

see that all bleeding has stopped and the wound covered with collodian wool, otherwise sores from flies will result. No immediate ill effects from the bleeding have been noticeable. In the course of 2½ years I have obtained some 400 tubes of blood and in none has initial contamination occurred. In contrast with this I have repeatedly tried the same technique in the slaughter-house and farm buildings but with 50 % - 70 % contaminations. Scrupulous cleanliness of the floor, ceiling, trestle and walls of the hut are essential, crude creasol and water being used for this purpose after each occasion.

EQUIPMENT DETAILS FOR SHEEP BLEEDING.

Hut 10' x 8' with wooden floor, side windows, and two sky-lights to ensure efficient lighting; interior painted white.

Small hurdle pen 8' x 5' with gate, attached to side of hut.

Two tables 3' x 2' (about).

Four coat pegs on wall for white coats, etc.

One stool for operator.

One wooden trestle for sheep.

Wooden box with two blocks having twelve holes in each to take the twenty-four 8" x 1" sterile blood tubes.

Six large bore steel needles about 30 x 2 mm. (Sterilise by placing in hot vaseline for about ½ min., remove with forceps and shake well before dropping into sterile 5" x ⅝" test tubes containing wool pad. Plug

with cotton wool to exclude air.)

Two hollow-ground razors.

Shaving soap and brush.

Spirit lamp and bottle of methylated spirit.

Absorbent cotton wool.

Carbolic acid liq.

Two large enamel basins.

Four towels.

One large can of hot water (if water is not laid on)

Two large sized artery forceps.

Bottle of collodian.

It is essential that the hut should only be used for bleeding purposes.

PREPARATION OF THE HAEMOGLOBIN AND SERUM. The clots are loosened the same day; usually sharp rotation of the tube between the hands is sufficient to do this. The tubes are then stored in the ice chest or refrigerator for 24 hrs. The serum is next decanted into sterile 5" x $\frac{5}{8}$" tubes and spun to clear of corpuscles. Sufficient serum should be left in the tubes so that the clot is just covered. The serum, when free from cells, is stored in 5" x $\frac{5}{8}$" sterile tubes the plugs of which have been soaked in hot paraffin to seal, and is found to keep almost indefinitely although in time slight precipitation occurs. The clots are then placed in a freezing mixture of crushed ice and common salt for 3 - 4 hrs. being covered with a small blanket. This is usually sufficient but some tubes may however require longer.

When freezing is complete the tubes are placed in a basin of water which is gradually brought up to 60°C (not higher). If plunged into water at 60°C many tubes crack under the sudden change of temperature. If the freezing of the clot has been complete, haemolysis is produced and, once started, continues for some weeks at room temperature, or preferably, in the refrigerator. The haemoglobin is sufficiently concentrated for use in 4 - 5 days time. Provided the tubes are well plugged the haemoglobin and clot will keep for some months. The haemoglobin is decanted into 6" x ¾" tubes as required.

STANDARDISATION OF THE HAEMOGLOBIN. This has been found necessary owing to the variability of concentration, which not only materially affects the colour of the agar or broths, but the growing properties of the media. By the Fleischl-Miescher haemoglobinometer method it has been found that the concentration varies between 7 and 14 grams %. The tubes are pooled in such a way as to standardise to 10 grams %, a final check being made. In practise it is usual to decant 5 - 6 haemolysed clots at a time and, when standardised, they are ready to undergo the extraction process in the vacuum chamber.

CULTURAL METHODS.

COLLECTION OF MATERIAL. For tonsillar material I have employed a platinum loop which has been inserted into the crypts, care being taken that no contamination from the mouth occurs. During the past two years however I have made use of Eve's suction pump which, if employed in a certain way, ejects the contents of the crypts into the interior of the glass rod and thus obviates all risk of external contamination and obtains pus or cheesey debris lying deep in the substance of the tonsil. Since adopting this method a more representative flora has been available for study.

Dental cultures have been made from either the apices of extracted teeth or from pus expressed from periodontal pockets. In the former instance care has to be taken to eliminate as far as possible contamination from the gum surfaces by two applications of tincture of iodine some minutes prior to extraction. From time to time granulomatous sacks have come away attached to the apex of teeth and these have been opened and the contents cultured. Recently, to eliminate altogether the possibility of outside contamination, large sacks have been soaked in 1/20 carbolic for 5 mins. and small sacks for 2½ mins. The sack is then opened with a sterile scalpel and the contents and wall-scrapings cultured. By this method it has been shown that anaerobic diphtheroids sometimes form part of the infecting organisms.

Study of the bacteriological flora of the stomach has been carried out with strictly sterile precautions, the Ryle's tube, syringe, and tubes being sterilised by boiling immediately before use. As soon as the tube is passed and the resting juice drawn off, a portion, 5 - 10 cc., is placed directly into a sterile tube and immediately cultured. The resting juice is then divided into two portions, one remaining at room temperature, the other incubated at 37°C for 24 hrs. when cultures from both tubes are repeated. In this way it has been possible in a large number of cases to correlate the bactericidal power of the resting juice with that of its free hydrochloric acid and pepsin content. It has been striking to note the number of cases in whom the gastric mucous membrane has proved to be infected and in many instances proved a more fertile source than either teeth or tonsils.

The collection of faecal material suitable for cultural purposes is of the greatest importance and has to receive special consideration. This would perhaps seem unnecessary and laborious but unless care is taken bacteriological failures and wrong diagnoses of underlying infections is inevitable. The average sample of stool collected from mental patients is not infrequently quite useless for the study of the true intestinal flora either aerobic or anaerobic. Experience has shown that scybalous and even semi-formed stool passed after admission where there is 2 - 3 days delay in the colon, contains as a rule only coliforms

and small numbers of streptococci. Anaerobes are usually absent or very scanty. Hence it has been found necessary to accept only samples that from observation are known to have passed through an empty colon which has, 24 hrs. previously, been thoroughly cleared out by Plombiéres lavage or enemas. A mild laxative is usually given to ensure that at least the intestinal content is not more than 24 hrs. old before evacuation. In this connection it is as well to mention that the Plombiéres lavage water contains as an antiseptic 1 - 2 ozs. of H_2O_2 per pint, thus excluding the doubtful circumstance of universal infection by possible anaerobes after admission. In cases where a good daily action occurs it is the rule to take that which is passed last; care has also to be taken to avoid urinated specimens as these are frequently misleading. Uniformity of the samples and the minimum lapse of time before culturing are imperative if any cognisance is to be taken of the intestinal changes that may take place from time to time. In spite of these precautions, some cases do not at first show the full extent of their infection, and it is only later, after local treatment and sometimes specific focal reaction by vaccines, that a true assessment can be made. Further reference will be made and the reasons for this will be given in another section.

METHOD OF INNOCULATION FOR PRIMARY CULTURES. Material from the tonsil, teeth, stomach, cervix or vagina are

inoculated on the aerobic chocolate agar media and two anaerobic glucose agar tubes. In order to obtain sufficient discretion of colony growth one loopful of the material to be inoculated on the anaerobic media is first mixed and spread on the surface of an aerobic nutrient agar slope, and from this serial inoculation is made on to the anaerobic media.

Primary cultures from the stool are made as follows. MacConkey's bile salt agar and chocolate agar are used for the study of coliforms and the streptococcal group respectively. With a standardised platinum loop one "flat" loopful is placed near the margin of the media and spread over with a clean bent glass rod previously sterilised in the bunsen flame. In inoculating the chocolate agar, especially with newly made plates, great care should be taken not to break the surface, but at the same time the material should be well rubbed over to ensure even spreading. Until recently the method of inoculating the anaerobic glucose agar tubes has been to take a "flat" loopful and spread this thoroughly over the surface of the nutrient agar slope. The loop was then flamed and, when cool, was rubbed on to the films at the top of the tube where it is thinnest. This was then transferred to three anaerobic agar tubes by serial inoculation, care being taken to rub the material well over the whole surface of the agar before carrying the loop on to the next tube. It was found advisable to open the anaerobic tubes just prior to making the culture, rotating the top of the tube and cork in the bunsen

flame for 4 - 5 secs. this being sufficient to allow the cork to be removed without difficulty. The wool plug was picked out with the dental tweezers before mentioned, and discarded. To seal the tube a plug of ordinary clean absorbent wool was used, as, if dry sterilised, it became brittle and powdered the surface of the agar. No contamination has ever occurred on account of using unsterilised wool for this purpose. The chemicals were added and the tubes sealed in the usual way, and incubated at 37°C.

In order to obtain additional uniformity the following method is now being tried. One loopful of faecal material is rubbed into 5 cc. of sterile normal saline until the suspension is approximately equal to that of a 2000 mill. comparator vaccine standardisation tube. Of this suspension one loopful is transferred to the first anaerobic agar slope, and serial inoculation carried out as in the other method.

METHOD OF SETTING UP CULTURES FOR CEREBRO-SPINAL FLUID AND SUBSEQUENT TECHNIQUE. The whole of the fluid, usually 10 cc., is spun at 1700 revs. per minute for 5 mins. in order to collect any cells and bacteria for microscopical examination. All but 0.5 cc. is decanted into another tube, the residue shaken and smears made by transferring several loopfuls on to clean slides. One nutrient aerobic agar tube is inoculated with several loopfuls of the deposit and the whole of what remains is transferred into an anaerobic glucose haemoglobin agar tube which is then

sealed. After 24 hrs. incubation the tubes are examined and any colonies developing within this time are identified. If they have remained sterile the fluid in the anaerobic tube is carefully washed upon the surface of the agar to allow any developing bacteria to be deposited on the agar, this being done without opening the tube. This process is repeated each day for seven days, and if, by the end of this time, no sign of growth appears, the cerebro-spinal fluid is considered to be sterile. If however bacteria of the diphtheroid group are present, minute transparent colonies can usually be seen growing on the surface of the agar on the second or third day, where the fluid has been washed up. They are usually attenuated but will survive one or two subcultures as a rule. In some thirty cultures by this method, growths which have appeared on the aerobic or anaerobic media within 24 hrs. have been found to be either diplococci or staphylococci which subculture readily and become vigorous, but in only two cases have such growths occurred and these were accepted as contaminations.

THE STUDY OF PRIMARY CULTURES. The MacConkey and anaerobic tubes are examined after 24 hrs. incubation but the chocolate agar plates are left for 48 hrs. before making subcultures; this allows time for better differentiation of colonies. The colonies on the MacConkey plates are examined and subcultured in the usual way and the anaerobic tubes are next

studied in full daylight under a X 10 aplanic hand lens. All three tubes are examined and compared, the differences in colony characteristics being made out mainly according to their shape, colour and surface appearance. The latter is best ascertained by rotating the tube slightly so that the light may be reflected on the surface. Some colonies can only be differentiated by a close study of their light reflecting properties which may vary considerably. When the different types of colonies and their numbers are roughly assessed, the tubes are opened and the colonies picked off under the lens with a fine platinum point. At this stage further differentiation can often be made as the consistency of the colonies varies considerably; some are intensely sticky (adherent), some soft but cohesive, while others again are watery and melt under the needle. As each colony is picked off and drilled, its characters are carefully noted in a book specially kept for the purpose, so that subsequent growth can be correlated with the primary characteristics. In the size of tube used it is possible with care to make 4 - 6 drills on one slope, the primary and subculture tubes then being re-plugged, sealed, and incubated for another 24 hrs. It is important to study the primary colonies after 48 hrs. growth since many undergo changes which more clearly differentiate them from any other species that may be present. The drills are usually sufficiently grown in 24 hrs. for them to be stained by Gram for microscopical

differentiation. Where the third, and sometimes the second primary tubes show little or no growth after 24 hrs. incubation, it is advisable to leave them until next day. In this way the more delicate and slower growing diphtheroid species can be isolated, such colonies as a rule subculturing readily. When however growth is good at 24 hrs. and the colonies are close together, subculture at 48 hrs. very frequently fails. This is partly owing to failure of food supply and also to the relatively limited survival capacity of these anaerobic species of bacteria.

THE USES OF THE ANAEROBIC SERUM HAEMOGLOBIN GLUCOSE BROTH.

A. PRIMARY CULTURES. This media has been in use for the past six months and has proved of great service in the study of anaerobes for all material except the intestinal content. In the latter the rapid growth of the coliform utilises too much nutriment and causes a rise in the Ph. which inhibits the growth of the more delicate and slow-growing diphtheroid or leptothrix species. In some instances, particularly where the dental, tonsillar or cervix cultures on the anaerobic agar slopes have not shown diphtheroid growth, the broths, after 48 hrs. incubation, have been found to contain small numbers of polar-staining diphtheroids which, if incubated for 3 - 4 days, continue to multiply. Anaerobic and facultative streptococci also grow well but, owing to the longer survival capacity of the diphtheroid species, subculture on the third day on to anaerobic haemoglobin

glucose agar as a rule yields a pure growth of the diphtheroids. In cervix cultures this broth almost entirely eliminates the largely saprophytic aerobic flora of cocci and large types of diphtheroids. This cultural selectivity has made it possible to obtain growths of attenuated anaerobes which can then be further differentiated, according to their aerobic adaptability, by subculture on to aerobic and anaerobic haemoglobin agar. My experience of the value of this media allows me to predict with some measure of confidence that bacteriological research, if carried out by these methods, will yield results not hitherto possible in the study of chronic and latent infections of the cervix and endometrium, especially in relation to neurotoxic states and puerpueral fever.

B. SUBCULTURES. Good growths of the anaerobic diphtheroid and leptothrix species can usually be obtained either from well-grown colonies picked off from the primary anaerobic haemoglobin agar, or from pure isolations in subculture. Growth is usually apparent in 24 hrs. but some delicate strains take 48 to 72 hrs. before a visible deposit forms. Facultative bacteria belonging to both species grow very strongly and differentiate well. With the diphtheroid group vigorous growth may form a heavy deposit with the production of cloud in 24 hrs., but as a rule most strains require 48 hrs. Strong growth is usually accompanied by the disappearance of the cloud and the precipitation of the haemoglobin owing to the rise of

Ph. largely due to carbohydrate decomposition.

Observations on the behaviour of a large number of strains would show that the maximum period of vitality is usually at the fourth day, growth after that time virtually ceasing. This has been tested by subculturing on to anaerobic agar every day up to the sixth, and noting the vigour of the growths. By the fourth day growth appears to be spent as shown by failure of most strains to undergo further propogation. Those that survive grow sparcely, and usually die out in another 24 hrs. This finding bears directly on the study of their fermentation reactions which will be discussed in another section. Up to the present no production of gas has ever been noted with any of the diphtheroid species isolated. The anaerobic leptothrix group adapt themselves much more readily to broth than to surface conditions. They can either be subcultured from the primary haemoglobin agar slopes or from drills. Their growth in broth is characteristic as they form a cohesive or filamentous ropelike mass without cloud. Growth is slower than that of the diphtheroid group and their vitality is maintained a little longer, but this has not been found to alter their sugar reactions beyond the fourth day.

METHOD OF INNOCULATING THE ANAEROBIC SERUM HAEMOGLOBIN SUGAR BROTHS FOR TESTING THE FERMENTATION REACTIONS OF THE ANAEROBIC DIPHTHEROID AND LEPTOTHRIX GROUP. The sugars can be innoculated either from good surface growths on the anaerobic glucose agar slopes, or preferably from a well-grown 24 or 48 hrs. old anaerobic broth culture. In the series of reactions given, the latter method has been employed with only a few exceptions. Here it is necessary to enter into some detail as to the technique since there are various sources of error which cause failure and misinterpretation of results. Nine test substances have been used, viz. dextrose, lactose, saccharose, maltose, mannite, galactose, dextrin, glycerin, and laevulose. The nine sugar tubes are placed in a rack in the order mentioned. Each tube is unsealed but the wool plugs left untouched during the period of colour reviving which has already been referred to. This is necessary so that the conditions may be the same for all the sugars, and takes a few minutes, the tubes being very gentle agitated to hasten the process. When this has been done the plugs, which tend to become more moist as the tubes keep, are removed. It will be noticed that in the tubes in which the plugs are almost saturated a ring of brown alkaline fluid adhers to the glass. Great care must be taken that this does not trickle down into the broth unobserved. If this happens the media is completely spoilt, but may only be noticed later by the absence of growth and an orange discolouration of the

broth. The growth deposit in the anaerobic broth is then carefully sucked up into a sterile glass pull-out pipette with I R teat, and 2 drops transferred to each sugar broth, great care being taken not to touch the inside of the tubes or chemical contamination will occur. When completed, fresh plugs of absorbent cotton wool are inserted, in doing which it is necessary to push the plug sufficiently far down to soak up the residue of brown chemical adherent to the glass and to withdraw this a little if too close to the broth. Once the plugs are introduced, the pyrogallol and 2 drops of 10% NaOH are added, and sealing up proceeded with. The tubes are incubated and read each day on four subsequent occasions, and any tubes which, after 24 hrs. at 37°C, exhibit a brown or orange colour should be discarded as this is either caused by access of air due to an improperly fitting cork, or to the chemicals having reached the media. After a few weeks experience, errors such as those described rarely occur, the technique having been found reliable and the results easy to interpret.

GROWTH CHARACTERISTICS AND DETERMINATION OF FERMENTATION REACTIONS OF ANAEROBIC DIPHTHEROID AND LEPTOTHRIX BACTERIA.

A. THE ANAEROBIC DIPHTHEROID GROUP. The present combination of serum and haemoglobin in the proportion of 3 : 1, and 4% of the total constituents of the broth, appears to satisfy the requirements of all the anaerobic diphtheroids which have been studied up to the present, including some of the most fastidious

and delicate species. In determining the fermentation reactions the following observations are made; change of colour, amount of growth (referred to as deposit), presence or absence of cloud, and, at a later stage, decolourisation indicating maximum fermentation. Carbohydrate splitting power and the amount of growth produced runs practically parallel. In four days with vigorous strains the deposit formed in the sugars that are strongly fermented may amount to a quarter of the total bulk of the media. With these, fermentation frequently commences in 24 hrs. and always in 48 hrs. In contrast to this the sugars that remain unfermented, although always showing growth, do so in a relatively smaller degree. Less active strains may not show definite fermentation until the third or fourth day, growth again corresponding, but sometimes slightly preceeding the production of acidity, which may occur suddenly between the third and fourth day. The fact that the media is transparent makes such observations possible, and with experience one has been able in some instances to predict the ultimate reaction, an advantage not obtained by the Kiss's serum sugars which have also been studied under similar anaerobic conditions. During the period of incubation bleaching of the litmus already referred to occurs, the tubes turning a cherry pink colour. In tubes that are fermenting this change is masked, but in the others bleaching may be mistaken for acid production except that the relatively small deposit acts as a guide.

Confusion is avoided however and the reactions made perfectly clear by the simple expedient, mentioned previously, of opening the tubes after the preliminary notes of the final reactions are made. This has been termed the "Air Test" and is carried out as follows. The tube which, before opening, shows the maximum bleaching with the minimum of growth is chosen for a control. Restoration of colour is observed in the tubes after opening until the control tube returns to its normal colour. During this period it is necessary to agitate the tubes gently. It will be found that only those tubes in which considerable deposit has formed remain acid, there being rarely any difficulty in deciding the degree of acid reaction. Maximum fermentation is noted by a light straw colour of the broth while the growth deposit, which is heavy, is also seen to be augmented by a brown precipitation of haemoglobin. A light red colour with heavy deposit denotes a strong reaction, cloud being either slight or absent. A medium reaction is darker in colour, with less deposit and varying degrees of cloud. A deep red with or without slight cloud is taken as a slight reaction. Indefinite or trace reactions are only occasionally met with. The formation of gas has never been observed.

B. ANAEROBIC LEPTOTHRIX GROUP. The same technique and observations apply, growth however rarely being so vigorous. It appears as a cohesive or filamentous ropey mass, cloud formation being infrequent and

and occurring only in sugars fermenting very strongly. On the whole their carbohydrate splitting power is much less marked; nevertheless it is possible to determine their reactions in the same way as for the anaerobic diphtheroid and Klebs-Loffler group.

The significance of the reactions of the two groups of bacteria is discussed in the section dealing with the classification of the anaerobic diphtheroid and leptothrix group.

STAINING AND FIXING METHODS.

A. GRAM'S STAIN. (Jensen's Method).

1. Methyl Violet — 0.5 % aqueous solution

2. Lugol's Iodine.

Iodine	1 gram.
Pot. Iodide	2 "
Dist. Water	100 cc.

3. Absolute Alcohol.

4. Neutral Red Solution.

Neutral	1 gram.
1% Glacial Acetic acid	2 cc.
Dist. Water	1000 cc.

Method. Methyl Violet, one minute. Lugol's Iodine, one minute. Absolute Alcohol, until decolourising ceases. Neutral Red Counterstain, one minute.

Good results for the diphtheroid group can be obtained by heating the slide on warm stage before staining, and applying stain for two minutes. The time required for decolouration is longer than for the first method, the granules and metachromatic properties however coming out more clearly.

B. NEISSER'S STAIN (modification of Coles' Method)

1. The films are warmed on the stage and, while warm, are stained with Neisser's blue for one minute.
2. Wash in water.
3. Stain with Lugol's Iodine for 30 seconds.
4. Wash in water.
5. Stain 10 - 15 seconds in Neutral Red Solution.

Neutral Red	0.25 grams.
Dist. Water	100.0 cc.

The body of the bacillus is stained a brick red and the granules black.

The above method has been used for differentiating the polar bodies in the diphtheroid and leptothrix groups and has given excellent results.

C. LÖFFLER'S METHYLENE BLUE. (Recognised Technique.)

D. CARBOL-FUCHSIN. Method for staining spores.

E. FLAGELLA.

Tannin	2 grams.
Dist. Water	20 cc.
Ferrous Sulphate Solution 1 : 2	4 "
Saturated Alcoholic Sol. Fuchsin	1 "

Method. Pour mordant over film and heat without boiling for one minute. Wash in water, Stain with carbol fuchsin. Wash in water. Dry and mount.

FIXING SOLUTION. For anaerobic broth deposits and growths in sugar broths it has been necessary to fix slide preparations in 1% formalin in absolute alcohol for ½ hr. For all other preparations the method of fixing by heat has been employed.

V. THE ANAEROBIC DIPHTHEROID AND LEPTOTHRIX GROUP. CULTURAL MORPHOLOGY, MICROSCOPICAL CHARACTERISTICS, AND CLASSIFICATION, WITH TABLES.

THE ANAEROBIC DIPHTHEROID GROUP.

It is necessary, before entering into the details of microscopic and cultural morphology, to define what criteria of growth habit has classified species of the diphtheroid group as anaerobes. The term anaerobic in this research implies primary conditions of cultural growth in a media from which the nascent and combined oxygen has been entirely removed. I have emphasised this because I believe that no method employed at the present time fulfils so closely the conditions of complete anaerobiasis. No diphtheroid has been included in the 312 anaerobic species unless it has appeared only on or in the primary anaerobic glucose haemoglobin agar or broth. Parallel aerobic cultures have been carefully studied to exclude facultative anaerobes. Although the full numbers have not been tested for their aerophilic adaptability, numerous members of each classified group have been tried at various stages of anaerobic growth. It is a remarkable fact that very few have survived the presence of oxygen, with the exception of two groups, even if given identical conditions of nutriment. A number of aerobic and facultative anaerobic species of diphtheroid have been isolated also, but it is readily seen from comparison of their numbers that they form a much less wide and numerous group than do the strict anaerobes; this

applies at least to case groups 1, 2, and 3, from which the species are derived. It is probable however that some species can be grown primarily under partial anaerobiasis and may even, under favourable conditions of growth in vivo, survive the presence of full atmospheric oxygen. It has been proved on the other hand that the majority of the species under consideration are amongst some of the most fastidious bacteria known, both as regards suitable nutriment and conditions of gaseous metabolism. It follows therefore that unless such conditions of growth are strictly fulfilled, there must remain a considerable gap in our knowledge of bacterial species in mental disorders, and especially in the study of chronic and latent infective foci.

In attempting to make a classification of the anaerobic diphtheroid species, it is realised that it is by no means complete and will in time undergo amplification and modification as knowledge is gained. It is hoped however that it will serve as a guide for those who may wish to take up the work.

Owing to the properties of the anaerobic haemoglobin glucose agar, it has been possible to differentiate broadly their cultural morphology and to divide them into six groups. There is however no hard and fast line and in many instances only long experience will enable the observer to note the subtle differences that distinguish one colony from another. The grouping has been based on appearances after 24 hrs. incubation,

since any change of growth character is, with one exception, merely an intensification of those already noted. The exception relates to the development of haemoglobinolysis which may only appear and alter the appearance of a colony at a later stage of growth. Haemoglobinolysis, or the precipitation of the unaltered haemoglobin to form an opaque circle round each colony, would appear to be due to some extent at least to fermentation of the glucose in the media, and local rise in Ph. to the point at which acid haematin is precipitated. Some colonies however which remain perfectly transparent, ferment glucose readily under broth conditions, therefore it does not seem entirely dependent upon sugar fermentation. The size of the colonies of course varies according to the discretion or otherwise of the culture, but average characteristics only have been given. Shape as a rule is constant except for two groups which, more especially if isolated, tend to become elevated or heaped at 48 hrs. with sometimes a slight depression in the centre; the base of the colony may also expand irregularly. Such colonies are either deep brown or cream in colour and show marked degrees of haemoglobinolysis. The term pin-head has been adopted to describe a surface contour which is either raised or only slightly flattened in distinction to the definitely flat colonies of some species.

The differences in texture of surface appearance can be made out under a hand lens and this forms for some the only means of differentiation. The surface

may be intensely reflective as in transparent watery colonies, or be granular and dull in appearance. Their colour, or the lack of it, is partly dependent upon texture and thickness, and also upon haemoglobinitic properties. Some, by transmitted light, are completely colourless and transparent, while those that are translucent are usually grey by reflected light. Beyond this point the colonies become denser and finally opaque. In these, colour serves as a distinguishing feature and this may vary from grey, yellow-grey, cream, yellow-brown to deep brown according to the degree of haemoglobinitic reaction. The consistency also varies and forms a distinguishing feature of some value. More than half are watery and melt on the point of the needle. From this they vary from being soft and cohesive to rather firm, none being markedly sticky and adherent as is the case with the anaerobic leptothrix group. On subculture much less differentiation is apparent, many of the haemoglobinitic colonies losing this property although it may reappear when the tubes are incubated for 48 hrs. or longer. This applies especially to pure growths of group 1 whose haemoglobinitic action often causes precipitation of the whole media, rendering it brown and opaque. Only first subcultures not more than 24 hrs. old have been relied upon for the differentiation of microscopical characteristics. The grouping of their cultural morphological characteristics has been for the present based on the frequency with which they have occurred;

thus it will be seen that group 6 has been placed last although it conforms much more to the first group than those that preceed it.

The main distinguishing morphological characteristics of the six groups, when grown on anaerobic haemoglobin glucose agar, are as follows;-

Group 1. (40.7%) are colonies with the following appearances after 24 hrs. culture. They show as medium sized colonies varying from 1 - 1½ millimetres in diameter and in contour form pin-head shaped elevations with smooth, shiny surface. The centre of the colony is denser, forming a contrast to the margin which has a slightly watery semi-transparent look. This appearance is characteristic of the group and enables them to be readily identified when associated with their yellow-grey colour. Some species, owing to their greater haemoglobinitic power, are denser and to transmitted light are opaque, with or without an area of surrounding media which is fogged owing to action on the haemoglobin. Such colonies are usually yellow-brown to brown in colour and their consistency is always soft but cohesive which enables them to be picked up quite readily on the point of the needle.

After 48 hrs. growth all in this group become denser and many develop clearly defined haemoglobinitic action which is associated with deepening of colour, increase in size, and frequently alteration in shape. The colony becomes elevated and often shows a puckered depression in its centre; the margin may remain

smooth, irregularities however sometimes occurring. In examining a 48 hrs. culture this development of growth is readily made out, confusion possibly arising however unless it is appreciated that all the colonies on one tube belonging to this group do not necessarily undergo this change; otherwise one may be led to believe they are two separate species.

Group 2. (31.1%) After 24 hrs. growth they appear as small to very small colonies, being usually ½ - ¾ millimetres in diameter and rarely larger than 1 millimetre. In contour they form either smooth or slightly flattened pin-head shaped elevations with even margin. Their surface is usually highly reflective in a good light, forming a miniature mirror, a feature characteristic of most of this group. By transmitted light they are mainly uniform in density whether they are completely transparent or slightly translucent. None of this group show haemoglobinitic changes, this absence of colour forming another diagnostic feature. In consistency they are almost invariably watery, melting at the touch of the needle.

After 48 hrs. culture only a slight increase in size is apparent except in isolated colonies, and no alteration in shape or margin occurs. The general tendency is to become less transparent but some retain this feature. Development of colour is inconspicuous and in the majority the most evident change is a slight greyness. As a rule their light reflective

properties remain unaltered, but they are usually rather less watery in consistence.

Group 3. (11.6%) After 24 hrs. growth the size of the colonies in this group is usually larger, varying from 1½ to sometimes 2½ millimetres in diameter according to isolation. They have a definitely flat contour with a shiny or semi-matt surface. By transmitted light they are either transparent or translucent. They are usually colourless but a few are sufficiently dense to appear grey.

At 48 hrs., growth, in isolated colonies, usually continues, but as a rule little alteration in shape occurs. Some strains however develop a small, centrally placed elevation, giving the colony a limpet-like appearance. No haemoglobinolysis occurs. As a rule they are firmer, and some may become brittle, breaking up on contact with the needle.

Group 4. (9.9%) In 24 hrs. these colonies vary from about 1 millimetre to 1½ millimetres in diameter. They are distinguished from others by their somewhat matt surface which, by reflected light, gives them a watered grey colour. Except for shape they are similar to group 3 colonies, being watery in consistence.

In 48 hrs. the colonies become less transparent but otherwise undergo very little change. No haemoglobinolysis occurs with this group.

Group 5. (5.8%) After 24 hrs. growth these are usually more uniform in size, being about 1 millimetre in diameter. In contour they are similar to group 1,

forming pin-head shaped elevations but with a less smooth, shiny surface. Their density is more uniform and colour is either grey or grey-white. Consistence is almost invariably cohesive.

After 48 hrs. increase in size occurs uniformly, heaping up not having been observed in this group. The colony becomes denser and its colour more prominent. Definite haemoglobinitic change has not been observed but occasionally a colony may turn to a definite cream colour in which case very slight alteration of the media can be seen.

Group 6. (0.9%) At 24 hrs. these colonies are usually large, varying from 1½ to 2 millimetres in diameter. In contour they may either be pin-head and definitely elevated, or show distinct heaping and irregularity of the circumference, while the surface is usually dull. Colour is yellow-brown to yellow and haemoglobinolysis apparent, although not so developed as in group 1.

After 48 hrs., continued growth is most usually to be noted in increased size with heaping and irregularity of surface and margin. Colour changes according to the amount of haemoglobinitic action, some colonies deepening to a red-brown. Consistency remains unchanged.

MICROSCOPICAL MORPHOLOGY. Of all the bacteria known to science none more than the Klebs-Loffler bacillus and allied species can claim the distinction of having stimulated so much research or of having raised so

much variance of opinion as to their morphological classification and biochemical reactions. From my study of the anaerobic species this reputation has unfortunately been maintained, and the difficulties of classification have been such that I hope it will be appreciated that in their present form many of the groups are to be regarded as tentative, and must in due course undergo modification. From the point of view of practical laboratory work however the table has proved useful and may be so to others.

Although it is generally accepted that Loffler's methylene blue fulfils most needs in the differentiation of microscopical characteristics, in practice I have found it better to rely on the combination of Gram's stain (Jensen's method), and Coles modification of Neisser's method, using neutral red in place of Bismark brown counterstain. In adopting a method of classification I have followed fairly closely that of Westbrook, Wilson and McDaniel (Ref. 1) referred to by Graham-Smith (Ref. 2) as insufficient data has as yet been obtained to attempt to classify them by their biological and pathogenic properties. The first 24 hrs. old subculture from the primary colony on the anaerobic haemoglobin agar has been taken as being most representative, since experience has shown that misleading involutional changes take place very soon on subsequent subculture. Polymorphism is, in some groups at least, not quite so marked as with the Klebs-Loffler bacillus, a few species however being highly

pliomorphic. These seem to have no fixed morphology, a fact which has rendered their accurate classification a matter of considerable difficulty and made it necessary to include an indefinite or unclassified group. The capacity of all the groups to take on Gram's stain is exceedingly variable but on the average they are what might be termed half Gram positive. Some species occur however that stain very deeply, while others again are completely Gram negative. With Gram the metachromatic granules as a rule stain clearly in the half Gram positive species and those that are Gram negative. The presence or otherwise of granules has always been confirmed with Neisser's modified method which shows them up as inky black dots against a brick red background. They form themselves at present into eleven groups including those classified as indefinite, subdivision having been made according to the granular, barred, or solid forms of Westbrook. Additional to these are coccoid rod types forming definite chains, completely coccal or diplococcal forms, threading segmented forms, and those having characters approximating to the Hofmann morphology without however being classifiable with that bacillus. The last mentioned group form only 1.9% and are very similar morphologically to the more common aerobic forms of genito-urinary and nasal types of diphtheroid. The first five groups fall under the term granular, while groups 6 and 7, although being sparsely granular, have had to be classified separately on account of

very distinctive characteristics, the former being Gram negative and the latter coccoid and chained. Groups 9 and 10 are non-granular. Subdivision of the granular forms into five groups has been possible owing to the relationship of the degree of granularity, and the size, Gram positiveness, and outline of the individual rods.

DESCRIPTION OF MICROSCOPICAL GROUPS.

Group 1. These are generally half Gram positive, rather thin, curved, regular shaped rods with small but definite bi-polar granules. Neisser's stain shows the granules to be larger than with Gram but their disposition remains usually bi-polar. There are grounds for believing that this group and group 2 are in reality the same, but owing to the distinctive appearances on first subculture of a large number, they have for the present been kept separate. In older cultures group 1 tend to take on the primary characteristics of group 2.

Group 2. This group are more Gram positive and are longer, thicker, and with an irregular beaded outline, the ends being definitely rounded. The granules are distinct, larger, and more numerous than in group 1. Neisser's stain shows numerous granules which are as a rule regular in size and distribution, three in each rod being the usual number. Some species tend to show pliomorphism, especially clubbed and bottle shapes.

Group 3. These are usually very strongly Gram positive, some having a barred appearance similar

to the barred forms of Westbrook. The condition is not parallel however as in this group granules are most numerous. They are irregular in size and distribution and many are very large if stained by Neisser. The rods are larger and longer than any of the other groups. Pliomorphism is the rule, clubbed and coccoid forms predominating, while pseudo-branching due to partial longtitudinal fusion is a distinctive feature of most.

Group 4 are characterised by being variable in taking on the Gram stain but none are Gram negative. Pliomorphism is a marked feature, clubbing, coccoid formation being the rule, and occasionally short chains formed of rods are to be seen. The rods are much shorter than in any of the other granular groups. Neisser shows as a rule well marked, rather small, granules which vary in size and shape according to the degree of pliomorphism.

Group 5. This group are usually half Gram positive although some species are almost Gram negative. Neisser usually shows fairly numerous but small metachromatic granules, mainly bi-polar. The rods are characterised by being slightly curved, thin, and regular in outline with rounded ends. Two forms occur in the group, one having long rods and the other short, being usually half the length of the former. This group stands out quite distinctly from the other granular types.

Group 6. These are distinctive in being almost

completely Gram negative. Metachromatic granules are generally sparce but when present are moderately large and situated in the bulbous portion of the rod. The shape of the rod is characteristic and can be contrasted to a large-headed comma, the end of the rod usually tapering to a point although occasionally showing clubbing.

Group 7. The characteristics of this group are fairly constant, usually being Gram positive. The rods take on the form of moderately large coccoids, the longest diameter of the sphere lying parallel with the line of chain formation which is a diagnostic feature of the group. The size and shape of the coccoids varies, some being rod-like while others are large and ovoid. The length of the chain varies also but as a rule contains 6 - 20 coccoids. Neisser shows the presence of only a very few small granules, but under broth conditions this group may undergo very definite metamorphosis, lose its chains, and become definitely granular.

Group 8. This group, although small in number, are of interest in that they form wholly coccal elements in place of rods. The cocci are large and usually regular in shape but vary a little in size, being attached together in pairs, the envelope of the cocci apparently fusing at the point of union. Single cocci are not often to be observed. With Gram's stain they are usually completely decolourised but occasionally some elements retain a little of the methyl-violet.

As a rule a small granule can be observed lying in each coccus which is usually better defined by Neisser and takes on the black colouration of the polar bodies in the other groups. From this fact it is probable that they are analogous to metachromatic granules characteristic of the diphtheroid group, and for the present it has been necessary to classify them as belonging to it in spite of the wide divergence in morphology.

Group 9. The inclusion of this group amongst the anaerobic diphtheroids is perhaps open to doubt as their morphological characteristics place them somewhere between these and the aerobic leptothrices. They are usually strongly Gram positive and form large, thick, rather straight rods with slightly rounded ends. These elements vary very considerably; in length some are short and others may form long coarse threads either with or without segments. No branching has been observed. Neisser's stain shows them to be devoid of granules but in some species minute black particles can be seen scattered throughout the rods. In contrast to the preceeding groups, they are facultative aerobes and form characteristic colonies on aerobic haemoglobin glucose agar which are frequently different from those growing under anaerobic conditions. Some are most probably anaerobic species of the aerobic leptothrices to which they appear to be the most closely related in general morphology.

Group 10. This group are very strongly Gram positive and usually form medium to large, straight or curved rods having rounded or pointed ends; others again may appear as elongated cocci. Some species are rather similar to the Hofmann bacillus but tend to be larger. As with group 9 they are facultative aerobes although some of them do not grow well at first. Most of the members of this group are probably a few species of the very large and commonly met with aerobic group which are strongly facultative to anaerobic conditions of growth and, while only appearing under primary anaerobiasis, retain their power of reversion to aerobic habits of growth. Neisser's stain has shown no evidence of granules except for minute particles similar to those observed in the previous group.

TABLE 5.

CLASSIFICATION TABLE OF 312 ANAEROBIC DIPHTHEROID SPECIES OF COLONY AND MICROSCOPICAL CHARACTERISTICS, GRAM AND NEISSER STAIN, SHOWING THE RELATIONSHIP BETWEEN THE GROUPS, GIVING FIGURES AND PERCENTAGES.

Gram Neisser	Colony Characteristic Groups.						Microscopic Groups.	
Microscopic Grouping.	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Totals	Percentage.
Grp. 1 Granular.	44 35.8%	18 18.5%	3	6	3	0	74	23.7
Grp. 2 Granular.	44 35.8%	9 9.2%	0	4	2	0	59	18.9
Grp. 3 Granular.	17 11.0%	12 12.3%	3	4	4	0	40	12.8
Grp. 4. Granular.	3 2.3%	9 9.2%	1	4	0	0	17	5.8
Grp. 5 Granular.	1 0.7%	17 17.5%	8	2	1	0	29	9.2
Grp. 6 Pliom. Gram Negtv.	8	10	2	4	1	0	25	8.0
Grp. 7 Chained Type.	1	9	10	2	1	3	23	7.3
Grp. 8 Coccal	5	0	2	0	0	0	10	3.2
Grp. 9 Threading.	3	0	3	2	1	0	9	2.8
Grp.10 Aerobic Types.	1	4	1	0	0	0	6	1.9
Grp.11 Indefinite	0	9	3	3	5	0	20	6.4
Totals Cultural Groups	127	97	36	31	18	3	312	
Percentage. Groups	40.7	31.1	11.6	9.9	5.8	0.9		100%

The accompanying table has been compiled to indicate the main relationship between the colony types, which have been divided into 6 groups, and their microscopical characteristics which I have differentiated as far as possible on recognised lines of morphology.

Key to Colony Characteristics of the 6 Groups, 24 hrs. Growth.

Group 1. (40.7%) Medium sized, pin-head, raised, yellow-grey to yellow-brown, cohesive colonies, the latter showing slight haemoglobinolysis of the surrounding media.

Group 2. (31.1%) Small to very small, pin-head, raised or flattened, transparent or semi-transparent, watery colonies.

Group 3. (11.6%) Large to medium, flat, grey, transparent or matt, usually watery colonies.

Group 4. (9.9%) Medium, pin-head, raised, grey, transparent, watery colonies.

Group 5. (5.8%) Medium, pin-head, raised, grey or grey-white, cohesive colonies.

Group 6. (0.9%) Large, pin-head, raised or heaped, yellow, cohesive colonies showing slight haemoglobinolysis.

Note;- The percentage figures in brackets are calculated on the microscopic group totals.

ANALYSIS OF THE CULTURAL AND MICROSCOPICAL MORPHOLOGICAL TABLE. Examination of the table shows that correlation of cultural and microscopical morphology has not led to a completely clear division of species. This is hardly to be expected however since discrepancies in both methods of differentiation are inevitable owing to various factors which are partly beyond control, such as initial attenuation of growth, slight variation in cultural conditions, or the association of strongly growing colonies affecting the development of weaker species. Nevertheless certain broadly defined grouping can be made out which serves as a basis for further classification and study. Cultural groups 1 and 2 are the largest, forming 71% of the total, being 40.0% and 31% respectively. Group 3 falls far short but is next in numbers. The first cultural group contains a very large proportion of the granular types, groups 1 - 5 having 85.6%. Further subdivision of the microscopical characteristics show that groups 1 and 2 have the largest share and together form 71.0%. The second cultural group contains 66% of the five granular types but with a more even distribution of numbers. In contrast to this, cultural group 3 has 63% of non-granular types. The remaining cultural groups revert to the granular species but the numbers are scarcely large enough for comparison.

With regard to the microscopical group classification it will be seen that the granular types predominate, forming 70.4%, of which the closely-allied groups 1 and

2 form more than half.

THE RELATIONSHIP OF THE MICROSCOPICAL CHARACTERISTICS OF SOME OF THE GROUPS TO THE KLEBS-LOFFLER BACILLUS.

Owing to circumstances beyond my control I have not been able to examine at Wantage House many strains of Klebs-Loffler for purposes of comparison. However, from my experience of the hundreds of throat swabs from suspected cases and systematic searches for carriers in schools while at Southport, I believe I have acquired the necessary background of knowledge that enables the pathologist to give or to reserve a diagnosis of diphtheria. The microscopical morphological characteristics of groups 1, 2, and 3 are in my opinion identical with that of Klebs-Loffler, and, if they had been seen in smears from acute throats mixed up with other bacteria, no hesitation would have been felt as to a diagnosis of diphtheria. It will be seen later how closely in another respect some of the species examined conform to the Klebs-Loffler bacillus. Group 5 are also very similar in their general characteristics but belong to the less common Klebs-Loffler types. It is interesting too that the colony characteristics of group 1 resemble somewhat closely the growth of Klebs-Loffler on Loffler's medium and both types of colonies appearing on nutrient agar. Group 2 colonies however appear to have no analogous diphtheria colony prototype.

LATER MICROSCOPICAL MORPHOLOGY ESPECIALLY IN ANAEROBIC SUGAR BROTH. In common with the Klebs-Loffler bacillus, all the granular species of anaerobic diphtheroids undergo involutional changes which produce a multiplicity of size, shape, and aggregation of the metachromatic granules. Under broth conditions, especially in growths which have rapidly fermented sugars such as glucose, galactose, and laevulose, the interesting phenomenon of branching occurs. Careful observation shows that this process is identical with that described by Hill in 1901 (Ref. 3). L.Marten in 1924 (Ref. 4) describes this occurrence in Park and Williams No.8 strain, and Hill found it present in nine out of ten virulent strains. In 48 hrs. glucose broth cultures and fourth day sugar broths, branching, with and without polar bodies, has been observed in some species of the microscopical groups 1, 2, 3, and 6. I believe this feature, apart from others, helps to form a strong link which brings closer the relationship of some of the anaerobic diphtheroids to the bacillus of diphtheria. An investigation of the motility, presence of flagella, and spores has been made, and all have proved conclusively negative.

THE ANAEROBIC LEPTOTHRIX GROUP AND ALLIED FACULTATIVE AND AEROBIC SPECIES.

I have found that the literature referring to this group of bacteria contains only one reference to strictly anaerobic species and that text books give very little information. Ford Robertson, in his book

on Therapeutic Immunisation, pages 98 and 172, records the isolation of anaerobic streptothricees from the intestine in cases of diabetes, and in nervous or mental disorders. He refers to them as important neurotoxic organisms which proved to be pathogenic when fed to rats, causing emaciation and death. No mention is made however of the isolation of leptothrices. Bulleid (Ref. 5) in an experimental study of the relation of the leptothrix bacillus to tarter formation, describes its cultural and microscopical characteristics and gives the biochemical reactions of ten strains. It is Gram positive but variable, very pliomorphic, and a facultative anaerobe. No mention is made of metachromatic granules. Leptothrix Innominata and its action as a parasite is discussed by Miller (Ref. 6) who describes it as a microaerophile-anaerobe which hitherto, according to Goadby and Kuster, has never been cultivated. In morphology it has only a slight resemblance to some of the anaerobic leptothrices to be described, and is unlike those intermediate between them and members of the diphtheroid group. The latter are frequently facultative anaerobes. Although some of the anaerobic streptothrix group isolated by Ford Robertson may be closely allied, I have good reason to believe that the main members of the group now to be described present a type almost unknown to bacteriology. One of the grounds for this presumption is the fact that, even under the present

strict anaerobiasis, most strains are very delicate and do not readily survive subculture even in the anaerobic broth which has been found to suit them better than the surface culture. Further, none out of a very large number tried have ever grown when subcultured under aerobic condition; those that have adapted themselves to aerobic culture have shown quite a different microscopical morphology.

THE CULTURAL AND MICROSCOPICAL CHARACTERISTICS OF THE ANAEROBIC LEPTOTHRIX GROUP. The classification of this group has been based on the study of 141 strains isolated mainly from case groups 1, 2, and 3. The conditions of culture are those already described under the anaerobic diphtheroid group. They divide themselves into three main groups.

Group 1. 118 strains. After 24 hrs. growth the colonies of this group are readily distinguishable from those of the anaerobic diphtheroids. They are variable in size but usually large, being 2½ to 3 millimetres in diameter; smaller forms of 1 millimetre do occur but are not so common. Their contour is characteristic and may take one of two forms. The first and most frequent is like a broad cone. The second is flatter with a central point and raised margins; both have a rough surface giving them a sugared appearance which is absolutely characteristic. The margins of the colony are irregular, in keeping with the furrowed appearance of the surface. They are most commonly grey or yellow-grey in colour but transparent forms are met with. Some strains have a

distinctly haemoglobinitic action which gives the colony a yellow-brown colour. Their consistency forms another valuable means of differentiation, being intensely sticky, adhering to the media and, when picked up, pulling out to a long thread before taking the point of the needle.

At 48 hrs. most colonies are increased in size and density and the haemoglobinitic strains show a deep brown or cream colour. The roughness of the surface has usually increased and the margin is more irregular.

Group 2. 15 strains. After 24 hrs. growth members of this group are usually small, varying between 1½ and 2 millimetres. The contour of the colony is gently rounded and the surface, while being somewhat glazed, is also slightly pitted which gives its grey or grey-white colour a mottled appearance. The margin is much smoother than in group 1 but in consistency the colony is characteristically sticky and adherent.

After 48 hrs. culture growth has continued but is usually slow. Some strains develop a slight action on the haemoglobin, giving the colony a light yellow colouration, the surface appearances remaining unchanged.

Group 3. 8 strains. At 24 hrs. the colonies vary between 1 and 2½ millimetres in diameter and are usually quite flat, having a finely grained surface. They are colourless, being either transparent or slightly opaque. Consistency varies, some being very slightly sticky while others are brittle and a few are watery.

After 48 hrs. culture growth continues but no definite changes are apparent.

MICROSCOPICAL CHARACTERISTICS. As these are relatively stable when compared with the anaerobic diphtheroid group the following description has been taken from smears of primary growths and of subcultures.

Group 1. The members of this group form a tangled mass of long, thin, even, filaments with only very occasional segmentation of the protoplasm. In only two, bulbous dilatations have been observed appearing at wide intervals along the filaments, while thicker filaments occur in some species. In old cultures a definite sheath can be made out but this is not well defined in the earleir stages of growth unless segmentation has occurred. Staining is most variable with Gram, a few being Gram positive but the majority half Gram positive with filaments which do not take on the stain. Others again tend to be completely Gram negative. About 80% of the strains in this group have the distinguishing feature of containing numerous metachromatic granules; these are best seen on smears stained by Gram or Neisser made from primary growths on the anaerobic agar slopes. By Gram they are either purple or deep red in colour while Neisser's modified stain gives them the inky black colour of the diphtheroid group.. Their arrangement varies, in some strains being close together just as they would appear if short bi-polar rods were strung together and held by a sheath, in others some

distance apart. Their size varies also but is usually small and rarely causes beading. In older cultures the granules tend to disappear and rarely become irregular or larger. The question of branching has been carefully studied and it has been found difficult to distinguish apparent branching due to artifact, and that which is true dichotomy. None of the strains in this group exhibit true branching, a fact which merits their relegation to the leptothrix group of bacteria.

Group 2. 15 strains. The general characteristics of group 1 apply to this group with however the following differences. The filaments are thinner and more delicate, segmentation is more frequent but without fragmentation of the sheath, and in some the filaments are made up of short tapered rods enveloped in a thin sheath. Nearly all the species examined are Gram negative, and metachromatic granules are either absent or very sparce. Branching does not occur.

Group 3. 8 strains. This group exhibit quite different characteristics from the two preceeding ones. They form short, thick, often irregular filaments which frequently show segmentation resulting in breaking of the sheath; thus rod forms of varying lengths can be found. The larger filaments are characterised sometimes by showing curled-up slightly bulbous ends, but only two suggested that true branching was present. Granules have not been found in this group. There is reason to suspect that some of the threading species of diphtheroids in group 9 should

be included here; only three however have colony types which approximate to the present group in character.

THE RELATIONSHIP OF THE ANAEROBIC DIPHTHEROID TO THE LEPTOTHRIX GROUP. On a morphological basis alone it will be seen that there are structural features common to both groups, the first and perhaps the most striking being the presence of metachromatic granules, both groups alike containing species in which these are either numerous or absent. The second is that certain species of both groups at some stage of development have filaments and rod forms together. Lastly, under certain conditions the Klebs-Loffler and the anaerobic diphtheroids develop filamentous forms which some observers hold show true dichotomy. In regard to pathogenic action there are good grounds for asserting that the leptothrices produce a neurotoxin very similar to the Klebs-Loffler and the anaerobic diphtheroid group.

VI. BIOCHEMICAL REACTIONS OF ANAEROBIC DIPHTHEROID AND LEPTOTHRIX GROUP.

ANAEROBIC DIPHTHEROID GROUP.

A description of the technique has already been given but it is necessary to add that the purity of the growths in each series of tests has been carefully checked for possible contaminations and singleness of species. All the reactions have been noted on the fourth day, after application of the air test. Of the twenty-one anaerobic diphtheroid strains all but two fermented glucose strongly, the reaction usually starting on the first or second day. The two giving weak reactions also showed only slight change in other sugars, probably owing to intial attenuation. Anaerobic strains 14, 16 and 18 gave strong acid reactions in Hiss's serum broth with clot formation in one. Strain 17, the only facultative anaerobe, produced strong acid and clot, anaerobic conditions similar to the other sugar broths being applied.

Only one strain fermented lactose strongly. Four gave plus reactions and a similar number produced only slight change, usually on the fourth day. Twelve were negative.

Saccharose fermenters were found in four strains; two gave slightly acid reactions and one a doubtful trace, the fermentation power of the last strain being strong in three diagnostic sugars, including saccharose. No change occurred with seventeen strains.

Maltose was fermented by all strains, twelve giving markedly acid reactions and six strains plus acid, while three showed slight change. Two of these were the attenuated strain referred to previously.

In mannite acid reaction failed to appear in all but one which gave plus acid reaction on the fourth day.

Galactose was tested in fourteen strains. Seven produced marked fermentation, one of these causing decolouration. Five gave a plus acid reaction. Two were negative, one of these being the weakly fermenting strain referred to. The other was tested twice and remained negative but fermented glucose, maltose and laevulose markedly.

The same strains showed variable reactions in dextrin, seven being negative, three slightly acid, two definitely acid, and two markedly fermented.

Of eight strains tested in glycerin none showed any fermentation reaction.

Laevulose gave marked acid reaction in seven out of eight strains tested, the remaining one producing definite but less fermentation.

The facultative anaerobic diphtheroid No. 17 produced strong fermentations in glucose, maltose, galactose, and laevulose, acid reactions in lactose and dextrin, and negative results in saccharose and mannite.

The aerobic diphtheroid and Klebs-Loffler fermented none of the sugars, the latter producing very

slight growth but not appearing able to adapt itself to the strict anaerobic conditions. Unfortunately circumstances did not make it possible for me to obtain further strains of either virulent or avirulent Klebs-Loffler as the reactions would have formed an interesting comparison.

TABLE 6.

ANAEROBIC LITMUS BROTH REACTIONS OF 21 ANAEROBIC DIPHTHEROID STRAINS.

NUMBER	PATIENT	CASE GROUP	SOURCE OF INFECTION.	COLONY GROUP	MICRO GROUP	1 GLUCOSE	2 LACTOSE	3 SACCHAROSE	4 MALTOSE	5 MANNITE	6 GALACTOSE	7 DEXTRIN	8 GLYCERIN	9 LAEVULOSE
1.	A	1	Stool	2	1	+	±	-	+	-	.	.	.	.
2.	B	1	Stool	1	1	++	-	-	++	-	.	.	.	.
3.	B	1	Stool	3	4	±	-	-	±	-	.	.	.	.
4.	B	1	Stool	2	6	+	±	-	++	-	.	.	.	.
5.	C	1	Stool	1	2	+	-	-	±	-	.	.	.	.
6.	D	3	Tonsil	1	5	++	-	-	+	-	.	.	1	.
7.	D	3	Stool	1	1	++	-	-	++	-	.	.	.	.
8.	E	1	Tonsil	2	5	++	+	-	++	-	++	-	.	.
9.	F	1	Stool	1	1	++	-	-	++	+	++	-	.	.
10.	G	1	Stool	1	1	±	-	-	±	-	-	±	.	.
11.	H	2	Stool	1	1	++	+	Tr	++	-	++	±	.	.
12.	A	1	Stool	1	1	+	-	-	+	-	+	-	.	.
13.	B	1	Stool	2	6	+	-	-	+	-	+	-	.	.
14.	B	1	Stool	2	6	D	±	±	++	-	++	++	-	++
15.	J	1	Stool	1	1	++	±	-	+	-	D	-	-	++
16.	K	1	Stool	1	6	+	-	-	+	-	+	-	-	+
17.*	K	1	Stool	-	-	D	+	-	D	-	++	+	-	++
18.	L	1	Stool	1	2	++	++	++	++	-	++	+	-	++
19.	J	1	Stomach	1	3	++	-	-	++	-	-	-	-	++
20.	B	1	Stool	1	1	++	-	-	++	-	++	±	-	++
21.	B	1	Stool	3	7	++	+	-	++	-	+	++	-	++
22.	D	3	Stool	5	4	D	+	±	++	-	+	+	-	++
23.	M	1	Stool	-	-	-	-	-	-	-	-	-	-	-
24.	-	-	Throat	-	-	-	-	-	-	-	-	-	-	-

KEY.

- = No change in reaction.

Tr = Trace acid.

$\pm$ = Slightly acid.

\+ = Acid.

++ = Markedly acid

D = Strongly acid (decolourised).

. = Not tested.

* = Facultative anaerobe.

NOTES ON REACTIONS AND CASES.

No. 1. Tested twice with same reaction.

No. 2. Tested three times with same reactions; this case has five different anaerobic diphtheroid species in the intestine.

No. 6. Slight post-nasal catarrh, history of recurrent septic throats in childhood for many years, assigned cause bad drains. Two species of anaerobic diphtheroid in intestine.

No. 8. Acutely inflamed and odematous throat in very toxic, confused patient.

No. 9. Mannite acid fourth day, broth culture markedly toxic to guinea-pigs.

No. 17. Aerobic diphtheroid from chocolate agar, strongly facultative anaerobe, non-metachromatic, sheath type?

No. 18. Reactions tested twice with same result.

No. 19. Galactose reaction tested twice with same result.

No. 21. Chained type which developed rod forms and

numerous metachromatic granules in the sugars.

No. 23. Aerobic diphtheroid from chocolate agar, metachromatic and non-facultative.

No. 24. Klebs-Loffler bacillus non-virulent.

Strains 14, 16, and 18, gave strong acid reaction in Hiss's serum broth on the fourth day under anaerobic conditions. In one, No. 16, clot was produced.

Strain 17. The only facultative anaerobe tested, gave strong acid and clot.

ANALYSIS AND SIGNIFICANCE OF RESULTS. Most authorities are now in agreement as to the fermentation reactions of the Corynebacterium diphtheriae; some nevertheless still doubt the sharp distinction made between the bacillus diphtheriae, virulent and non-virulent, and the diphtheroids, on account of the former's inability to ferment saccharose, Hewlett and Bulloch (Ref. 7) in the Medical Research Council "System of Bacteriology" No. 5 for 1930, pages 81 and 138, state that the diagnostic reactions in Hiss's serum-water on the fourth day are acid in glucose and galactose, and negative in saccharose. The bacilli giving this reaction may be either virulent or non-virulent. The other sugars which the bacillus diphtheriae ferments are maltose, glycerin, and dextrin, mannite never being fermented. In 1924 Barratt's (Ref. 8) and O'Kell and Baxter's (Ref. 9) careful work showed that the anomalous results with lactose in the past were due to alteration by heating, and that unheated lactose is never fermented. Barratt further considered that dextrin is a substance too uncertain in composition to constitute a reliable reagent. Kliewe in 1926-7, on the other hand, in examining one hundred and thirty strains of the diphtheriae bacillus and other corynebacteria found some that were saccharose fermenters. Graham-Smith (Ref. 2. page 160) in testing a number of bacilli, obtained from outbreaks of diphtheria in six villages, found that nineteen out of twenty-two strains from one village fermented saccharose. The other

sixteen strains from the remaining villages did not ferment this sugar. From a large number of fermentation tests he concluded that all strains of diphtheria bacillus produce acid from glucose, galactose, laevulose and maltose, and the majority from dextrin and glycerin. The action on lactose is very variable, and only a few strains act on saccharose. All his tests on mannite yielded negative results.

I have outlined the biochemical investigations of different authorities as their views, especially those relating to the more controversial sugars, have a bearing upon the probable significance of some of the anaerobic diphtheroid group under consideration. In this series of tests lactose has been sterilised by heating and is thus unreliable, according to Barratt, In spite of this, only nine strains produced acid, and of these, four were slight reactions. Of the twenty-one anaerobic strains tested it will be seen that Nos. 8, 12, 13, 15, 16, 20, and 21 gave the fermentation reactions of the corynebacterium diphtheriae. The facultative anaerobe examined, No. 17, likewise gave similar reactions. With regard to the other sugars, all seven fermented maltose, and of four tested in laevulose all produced marked fermentation. In dextrin only two acted on the sugar, and it is interesting to note that none fermented glycerin. The last two results may be significant in distinguishing them as anaerobic variants of the Klebs-Loffler bacillus. When these species are relegated to their cultural

and microscopical groups it is noteworthy that four have the colony characteristics of group 1 which correspond somewhat closely to those of the Klebs-Loffler bacillus on other media. The remainder belong to either group 2 or 3, a fact which at present leaves them without possible aerobic antecedents. Their microscopical morphology places four strains amongst the definitely granular types, three belonging to group 1 which has characteristics of the more commonly met with forms of Klebs-Loffler. The one strain in group 5 belongs to a less common type , while the chained form of group 7 is comparatively rare. The last strain belongs to group 6 whose characteristics conform to no known variant of the Klebs-Loffler, and which therefore must for the present be considered as an exclusively anaerobic species. Strains 11, 14, 22, and 18 gave Klebs-Loffler reactions except for their fermentation of saccharose. The first three gave some or very slight reactions, and it is therefore a matter of opinion as to their classification. Two have Klebs-Loffler types of colony, and three belong to the granular forms. The remainder of those tested on the nine sugars must be classified with the diphtheroid group. One fermented mannite which is a rare occurrence, only one out of one hundred and fifty diphtheroids examined by Barratt acting on this sugar. Two gave negative reactions in galactose, one however being a feeble strain which fermented glucose and maltose only slightly. The other, after being tested

twice in galactose, remained negative. Strains 1 - 7 were only tested in the first five sugars and are not conclusive; all however fermented glucose and maltose, and none saccharose. It is possible that some would have given Klebs-Loffler reactions had they been tested in galactose.

While it is necessary for further work to be carried out in order to give additional support to the evidence already obtained, it is, I believe, an important fact that strictly anaerobic Klebs-Loffler species exist and that the majority of them have their nidus in the intestine. Proof of this assertion will be given when the findings in the four groups are compared.

TABLE 7.

ANAEROBIC LITMUS BROTH REACTIONS OF 11 ANAEROBIC LEPTOTHRIX STRAINS.

NUMBER	PATIENT	CASE GROUP	SOURCE OF INFECTION.	COLONY GROUP	MICRO GROUP	1 GLUCOSE	2 LACTOSE	3 SACCHAROSE	4 MALTOSE	5 MANNITE	6 GALACTOSE	7 DEXTRIN	8 GLYCERIN	9 LAEVULOSE
1.	D	3	Stool	2	2	-	+	-	+	-	.	.	.	.
2.	F	1	Stool	1	1	++	-	+	-	-	+	++	.	.
3.	G	1	Stool	1	1	++	-	+	+	-	++	++	.	.
4.	N	1	Stool	1	1	+	+	++	+	-	+	++	.	.
5.	J	1	Stool	1	1	-	-	-	-	-	+	+	-	+
6.	O	1	Stool	1	1	+	±	-	-	-	±	++	+	++
7.	P	1	Stool	1	1	+	+	-	±	-	+	+	-	+
8.	D	3	Stool	3	3	D	+	±	D	-	+	+	-	+
9.	D	3	Stool	1	1	+	+	+	+	-	-	+	-	+
10.	R	1	Stool	1	1	±	±	-	±	-	-	-	-	-
11.	M	1	Stool	1	1	-	-	-	-	-	+	-	-	±

KEY.

Sugars read on fourth day.

- = No change in reaction.
± = Slightly acid.
+ = Acid.
++ = Markedly acid.
D = Strongly acid (decolourised)
. Not tested.

THE ANAEROBIC LEPTOTHRIX GROUP.

The fermentation reactions of this group are on the whole slower to develop (3 - 4 days) and are less vigorous. Even when allowed up to ten days for development practically no further change in acid production occurs. Owing to the relatively small numbers tested and the somewhat variable reactions, no definite grouping is as yet possible. A fairly large number have been tested, but, owing to the spreading nature of the colony, absolutely pure growths are difficult to obtain. Examination on the fourth day has shown in many the presence of small numbers of diphtheroids which are only to be detected on microscopical examination. Such tubes have of course had to be discarded.

Glucose is acted on strongly by the majority of strains, lactose and maltose to a lesser degree and only by seven out of eleven tested.

On saccharose less than half produced acid reaction, while none touched mannite.

Up to the present dextrin appears to be the sugar most readily and strongly fermented, eight out of ten strains producing acid and four of these being strong reactions.

Galactose was fermented by the same number of strains but not all to the same degree, while six out of seven strains acted similarly on laevulose.

Glycerin was only acted on by one strain. Only two strains, Nos, 6 and 7, produced changes in any way similar to the Klebs-Loffler bacillus.

The biochemical reactions given by Bulleid for ten strains of the leptothrix Buccalis in aerobic nutrient sugar broth, compare with my own series only on galactose, seven out of the ten strains producing slight to marked reactions. For the present at least, there is no indication that the anaerobic leptothrix species are derived from the leptothrix Buccalis, in spite of the fact that from my own observations tarter and caries in mental patients are remarkably common. Attempts to establish the relationship between the two bacilli are being made.

VII. ANIMAL EXPERIMENTS.

THE ANAEROBIC DIPHTHEROID GROUP.

With the time at my disposal it has only been possible to carry out a limited number of experiments. In all of them the hypodermic method has been used with the purpose of ascertaining the virulence and toxicity of both anaerobic groups of bacteria. Well-grown guinea-pigs of about 400 grams weight from exceptionally healthy and vigorous stock have been employed.

Bacterial emulsion, whole sugar broths, and supernatant broth fluids have been used. Seven animals have been innoculated subcutaneously with two strengths of bacterial emulsion from the anaerobic agar slopes, some being given 2000 mill., others 4000 mill. In all subsequent experiments the intraperitoneal route was adopted. One and two cc. supernatant fluid from 48 or 72 hrs. anaerobic broth cultures were injected into two animals. The effect of this fluid heated at 60°C for half an hour was also tried in two other pigs. Later four animals were infected with whole sugar broth cultures containing four different anaerobic strains, the amount given to one being 1 cc. and to the others 4 cc. A Klebs-Loffler type culture from the Lister Institute grown in aerobic sugar broth was tested, 1 cc. of the broth having no effect. Two control experiments were made with sterile uninnoculated sugar broths, intraperitoneal innoculation having negative results. In all, eighteen different experiments were made with eleven strains of anaerobic

diphtheroid, two of which gave Klebs-Loffler sugar reactions. None proved virulent but one was toxic and another produced later pathogenic effects. The former strain (No. 9, Table 6) fermented mannite, and the contents of the tube, amounting to 4 cc., was injected. In about four hours the animal was acutely ill and remained so for 36 hours during which time it refused food and lost 120 grams in weight. In another 24 hours it had recovered and has remained healthy. One of the early experiments had an interesting sequel which, although not conclusive, raises questions bearing upon the pathogenic action of some of the anaerobic species.

A female guinea-pig, injected subcutaneously with a 2000 mill. emulsion of strain 12 isolated from the stool in Case A, (see Table 6) suffered no immediate toxic effects and remained apparently well for nearly four months. It then dropped a small but healthy pig. Quite unexpectedly both were found dead a month later, death having occurred during the night. On examination the following morning there was no evidence of injury and both appeared to be in good health. The only pathological lesion found on post-mortem in both animals was in the colon, the proximal coils of the large intestines being greatly distended, while in contrast to this the distal portions were empty and contracted. The appearances suggested obstruction at one of the flexures. On opening the distended portions by cautery they were found to be loaded with soft

faecal material. Both stools were cultured, aerobic growth yielding only coliforms and sporing bacteria. The anaerobic tubes however showed, in both, large numbers of metachromatic diphtheroids almost identical in cultural and microscopical characteristics to that innoculated. In the case of the mother at least, it is obvious that the organism, in reaching the colon, may have caused marked changes and paresis of the muscular wall resulting in acute toxaemia and death. The manner in which the offspring became infected is a matter for conjecture. Unknown to us the mother was pregnant when injected, and it is just possible that infection may have occurred in utero, or, what is more probable, from the mother's milk. The fact that they died simultaneously was most probably chance, but, on the other hand, there is no doubt as to the cause of death in both. The main observation to be made from this experiment is that it suggests the affinity of this organism for the lower alimentary canal which it must have reached via the inguinal and pelvic lymphatics. I have carried out bacteriological examinations by anaerobic methods in a number of guinea-pigs both at Wantage House and at Southport, and in none were anaerobic diphtheroids isolated.

To summarise the results, none of the anaerobic diphtheroid strains proved virulent, while the toxins from two likewise had no effects. Only one caused toxic symptoms, and another strain apparently caused death by toxaemia and marked changes from infection

of the colon. It is essential to add however that my cultural technique did not, for two reasons, fulfil the conditions necessary to obtain the maximum production of toxin. The fact that sugar broth and glucose agar cultures were used, must have reduced the available amount of toxin, and also the conditions of free aeration during incubation of the growing broth culture, which are so essential to toxin production, could not obviously be fulfilled. This latter fact may however have an interesting bearing upon the question of toxin liberation by strict anaerobes both growing in culture and in vivo.

These findings are in agreement with Ford Robertson's experimental work with the bacillus paralyticans isolated from cases of general paralysis. Guinea-pigs were immune, although some strains were however virulent and toxic to mice. His other experiments, especially those in which rats were fed with cultures of the bacillus paralyticans, were very striking, not as evidence of their toxicity but as proof that this organism was pathogenic to most of the animals, symptoms and morbid changes corresponding to the disease being reproduced and verified later on histological examination.

The collective experimental evidence therefore shows that neither aerobic or anaerobic diphtheroids, nor those having Klebs-Loffler reactions which are isolated from mental patients, can produce lethal quantities of toxin. The fact remains however and it

has not yet been refuted, that under suitable conditions of experiment, some are slowly virulent to rats and rabbits and produce effects closely related to nervous and mental disorders leading ultimately to death.

It is common knowledge that the pathogenic action of the diphtheria bacillus is dependent upon two factors, virulence and toxicity, and, although to some extent always related, it is however the latter that decides its pathogenic importance in man and in experimental tests. This criterion has unfortunately taught medical opinion to believe that the non-virulent B. diphtheriae are only to be regarded as potential pathogens, and, what is more important, that the allied diphtheroid species with a few exceptions have no pathogenic significance. The experience of this research, and the evidence which has been obtained, lead me to hold that these views must undergo very considerable modification. More suitable methods of cultural technique will, I believe, prove that many anaerobic species of diphtheroid and Klebs-Loffler can produce small but estimable amounts of exotoxin, although this must be minimal owing to the fact that oxygen has little or no part in their metabolism. In other words they are biochemically incapable of being highly toxic. Another factor, that of attenuation, may contribute to a lower level of pathogenicity. It has to be appreciated however that this factor is partly relative to artificial conditions of growth and may be a

misleading criterion when assessing pathogenic activity in vivo where the susceptibility of the individual plays an important part. The endotoxic properties of the B. diphtheriae are known to be distinct from its exotoxin, but experiments on guinea-pigs by Hewlett in 1912 have shown that endotoxin can confer a considerable protection against innoculation with living diphtheria culture. This fact I believe constitutes the link between the highly exotoxic and relatively non-toxic groups, as it is a feature common to both. Unfortunately most authorities claim that endotoxic power is of little pathogenic importance. This belief is contrary to the evidence I have obtained with regard to the anaerobic species, whose loss of exotoxic action would appear to have greatly enhanced their endotoxic powers. From my personal experience this statement receives support from a mass of accumulated fact relating to the results of therapeutic immunisation in mental cases in the study of their focal, systemic, and specific reactions to the diphtheroid group. Having a vital bearing upon this problem is the question of the extent of the focus of infection. It can be shown that the chief nidus of these anaerobes is the large intestine, a focus which is incomparably larger than the confined area of the fauces. Further, anatomically and physiologically the colon has a much greater potentiality as a chronic focus of infection from which special toxins can be absorbed. From this

fact, were the anaerobic diphtheroid group endowed with similar virulence and toxogenic action as the epidemic Klebs-Loffler, it is inconceivable that the economy would survive more than a few hours. That they are not so is in harmony with their special toxic effects which will be discussed later. In place of virulence, we are faced with a sub-lethal poison, coming from a wide focus and having a specific neuro-toxic action, whose place in time is not to be estimated in terms of hours, or even weeks, but of years. I firmly believe that the present views on the non-pathogenicity of most of the diphtheroid group is untenable, and that the anaerobic species of diphtheroids, including those that have now been ascertained to be Klebs-Loffler, are amongst some of the most important etiological factors in mental disorder and allied states. This assertion is only made having due regard, not only to the vital importance of the soil, but also to the situation and extent of the focus of infection.

THE ANAEROBIC LEPTOTHRIX GROUP.

Only four strains have been tested experimentally, either bacterial emulsion or whole sugar broths being used and the amounts varying from 1 cc. to 6 cc. No lethal or toxic effects were noted in the four animals innoculated by the intraperitoneal route. The same factors militating against the development of toxicity enter into these experiments however, although it is probable that, in keeping with the anaerobic diphtheroid

group, they are endowed with only slight exotoxic properties. In spite of this non-toxicity, Ford Robertson's feeding experiments on rats with the closely related anaerobic streptothrices isolated from the intestine, show very definitely that they are subvirulent and pathogenic, producing severe morbid changes of a character which ally them very closely to the anaerobic diphtheroids in their toxic action.

VIII. ANALYSIS OF THE AEROBIC AND ANAEROBIC BACTERIOLOGICAL FLORA OF THE MINOR AND MAJOR FOCI IN THE FOUR CASE GROUPS.

In order to ascertain the significance of the bacteriological findings in the large number of cases under review, it has been necessary to resort to a number of tables which will I hope form a sufficiently clear explanation of the facts they are intended to convey. With regard to assessment of the bacterial flora, any method of estimation in numerical terms is open to a certain amount of fallacy. In this research experience obtained from culturing fresh samples of the stool at intervals of a day or so in the same cases, showed that, within certain limits, variations in the numbers of bacterial species almost always occur, although each may be consistently present. It was therefore obvious that any very exact method of enumeration was unnecessary so long as the total value of the cultures was constant and the method of innoculation uniform. The terms in signs of plus and plus-over-minus have been adopted, these adding up to the maximum figure of eight plus in any one culture. In the case of anaerobic cultures where three tubes are used, the flora of each is assessed and the average taken. The maximum figure of eight was chosen in order to allow for adequate expression where a large number of species were found. The following scheme shows the divisions and the relation of the numerical values to the terms expressing the degree of infection;

approximate figures in percentage of the maximum culture are also given for those who may be accustomed to assess cultural flora by this method.

Estimation of One Bacterial Group.

$\pm$ and 1+	= Mild infection.	20%
1½+ to 3+	= Moderate to moderately severe infection.	40%
3½+ to 6+	= Severe to very severe infection.	80%

Estimation of Two Combined Bacterial Groups.

$\pm$ to 2+	= Mild to moderate infection.	25%
2½+ to 4+	= Moderate to severe infection.	50%
4½+ to 6+	= Severe to very severe infection.	75%
6½+ to 8+	= Exceptionally severe infection.	100%

In practise this method has worked satisfactorily and provides a means of estimating the cultural flora, which, while easily carried out, determines I believe with sufficient accuracy the numbers of bacteria in any focus.

THE MINOR AND MAJOR FOCI OF INFECTION AND THEIR RELATION TO EACH OTHER. The term minor foci of infection has mainly an anatomical significance and does not imply that such are necessarily relatively less important as pathogenic entities. The large and

small intestine have been called the major foci in distinction to more circumscribed regions such as the tonsils, teeth, etc. for which see Table 8. This arbitrary division has been made with the purpose of conveying a clear impression of the pathological relationship of one focus to another, and is the logical outcome of much systematic bacteriological research in the tracing of infection from one focal source to another. Oral sepsis, which, broadly speaking, includes tonsils, antra, sinuses, etc., in relation to the development of infective cranial lesions, has been the subject of a noteworthy piece of research by Reynolds. In the pathology of mental diseases bacterial dissemination in the opposite direction has received however relatively little attention. The researches of Ford Robertson and McRae (Biblio. 8 - 17) in general paralysis form one of the first contributions on this subject. During the past four years my efforts have been concentrated upon tracing the paths of bacterial spread from oral sources to the large intestine. Between the two foci is the stomach whose mucous membrane may become the site of severe accumulative infection from the mouth. The bearing that lesions of this organ may have upon the state of the intestine has been studied in 116 mental cases with a view to ascertaining the relationship.

I have found that even severe infection of the stomach such as occurs especially in achylics and achlorhydrics, although often more severe than that existing in the mouth which has frequently been

relieved of its sepsis, does not seem to increase the incidence of bacterial invasion of the large intestine. There is only one exception to this in my experience and this relates to the severe secondary and pernicious anaemias. The next fact elucidated has an important bearing upon the appreciation of how focal infections may reach the lower bowel. It was found that, in spite of the strong bactericidal action of the high acid content in hyperchlorhydrics, that the incidence of oral streptococci, and in some cases anaerobic organisms, was much higher than in the anacid groups. There seems to be only one logical explanation for this; that oral infection, to reach the intestine in these cases, must travel via the lymphatics of the mediastinum and mesentery. Blood-borne infection is probably less frequent but is instanced in the production of gastric and duodenal ulcer, which developes as a natural result of the highly autolytic qualities of the gastric secretion. Dissemination of infection via the lymphatics has long been recognised as the common pathway of tubercle, the early appreciation of which has been due to the readily identified morbid changes in the glands and the peculiar staining qualities of the tubercle bacillus. With other bacteria however, evidence of this nature is much harder to obtain and it has been necessary to build up a similar hypothesis by less direct methods of research. In spite of these difficulties the observations of others as well as myself leave little doubt I believe

that pathogenic bacteria of the more common type take exactly the same pathways and can be disseminated throughout the body. If this is true of physical diseases, experience leads me to believe that in mental disorders it has an amplified significance, for, in addition to providing a channel for the well recognised aerobes, the special type of bacteria peculiar to insanity, by taking the same paths, are enabled to reach the intestine which, from all the evidence obtained, is especially suited to their growth. Three other sites of focal infection, though occurring less frequently, may however take precedence.

The first are the brain, spinal cord and membranes. Ford Robertson and McRae (Biblio. 8 and 19) have shown that in general paralysis diphtheroid bacilli can be grown from the brain and cerebro-spinal fluid. My own observations in the same disease show the presence of living anaerobic diphtheroids in four out of six cases. In other mental states similar organisms were isolated in three out of seventeen cases. One was a well-defined post encephalitic and the others possessed symptoms suggestive of this condition. While much research is required to determine the exact pathogenic importance of these attenuated and anaerobic bacteria, the fact remains that they do exist and cannot be regarded as contaminations.

The second foci to be referred to are the uterus and adenexa. Their close anatomical relationship to the colon and constant state of physiological activity

render them a peculiarly vulnerable and suitable nidus. Ford Robertson's bacteriological researches (Biblio. 17 and 24) in general paralysis and in cases of neurasthenia show the prevalence of their infection by the anaerobic diphtheroid group. My own observations are limited, but two cases, both young virgins whose mental state was greatly exaccerbated by the onset of the menses, proved to have severe infection of the endocervix. This was almost exclusively anaerobic and, when compared with their intestinal flora, was virtually a reflection. I cite these two cases since the only possible paths of infection were either via the lymphatics or the blood stream.

The third foci are the sinuses. Only one case of sphenoidal infection has come under my observation, but there is no doubt that the draining of the one involved led to rapid disappearance of the physical signs and symptoms, and complete recovery mentally. An aerobic and anaerobic diphtheroid formed the main infective factor in the sinus.

CULTURAL EVIDENCE OF SPREAD FROM MINOR TO MAJOR FOCI OF THE ANAEROBIC DIPHTHEROID AND LEPTOTHRIX GROUP.

Obtaining evidence of spread of the strict anaerobes is much more difficult than with the streptococcal group. This is partly due to attenuation in the minor and original focus, and also to the fact that in the course of years the bacteria may be eliminated. In four cases however, anaerobic diphtheroids

have been isolated from the debris in the tonsillar crypts, the infection being severe in one, and in another two species being present, one a facultative anaerobe. In the throat, the organism was sparce. Examination of the intestinal flora at the same time showed in each case morphologically identical organisms growing as strict anaerobes. In all four cases they were present in large numbers, and were relatively much more vigorous than in the original focus. Further observations in two cases showed that, while treatment eradicated the organism from the tonsillar focus, it was possible only to modify the degree of infection in the intestine. The enucleation of tonsils or the extraction of teeth, helpful as it may be in relieving the economy of the initial source of infection, is, in the majority of mental patients, like pulling up a weed long after the seeds have been scattered in the surrounding soil. Examination of the tables will show how the colon, and possibly the small intestine, form the final and by far the most severe and extensive focus of infection for the anaerobic diphtheroid and leptothrix groups of bacteria. Further, the method of spread suggested by clinical and therapeutic observations indicate that these infections are deeply seated, being sub-mucous, lymphatic, and possibly lacteal.

Anaerobic diphtheroid infection of the teeth likewise exists, but not to the same extent as in the tonsils. Granulomas have been found to contain similar bacteria and in one instance the organism was traced to the intestine.

Efforts to identify the anaerobic leptothrix group in the minor foci, especially the tonsils and teeth, have not been so successful. No cultural growths have been obtained from the tonsils but evidence of another kind has been available. In smears of the cheesey debris from tonsils, long filaments which are definitely metachromatic have been observed. Except for being thicker, these are very similar to the metachromatic filaments of the group 1 anaerobic leptothrix found in the intestine.

From teeth and gums however, both aerobic and anaerobic species have been isolated in culture, but none of them belong to the delicate, strictly anaerobic, group 1 type. It is probable that group 1 organisms do exist in both tonsils and teeth but are too attenuated to be cultivated. The fact however that they are relatively easily isolated from the intestine is further proof of the suitability of the conditions of growth there.

One other question deserves consideration here. It may well be asked, how and when do these minor focal conditions originate, and what is their relationship to the development and onset of physical and mental illness? The answer to the first question is problematical and its study would involve bacteriological research from birth to the grave. From evidence I have obtained that anaerobic species of the Klebs-Loffler bacillus do exist, it is reasonable to suspect, amongst many instances that might be cited, that in

the course of diphtheria epidemics, whether those infected take the disease or not, the bacillus finds in some of those that become carriers a suitable soil in which to become firmly entrenched, taking on anaerobic habit of growth. The subsequent course of events must be dependent upon numerous agencies, some of which are largely beyond control. The factor of inherent somatic immunity in determining the varying degrees of resistance or susceptibility of one system and another in each one of us, must play a part in deciding the ultimate fate and distribution of anaerobic infection. In some, dissemination by the lymphatic paths will proceed until major focal infection is established. The age period at which initial invasion occurs is mainly circumstantial but I believe may partly determine, in psychopathic individuals, the physiological epoch at which physical breakdown and mental disorder eventuates. To cite what may be extreme examples of early major infection and its results, I have on record two children of about 3 years of age, both acutely ill and toxic, One, if its mentality had been adult, would I believe have been recognised as being mentally ill; this case had, apart from other organisms, severe anaerobic diphtheroid infection. In the other case large numbers of anaerobic leptothrix were isolated. Both children died without a definite diagnosis of their condition being made. The facts just mentioned are I believe closely related to the development and onset of mental illness,

to which it is hardly necessary to add the well-known, mainly precipitating cause, the effect of environment upon the psychic mechanism. It is unfortunate, in the light of our present knowledge, that so much stress is frequently laid upon this as being definitely and wholly etiological to so many mental disorders.

TABLE 8.

THE INCIDENCE OF AEROBIC AND ANAEROBIC DIPHTHEROID AND LEPTOTHRIX INFECTION IN THE MINOR FOCI. GROUP 1 CASES.

Focal Site.	Number of Foci.	Aerobic Diph-theroid.	Anaero-bic Diphth.	Aerobic Lepto-thrix.	Anaero-bic Lepto.
Tonsil	42	4 (9.5%)	17 (40.4%)	2 (4.7%)	-
Nasal	7	4 (57.1%)	-	-	-
Sphenoid	2	1	1	-	-
Antrum	6	1	-	-	-
Dental	44	10 (22.7%)	13 (29.5%)	2 (4.5%)	3 (6.8%)
Stomach Res.Juice	67	7 (10.4%)	9 (13.4%)	3 (4.4%)	3 (4.4%)
C.S.F.	22	-	7 (31.8%)	-	-
Ear	2	1	-	-	-
Breast Milk	1	1	-	-	-
Pleural Fluid	2	1	-	-	-
Sputum	1	-	1	-	-
Urine	5	-	1	-	-
Os Uterae	6	3	1	-	-
Total Foci	207	33 (15.4%)	50 (24.2%)	7 (3.3%)	6 (1.9%)
Intestinal Total and Percentage	145	(16.5%)	(88.8%)	(6.2%)	(58.2%)

TABLE 9.

THE INCIDENCE OF AEROBIC AND ANAEROBIC DIPHTHEROID AND LEPTOTHRIX INFECTION IN THE MINOR FOCI. GROUP 2 CASES.

Focal Site.	Number of Foci.	Aerobic Diph-theroid	Anaero-bic Diphth.	Aerobic Lepto-thrix.	Anaero-bic Lepto.
Tonsil	4	-	1	-	-
Dental	5	1	-	-	-
Stomach Res.Juice	6	-	1	-	-
Os Uterae	2	-	1	-	-
Totals	17	1 (5.8%)	3 (17.6%)	0	0
Intestinal Total and Percentage	22	(13.6%)	(81.8%)	(13.6%)	(36.3%)

TABLE 10. GROUP 3 CASES.

Tonsil	5	-	2	-	-
Dental	1	-	-	-	-
Stomach Res.Juice	4	-	-	-	-
Urine	2	-	1	-	-
Totals	12	0	3 (25.0%)	0	0
Intestinal Total and Percentage	22	(4.5%)	(90.9%)	(0.0%)	(63.6%)

ANALYSIS AND DISCUSSION OF THE TABLES. Table 8 has been compiled to show, in group 1 cases, the incidence of the aerobic and anaerobic diphtheroid and leptothrix group in the minor foci of infection. With regard to the focal sites, it will be seen that the tonsil, with 40%, carries the highest occurrence of anaerobic diphtheroids, and the teeth, with nearly 30%, are next in order. In association with oral sepsis it will be noticed that the stomach, as a focus of infection, harbours 13.4%. The figure of 24.0% for the total minor foci forms a contrast to the 88.0% occurrence in the major focus, a finding which is in common with the anaerobic leptothrix group. In twenty-two cases the cerebro-spinal fluid is striking in having the high figure of 31.8% of anaerobic diphtheroids, the seven cases including four, out of six cases of general paralysis examined.

The aerobic diphtheroid prevalence is highest in the teeth, if one excludes the percentages of the nose and cervix, their numbers being too small to be very accurate. Comparison between the major and minor foci shows the incidence to be about equal.

The aerobic and anaerobic leptothrix group shows a relatively small incidence of not more than 6.8% in any one of the three foci infected.

In contrasting the two anaerobic groups and their occurrence in the minor and major foci, it will be seen that in the latter the diphtheroids are 64% in excess and the leptothrices 56.3%. These contrasting figures will be found at the bottom of Table 8, and prove

conclusively how much more important the intestinal focus is to these two groups as a nidus of infection.

Tables 9 and 10 are similar to 8 but illustrate the same features in case groups 2 and 3.

TABLE 11.

INCIDENCE OF INTESTINAL ANAEROBIC DIPHTHEROID INFECTION ON ADMISSION IN THE FOUR CASE GROUPS.

GROUP	CASES	NUMBERS IN TERMS OF PLUS SIGNS. None	1+	2+	3+	4+	6+
1.	145	17 11.7%	12 8.3%	32 22.1%	45 31.0%	20 13.8%	19 13.1%
2.	22	4 18.2%	3 13.6%	6 27.3%	2 9.1%	4 18.2%	3 13.6%
3.	22	2 9.1%	0	5 22.0%	8 36.4%	5 22.7%	2 9.1%
TOTAL	189	23	15	43	55	29	24
COMBINED PERCENTAGES		12.2%	7.9%	22.8%	29.1%	15.3%	12.7%
GRP.4	261	136	72	28	19	6	0
PERCENTAGES GROUP 4.		52.1%	27.6%	10.7%	7.3%	2.3%	

Table 11 illustrates the incidence on admission of anaerobic diphtheroid infection in the major focus in the four case groups. A summary of the table will serve to contrast the great difference between the first three case groups and the controls. Reference to the scheme at the beginning of this section will indicate the relationship of the plus signs to the terms mild, moderate and severe infection.

	None	Mild	Moderate to Moderately Severe	Severe to Very Severe	Total Infected
Groups 1 2 and 3 Combined	12.2%	7.9%	51.9%	28.1%	87,8%
Controls Group 4	52.0%	27.6%	18.0%	2.3%	47.9%

These percentages show that mental cases and the two allied groups have a strikingly greater diphtheroid infection, both in severity and numbers of cases affected. It will be seen that the mental groups have virtually 40% more patients bearing infection than the controls, and this difference is in reality greater as fully half the cases on subsequent examination proved to have large numbers of diphtheroid and leptthrix organisms, the percentage showing an absence of this flora approaching about 5% in place of 12%. Taking the lower figure of 5% as being nearer to the real facts, approximately 13% of the mental groups have either mild or no diphtheroid infection, whereas 80.0% of the controls come under this category.

Forming a great contrast to this, 80.0% of the mental group have moderate to very severe infection against the controls' 20.3%. Here it is worth mentioning what physical diseases are most common amongst the control cases having moderate to moderately severe diphtheroid infection. These form 18.0% of the cases, and a diagnosis has been ascertained in 32 out of the 47 patients.

General debility with intestinal disorders, constipation in some	5
Gastro-intestinal disorder	4
Asthma	4
Rheumatism	4
Diabetes	4
Colitis, severe	3
Recurrent catarrh	3
Acute lymphatic leukaemia (Child of 3 years)	1
Pernicious anaemia	1
Megraine, severe	1
Furunculosis	1
Iritis	1
Psoriasis	1
	32

Six controls, or 2.3%, had severe or very severe infection and in these the diagnosis was as follows;-

Rheumatism with colitis	2
Acute lymphatic leukaemia (Child of 10 years)	1
Malignant endocarditis	1
Ill-defined nerve disorder (Wassermann C.S.F. negative)	1
Graves' disease, severe	1
	6

It will be seen that in the thirty-two cases twelve were suffering from chronic gastro-intestinal disorders including colitis. In five of these general debility was a definite feature. The asthma cases are interesting in that I have found a definite

relationship between their symptoms and intestinal findings, in the number who came under my care for vaccine treatment. From my observations of focal reactions, it was strongly suggested that the asthmatic condition was frequently a peculiar manifestation of neurotoxic action.

Three out of the six cases with severe infection were acutely ill, two having obviously inflammatory conditions of the colon. The relationship between the diseases and the presence of the infection is obscure except perhaps in the case of Graves' disease.

TABLE 12.

INCIDENCE OF INTESTINAL ANAEROBIC LEPTOTHRIX INFEC - TION ON ADMISSION IN THE FOUR CASE GROUPS.

GROUP	CASES	NUMBERS IN TERMS OF PLUS SIGNS.					
		None	1+	2+	3+	4+	6+
1	145	60 41.8%	26 17.9%	35 24.5%	18 12.4%	5 3.5%	1 0.7%
2	22	14 63.7%	2 9.1%	4 18.2%	1 4.5%	1 4.5%	0
3	22	8 36.4%	5 22.7%	4 18.2%	4 18.2%	1 4.5%	0
TOTAL	189	82	33	43	23	7	1
COMBINED PERCENTAGES		43.4%	17.5%	22.8%	12.1%	3.7%	0.5%
GRP. 4 261		237	13	7	4	0	0
PERCENTAGES GROUP 4		90.8%	4.9%	2.7%	1.6%		

Table 12 shows the same facts for the anaerobic leptothrix group. A summary of the table will illustrate the salient points.

	None	Mild	Moderate to Moderately Severe	Severe to Very Severe	Total Infected
Groups 1 2 and 3 combined	43.4%	17.5%	34.9%	4.2%	59.6%
Controls Group 4	90.8%	7.6%	1.6%	0.0%	9.2%

The general incidence of infection is lower than for the anaerobic diphtheroids but the contrast between the control and mental groups is even greater. The occurrence of infection is approximately 50.0% greater in the mental groups. In them 60.9% have either absent or mild infection as against 98.4% in the controls. The mental group also have approximately 40% with moderate to very severe infection, in distinction to the 1.6% in the controls. It would thus appear that anaerobic leptothrix infection is comparatively rare in physical diseases.

Of the eleven controls with anaerobic leptothrix infection the diagnosis was obtained in nine and these are as follows;-

Rheumatism, sub-acute	2
Chronic debility with gastro-intestinal disorder	2
Rheumatism with gastro-intestinal disorder	1
Asthma	1
Gastro-intestinal disorder	1
Psoriasis, severe	1
Lymphatic leukaemia	1
	9

It will be noticed that gastro-intestinal disorder again claims the largest number of cases. In this connection I may mention that, in most of the cases, the diagnosis was given by the physician in charge.

The subsequent tables deal with case groups 1, 2, and 3 only, and have been given to show the differences in numbers and character of the intestinal flora in the three groups.

TABLE 13.

INCIDENCE OF COMBINED INTESTINAL ANAEROBIC DIPHTHEROID AND LEPTOTHRIX INFECTION ON ADMISSION IN THE FIRST THREE CASE GROUPS.

GROUPS	NONE	ONE TYPE ONLY	2+	4+	6+	8+	TOTALS AND %
Group 1	10 6.9%	55 37.9%	4 2.7%	23 15.9%	41 28.3%	12 8.3%	80 55.2%
Group 2	2 9.1%	14 63.6%	0	2 9.1%	2 9.1%	2 9.1%	6 27.2%
Group 3	1 4.5%	9 41.0%	0	3 13.5%	9 41.0%	0	12 54.5%

Table 13 illustrates the incidence on admission of combined anaerobic diphtheroid and leptothrix infections. Case groups 1 and 3 have approximately the same percentage, 55%, whereas case group 2 has less than half, 27.2% although possessing a higher percentage of diphtheroid infection alone. In group 1 ten cases, or 6.9%, showed on admission an absence of anaerobic flora, although further investigation in eight

of these proved that in five either one or other type of organism was present in moderate or very large numbers. This brings the cases not exhibiting such infections as low as five out of one hundred and forty-five, or approximately 3.5%. Two cases were not re-examined and only two remained consistently negative. This finding illustrates the importance of not relying upon a negative result from one sample, as experience quite definitely proves that the numbers of anaerobes tend to increase in newly admitted cases as a result of treatment to the colon and focal reactions to vaccine therapy. This would lend further support to the view that these infections are deeply seated and are often only revealed when reparative processes are stimulated. One other fact to be deducted is that group 2 cases more frequently tend to have single infections on admission, showing 63.6% of the total.

TABLE 14.

INCIDENCE OF INTESTINAL ANAEROBIC DIPHTHEROID INFECTION WHERE LEPTOTHRIX IS ABSENT.

GROUPS	1+	2+	3+	4+	6+	TOTAL AND %
Group 1	6 12.5%	10 20.8%	16 33.4%	6 12.5%	10 20.8%	48 87.3%
Group 2	3 25.0%	4 33.3%	1 8.3%	2 16.7%	2 16.7%	12 85.7%
Group 3	0	3 42.8%	1 14.3%	2 28.6%	1 14.3%	7 77.7%

TABLE 15.

INCIDENCE OF INTESTINAL ANAEROBIC LEPTOTHRIX INFECTION WHERE DIPHTHEROID IS ABSENT.

GROUPS	1+	2+	3+	4+	6+	TOTAL AND %
Group 1	2 28.6%	4 57.1%	1 14.7%	0	0	7 12.7%
Group 2	0	1	1	0	0	2 14.3%
Group 3	1	1	0	0	0	2 22.3%

Tables 14 and 15 are given to show the incidence of either anaerobic diphtheroid or leptothrix bacilli as a single infection. It will be noticed on comparing the tables how very much higher is the total percentage in Table 14.

TABLE 16.

INCIDENCE OF NUMBERS OF TYPES OF INTESTINAL ANAEROBIC DIPHTHEROID INFECTION.

GROUPS	CASES	ONE TYPE	TWO TYPES	THREE TYPES	FOUR TYPES	MORE THAN ONE TYPE
Group 1	128	67 52.1%	56 43.9%	3 2.4%	2 1.6%	61 47.9%
Group 2	18	11 61.1%	6 33.3%	0	1 5.5%	7 38.8%
Group 3	20	11 55.0%	8 40.0%	1 5.0%	0	9 45.0%

TABLE 17.

INCIDENCE OF NUMBERS OF TYPES OF INTESTINAL ANAEROBIC LEPTOTHRIX INFECTION.

GROUPS	CASES	ONE TYPE	TWO TYPES
Group 1	84	72 85.7%	12 14.3%
Group 2	8	7 87.5%	1 12.5%
Group 3	14	10 71.4%	4 28.6%

Table 16 gives an illustration of the incidence of numerous types of anaerobic diphtheroid species in one culture. In groups 1 and 3 approximately 46% have more than one type, and as many as four types have been isolated from a few cases.

Table 17 is to show the same facts in the anaerobic leptothrix group.

In the control cases the question of streptococcal infection has not been minutely assessed with regard to incidence, so that strict contrast between them and the other groups cannot be made. From the records available however, it is a fair computation to say that a large percentage presented varying degrees of either excessive colon streptococci, or lesser numbers of types quite distinctive and usually associated with oral sepsis. This latter especially applies to the severe anaemias and rheumatic cases, of which there were a large number. The occurrence of aerobic normal and abnormal streptococcal types in groups 1, 2, and 3 has been studied in an attempt to trace them from oral and other sources to the intestine. Broadly speaking I believe their incidence runs fairly parallel with the control cases, except for the manic and manic-depressive psychoses which have a higher incidence. In reference to this last fact I have strong grounds for believing that in these two forms of insanity there is a relationship between streptococcal toxaemia and the manic type of reaction, and conversely, a predominating diphtheroid and leptothrix infection just preceeding and during the exhausted or depressive phase.

The following summary will show the occurrence and relative degrees of the streptococcal infection in the three mental groups.

	None	Mild	Moderate to Moderately Severe	Severe to Very Severe	Total Infected
Group 1	11.7%	14.0%	48.8%	25.5%	88.3%
Group 2	18.2%	8.9%	50.2%	22.7%	81.8%
Group 3	0.0%	25.4%	51.9%	22.7%	100.0%

It will be seen that the three groups are more or less parallel. In group 1 25.7% have either mild or no streptococcal infection, while 74.3% have either moderate or very severe invasion.

The following epitome of Table 13 will serve to contrast the differences on admission in the incidence of intestinal infection by the two anaerobic groups, in mental, borderland and neurotoxic cases.

	None	One Type Only	Mild to Moderate	Moderate to Severe	Severe to V.Severe	Excessively Severe
Group 1	6.9%	37.9%	2.7%	15.9%	28.3%	8.3%
Group 2	9.1%	63.6%	-	9.1%	9.1%	9.1%
Group 3	4.5%	41.0%	-	13.5%	41.0%	-

It will be noticed that groups 1 and 3 are almost identical in total incidence, but that group 3 cases tend to have a higher percentage carrying severe to very severe infection; none however are in the exceptionally severe category, of which group 1 have 8.3% of cases. Group 2 form a contrast in that more than half, 63.6%, have only one type of organism, and that there is a higher percentage with absence of both

species. The degree of infection in the group is noticably less marked, the other tables, especially Tables 11 and 12, bearing this out. This finding, as already stated, has a relationship to the many differences, psychological, physiological, and pathological, between the cases forming groups 2 and 3. The former are very much more inherently psychopathic both in their reactions to environment, and to physical disorders, of which, among many factors, neurotoxic infection is one. While it is true that those in this group present a wide range of psychic abnormality and some ultimately go over the borders of sanity, I believe it is also in keeping with known facts that the defensive, introspective, character of the mental reactions so common in this group, are in themselves a protection, in that they avoid the stress and strain of life much more than the majority of cases in group 3. Thus it would seem reasonable to submit that group 3 cases, because of this greater resistance capacity of the nervous system as a whole, can combat a much greater load of neurotoxic poison without manifesting the psychic disturbances common to group 2. Warning of impending breakdown of the mental processes is common in many, the symptoms of which I have described in section III of this paper, but it is all too common unfortunately for these patients to be reassured that they must carry on and all will be well. Their mental capacity and driving power enables them to do so, but in many the breaking- point is reached and may be precipitated by some serious environmental psychic or

physical stress and strain. The ultimate breakdown of the whole economy, I maintain, is thus much more serious when occurring in group 3 cases than in group 2, and the prognosis consequently less favourable for complete recovery. The circumstances I have described leading up to the onset of mental disorder in this former group are very common in the history of many chronic group 1 patients. Realisation of the importance of the prodromal mental symptoms of group 3 cases, combined with an appreciation of the underlying morbid physical and toxic processes at work, and the immediate application of the general principles of medicine, would without doubt greatly reduce the number of those who, at present, are destined to become a burden to the community.

In judging the incidence of infection in the seven sub-groups of group 1 cases, (see Table 2) the number of cases in each are not sufficiently large to make satisfactory comparison. One fact however stands out clearly, that there is no apparent differentiation of incidence in those that are classified in the so-called functional group, and those that are not.

Tedious as tables and figures are, I think they have served to show sufficiently strikingly the great difference of the cultural flora in mental and non-mental patients, and that anaerobic methods are essential in the recognition of this fact. With such evidence it is impossible for even the most sceptical to say their occurrence is mere chance, the numbers in each group preclude this criticism. The predominance

of these special bacteriological factors in mental disorder and allied states must, I believe, have a particular significance in etiology, especially when it is realised that, to these anaerobic infections, there is frequently superadded a streptococcal flora such as is found contributing to pathological conditions in control cases. Arising out of this anaerobic infection there is, I believe, a special type of toxin which, in its specific effects, materially alters the character of the morbid reactions to the resulting disease processes, for, in contrast to the controls, these are not manifested by the average mental patient. The vital importance of this fact will be referred to later.

IX. NEUROTOXINS AND NEUROTOXIC ACTION.

The term neurotoxin or nerve poison has been recognised by psychiatrists for many years, having been mainly applied to the group of toxic or infective psychoses in whom the physical condition and mental disturbances are more intimately associated. It is well known that such conditions as typhoid, malaria, influenza, and pneumonia can occasionally produce mental disorders superadded to the physical disease, with the exception of a systematised delusional state. Such cases form a valuable link with general medicine, and are particularly worthy of further study, especially in relation to their haematological and biochemical changes and the toxic processes at work, as affording an insight into the pathogenesis of allied mental disorders arising without obvious cause.

The nature of bacterial toxins, including those of the extra-cellular type, has not yet been fully ascertained, and their action in the development of toxaemia and immunity processes is only partially understood. On this account any attempt to formulate a hypothesis directly bearing on the subject of neurotoxaemia, in relation to the anaerobic bacteria under consideration, must in some respects be tentative, much research being required before the numerous problems are fully understood. In the following pages I have attempted to correlate from literature some clinical, bacteriological, histological and other facts,

some well established and others perhaps speculative which have a bearing directly or indirectly upon the subject of neurotoxins and neurotoxic action in relation to insanity, and to see in what way they link up with the evidence established in this thesis.

In spite of our rather limited knowledge I think it is worth while to enquire into and draw comparisons between the action of two known neurotoxins, and to see whether their effects in man and experimental animals have an analogy in the action of the anaerobes, which, from all the evidence produced, would appear to be very closely associated with the etiology of mental disorders. Thus it is necessary to refer briefly to the specific neurotoxin of the anaerobe, B. tetanus, which, like B. diphtheriae, produces an extra-cellular toxin of high potency. According to Sidney Martin's classification, its poison, unlike that of the diphtheria bacillus, is a secretion of the bacillary bodies per se, which, in broth culture, is formed, according to Brieger, of two basic bodies which he has termed "Tetanine" and "Tetano toxin". The former produces tetanic symptoms in mice, the latter only tremor, paralysis, and convulsions. Sidney Martin has extracted an albumose from the blood of a patient suffering from tetanus, which, when injected into an animal, produced a depression of temperature followed by progressive wasting. It will be seen that this part of the exotoxin, having combined with the tissues of the body, has no power to cause tetanus but nevertheless remains neurotoxic. Although differing in many respects

from the diphtheria toxin in its chemical constitution, nevertheless bodies are formed analogous to the toxoids of diphtheria toxin.

In distinction to the primary toxin of B. tetanus, the bacillus of diphtheria, according to Martin, produces an exotoxin secreted by the body of the bacillus which is only formed after the digestion and destruction of protein. Brieger and Frankel isolated in broth culture an albumose protein substance termed "tox-albumen" which is destroyed by a heat of 60°C but not at 50°C even with excessive acid. Hence they considered that probably it is not an enzyme. Warden, Connell, and Holly suggest that the toxin is a particular fatty acid in a peculiar emulsoid or colloid form. An artificial substance of this nature is toxic to a guinea-pig, reproducing the lesions of diphtheria toxin, and is neutralised by anti-toxin. Extracts of diphtheritic membrane contain only traces of albumose and the organic acid. The former, when injected subcutaneously, produced odema and irregular temperature; in larger doses, depression of temperature with paralysis and coma. Small multiple sub-lethal doses gave rise to similar but less marked symptoms, followed by weakness and less of weight. The organic acid is also a nerve poison but is not so toxic as the albumose. From the evidence so far obtained there are grounds for believing that the toxic substance, which Martin suggests is probably in the nature of a ferment, and which forms but a very small fraction of the toxin

developed in the broth culture, is the primary secretory product of the bacillus. This, by digestion and destruction of protein in culture and presumably in the tissues, forms two bodies, the more potent of which, as a neurotoxin, is the albumose form. The analogy of this is seen with the tetanus toxin which is recognised as being a primary secretion but which nevertheless forms an albumose in the body tissues.

In broth culture the primary toxin or ferment of the diphtheria bacillus combines with the protein of the media in much the same way as must occur in the tissues. It is known that a tox-albumen is developed in broth which, when newly formed, is highly poisonous and the lethal factor of virulence tests. What subsequently occurs in broths kept for some time has I believe a direct parallel, in spite of somewhat different conditions, in the more complex processes that result in the tissues when the primary toxin reaches them. The gradual loss of high toxicity of broth cultures is regarded as being due to the instability of the toxin, other substances being formed which are comparatively non-toxic and which vary in amount in different broths. These substances are known as toxoids and toxone, and combine in varying degree with anti-toxin, the toxone being specific to the primary diphtheria "ferment" or secretion. It is probable that the development of these relatively less toxic elements may come about by more complete binding with the residual proteins in the broth, a process which apparently

requires time. When contact with new protein is made, especially with anti-toxin which is charged with free receptors, a combination of the toxic elements takes place. In fresh broth the toxone however is always present in greater amount than the toxoids, and, having less affinity for anti-toxin, the greater part remains un-neutralised. This combination of neutralised toxoids and unaltered toxone, while not being highly lethal, produces morbid tissue changes and paralysis, and is therefore to be regarded as neurotoxic.

It has been mentioned previously that a parallel can be found between the known primary exotoxin of tetanus and that of diphtheria, in that both produce tox-albumen in the body tissues. Although the primary toxins of the two bacteria are materially different in the character and intensity of their neurotoxic effect, both, when combined with the protein of the body, form new poisonous derivatives which are almost similar chemically and which produce in experimental animals morbid neurotoxic symptoms which are almost identical, but which differ widely from those produced initially. This would point to a definite modification of their toxin action both in degree and character once the receptors and toxophile groups of the tissues have combined with the two sides of the original toxin. Subsequently however the degree of toxicity must undergo further modification through fixation and stabilisation, first in the tissues for

which it has an affinity, and later probably, in those for which it has less. This stage just referred to finds a parallel in that occurring in broth cultures, i,e. toxone and toxoid derivatives are formed through combination of the proteins in the tissues. While this action is proceeding, with it must run the gradual formation of anti-toxin, provided conditions are favourable. As anti-toxins are formed the toxoids combine with them, but the toxones, having less affinity, are neutralised much more slowly. I believe this residuum is a much more important neurotoxin than any of its more lethal precursors from the fact that it is considered to exist in fresh broth which necessitates its longer action on the nerve tissues of the animal, provided that it is not killed in the first instance by the more lethal portion of the toxin if present. As evidence of this view, animals dying from toxin in 48 hrs. rarely suffer from paralysis; on the other hand, the importance both of the time factor and the necessary toxone is shown by the fact that when guinea-pigs are given toxin anti-toxin mixtures which contain more toxone than any other toxic derivative, life is prolonged beyond 8 - 10 days and paralysis is often sufficiently severe to be general. The histological changes in these animals show typical Wallerian degeneration of the myelin sheaths in most of the peripheral nerves, while the brain and cord are hardly affected.

Quite analogous to the effects just described but considerably modified in degree are the experiments with the tox-albumen derived from the blood and tissues of the tetanus or diphtheria infected patients. Small doses in animals produced depression of temperature, weakness, and loss of weight, a condition which, from my own clinical observations, is a most common general manifestation of neurotoxic action in mental patients.

Lastly, to complete the line of discussion, the experiments of Ford Robertson on rats and rabbits infected by feeding with living diphtheroid cultures from cases of general paralysis of the insane, show very strikingly how alimentary infection by closely allied organisms to the Klebs-Loffler can produce nervous and even mental symptoms with corresponding morbid changes in the central nervous system. These experiments are given in the historical review in this paper. In the results from these animals we have without doubt the parallel to what is taking place in mental disorders in man.

To refer for a moment to the action of acute diphtheria intoxication in humans, 20% of all cases suffer from varying degrees of paralysis. The lesion is usually local but may be widespread, affecting even the spinal cord. The local lesion is an ascending neuritis, the more extensive morbid changes being recognised however by some authorities as being a selective neurotoxic action of the diphtheria toxin.

If this is the case, it implies that a certain proportion of those suffering from acute diphtheria have a neurotoxic susceptibility, which however must be influenced, as in animals, by the composition of the toxin and the length of time in which it acts. From my own personal experience of a palatal paralysis, the faucial lesion need not necessarily give rise to toxic symptoms, or even affect the heart, in order to produce a local nerve lesion of some severity.

Having referred to the effect of varying degrees of neurotoxic action in animals and man by the known lethal and sub-lethal toxins of diphtheria and tetanus, it is necessary to refer again to the train of events which leads up to what is I believe an entirely analogous process of toxic action in mental patients. The carrier mechanism in relation to diphtheria has been cited as probably forming the initial chronic focus in the tonsils or pharynx, and the same applies to allied diphtheroid infections, which, as with the former, become anaerobic in habit and undergo further modification in their toxic action. Unlike diphtheria, the tonsils as a focus of infection are in most mental cases relatively unimportant. It has however been seen how readily these bacteria find the intestinal nidus, which, when once established, enables them to exercise a greatly enhanced neurotoxic action. Experimental evidence of a very interesting kind having a direct bearing on the criticism so often raised that bacteria found in the stool are rarely pathogenic and can only

produce toxaemia by absorbtion of poisons formed outside the walls of the gut, is to be found from the observations on feeding mice and rabbits with virulent diphtheria toxin. It has been ascertained that even the ingestion of enormous quantities of toxin per os or per rectum has no effect even on susceptible animals. Much investigation has shown that the digestive ferments, pepsin, trypsin, and papain, exercise a destructive effect on the toxin, and that frequently, because of this and other minor factors, little or no toxin can be found in the intestinal content. In other words, diphtheria toxin, if it is to exercise a pathogenic effect in the alimentary canal, must do so by being produced within the mucous membrane, which necessitates the existence of the organism in the tissues. The method of lymphatic spread already mentioned would appear to form the ideal attainment of this end.

Having further established the fact that anaerobic diphtheroid and Klebs-Loffler infection must exist deep in the tissues of the lower alimentary canal, it is necessary to see how this morbid condition is effective in producing neurointoxication leading to widespread disturbances of nerve function, which, though similar in many respects to those induced experimentally, has many superadded features. Anaerobic habit of growth in the diphtheroid and Klebs-Loffler group does not necessarily imply loss of toxicity, vide the tetanus bacillus, especially in relation to the production of neurotoxin; in fact the converse of this can quite

reasonably be accepted. This type of infection however contributes I believe to the development in large amount of the special derivatives of toxin, the formation and neurotoxic action of which has been described. As has been suggested, such low-grade diphtheria and allied toxins are probably chiefly in the nature of toxones, and to these must be added I believe those which are even more specifically neurotoxic, and may be developed as an outcome of abnormal bacterial metabolism. In relation to this it is probable that neurotoxic susceptibility and reaction may cause changes in the tissues of the individual to provide certain substances especially productive of this abnormal bacterial metabolism. The analogy can be found in the evidence of the formation of toxin production in vitro. This view is quite consistent with the known tendency of the body to the formation of vicious circles of toxic action and reaction. A process such as described may partly account for the tendency to chronicity of mental disorders when once fully established.

One other factor remains to be mentioned, that of endotoxic production. Although most authorities consider this to be of no pathogenic importance in relation to the bacillus of diphtheria, such a view can hardly be maintained here, especially when the size of the intestinal focus and its ramifications are appreciated. Endotoxic action must, I hold, play an important part in pathogenesis but exactly in what manner has yet to be ascertained. I have sufficient evidence

on this point however to convince the most sceptical of the extraordinary sensitiveness of mental patients to anaerobic diphtheroid vaccines, with exaccerbations in mental state and aggravation of the manifestations of underlying physical disorders.

At this point it is necessary to refer briefly to the production of immunity in connection with neurotoxic action. The amount of toxin absorbed into the systemic blood stream from the lower alimentary canal will vary according to the activity of the infection, the power to produce anti-toxin, and the detoxicatory action of the liver which lies directly in the path of the portal circulation. This organ has, I believe on biochemical evidence, a strong protective action against systemic poisoning, but in mental disorder this function in a large number of cases is either overwhelmed or very defective. The production of antibodies in this type of intoxication would seem to occur relatively slowly, an assertion which is in harmony with the immunological evidence on the subject.

Before going on to outline the remote effects of neurotoxic action on the central nervous system, it is convenient at this juncture to mention some of the more local nerve lesions that are likely to result from these special infections. I have referred in the historical review to evidence, obtained by radiographic examination, of the frequency of morbid conditions of the colon, and to a less extent of the small intestine; these are mainly in the nature of spastic or tonic

conditions associated with enteroptosis in very marked stasis. My observations on a number of patients by the bismuth meal and barium enema amply confirm the evidence of others. It appears probable that mental disorder, above all other morbid conditions, is associated with severe pathological changes, particularly in the colon, which are in the first instance effect, and later a contributory cause in establishing a condition of chronicity. Stacey Wilson (Ref. 10) in his book on "Tonic Hardening of the Colon" presents a detailed account of this condition and describes its definite relationship to borderland and mental states. He uses the term "tonic elastic fixation" for this plastic form of static activity of the colon. The lesion is usually localised to the caecum and sigmoid but must be sought for elsewhere. The liability to severe constipation and proximal dilatation resulting from the constriction is mentioned. Tonic hardening is described as a reflex nervous phenomenon, dependent, not only on the amount of stimulus received from afferent impulses to the reflex centre in the segment of the cord affected, but upon the excitability of the centre itself. Thus the symptoms arising may be expected to vary according to the state of the central nervous system at the time. Referring to the relationship to mental disorder, the author states that his clinical observations show conclusively that the abnormal nervous impulses which are originated from the tonically

hardened colon segment may disturb the patient's sense of well-being and may take the form of mental depression, obsessions, phobias, etc., these sometimes being so severe as to carry the patient to the borderline of insanity. In other words, the reflex impulses, instead of seeking the afferent path via the sensory nerve routes, may effect direct transmission to the higher cerebral centres. With regard to etiology the writer suggests that the agent is a microbic toxin, a view founded on the results of his treatment. He also states that auto-intoxication, which is a chemical agency, is not responsible, and emphasises the point that tonic hardening is purely a nervous phenomenon, the ultimate cause of which is something which stimulates nerve endings. I have outlined Stacey Wilson's views as they have I think an important bearing, not only on the subject of this thesis, but as explaining one of the probable nervous mechanisms involved in the early stages of nervous disorder. His observations on many such cases are purely based on the symptomatology and the study of the abnormal reflex mechanisms involved. Tonic hardening of the colon and the abnormal paths which stimuli arising out of this condition will take is illustrative of perverted physiological nerve function, the psychological importance of which lies in the fact that the actual cause, which should be understood as pain, is hidden from the consciousness of the individual, being translated instead into

psychic disturbances without apparent cause. My own observations on this question in many cases lead me to believe that this mechanism exists and is of very considerable importance, especially psychologically, as there is no possible correlation of cause and effect by the patient. In the light of the facts presented in this thesis, and from the nature of this local condition of the colon, it would seem reasonable to postulate that this lesion is a specifically neurotoxic one with a direct effect on the Aurbach's nerve plexus which controls the involuntary muscular system of the intestine. For the past two years I have made careful clinical examination of all cases for this condition, on admission, later, and on discharge, and 100 cases mainly confirm his findings. In 58 of these the colon, especially the descending iliac and sigmoid portion, could be palpated. In marked examples the gut was very firm and sometimes no thicker than one's finger, varying in extent from a few inches to about twelve in extreme cases. In every instance constipation was persistent and severe, and the motion, in most, of the "sheep-dropping" type. On admission no tenderness as a rule could be elicited, but later, after mental improvement had begun, this was definitely noticeable in most cases. In those recovering mentally the condition was found to have either yielded or improved. The tonicity tends to disappear post-mortem, but where more or less permanent changes have occurred, segments of the colon can be seen, very

much resembling rubber tubing. On section, the circular muscular fibres are tightly contracted, and the mucous membrane rugose. Proximal dilatation above the constriction is frequently marked.

Of 141 cases admitted, 75% were more or less severely constipated, the remainder having fairly regular actions but frequently being found to have a well-loaded colon. When the fact is realised that it is in the splanchnic circulation that the concentration of neurotoxins is probably greatest, severe disturbances of the autonomic nervous system would appear to be almost inevitable. There are few patients indeed that do not manifest disturbances which can be classified as either vagotonic or sympatheticotonic, although in some, distinction between the two is ill-defined. What determines the type of reaction is at present obscure; it may have a purely physiological basis, or it may be dependent on the presence of different types of neurotoxins with selective affinity for the two systems. Certain observations on the point lead one to suspect that the latter theory may have some foundation.

Before considering the effects of neurotoxic action upon cerebral function and the morbid changes it may produce, it is necessary to refer briefly to the protective structures and mechanism that Nature has evolved to ensure that the vital controlling machine of the body is isolated as far as possible from other

tissues. L.Trotter,(Ref. 11) in a paper in 1926, emphasised the fact of how Nature has taken infinite pains to insulate the brain and nerves from direct contact with the somatic tissues, and that it is the maintainance of the integrity of the former that is so essential to its normal physiological functioning. He points out that in somatic disease, breaches frequently occur, and it is only then that the body is warned of impending trouble. To cite the best known example, in acute visceral disease, the action of the reflex arc mechanism centred in the segments of the cord, and the extension of this process to the basal ganglia, and hence to the cortex, brings about, through the consciousness, the warning that Nature intended. Arising from this conception of the isolation of the brain and nerves, I have adopted the term "internal environment" in distinction to environment which is only external (outside the body) in affectiveness, as expressing the relationship of pathological conditions arising in the somatic tissues and the manner in which they affect the nervous system as a whole.

In the brain itself the protective structures are more complex as they form principally a barrier or screen between the blood stream and the nerve tissues of which the cerebro-spinal fluid is a part. In addition to the highly selective filter action of the coroid plexus other units such as the glia, the

cerebro-meningeal capillaries, the ventricular ependyma, and the pia are also considered as part of this system. The question of permeability of the brain membranes in mental disorder has been the subject of much speculation and research. The bromide method of Walter (Ref. 12) shows that differences in permeability exist, the concensus of results showing that, with the exception of dementia praecox of whom about 36% have diminished permeability, it tends to be increased. No clear diagnostic significance for this test is however claimed. The relation of brain permeability to systemic neurotoxin must be of very considerable importance, but has to be considered in the light of their chemical nature, the poisons of which, if in a colloidal form, are much less diffusible than those which are not. Much must depend upon the effect of the toxin upon the barrier itself. In this connection Strecher, 1928, (Ref. 13) refers to the interesting fact observed by Stern that poisoning with diphtheria toxin, tetanus toxin, and tuberculin, is found to increase the permeability of animals. The correlation of this with the present research is obvious. As regards the entrance of neurotoxin by this pathway, its significance is, with out present limited knowledge, difficult to estimate.

Another possible route remains to be referred to; analogous with the tetanus toxin, which is absorbed by the nerve trunks, it is possible that a similar process may occur with the anaerobic Klebs-Loffler and

diphtheroid toxins in that they may travel up the cord and ultimately reach the higher centres. The mechanism referred to by Stacey Wilson may be in part the result of this process, which in time will extend until the more highly developed basal nerve centres and those of the cortex come under its effects. If we except dementia paralytica, and senile and arterio-sclerotic dementia, the brain changes found in cases of mental disease are uncharacteristic. Further, the histo-pathological data would, in the opinion of most, tend to point to degenerative rather than inflammatory changes of the parenchyma. In the so-called dementia praecox group I understand this particularly applies, where the localisation of morbid changes in relation to mental phenomena is still very obscure. The excessive fatty deposit found in the ganglion cells of the cortex in quite young persons who were cases of dementia praecox, is referred to by Goodall (Ref. 14) who stated that "this may be an instance of the phenomenon referred to by Leaths and Raper (Ref. 15) 'the conversion of fat normally present, which does not stain, into fat that does, because of a change in dispersion of the fat leading to aggregation.' The example they quote from Dudgeon is significant 'whereas normal heart muscle of guinea-pig and other animals shows no sign of fat in the cells when treated appropriately with Scharlach Red, these cells similarly treated 24 hrs. after the injection of diphtheria toxin

may be densely studded with deeply stained droplets and granules. Chemical analysis shows however, that the normal heart may contain as much fat as the deeply stained poisoned cells, or even more, though none of it is revealed by the staining.'" Apart from their interest, these observations have a definite significance when viewed in the light of the present research. Against this claim it may be asserted that the conditions are scarcely parallel in terms of toxin; it must be realised however that the central nervous system is infinitely more sensitive to diphtheria poison than the somatic tissues. Roux and Borrel found that in animals the central nervous system was approximately seven times more susceptible, and further, large doses of anti-serum had no protective action. Relative to this, chronic infective conditions supply this important factor, the prolongation of toxic action on any tissue it selects. This implies a wearing down process probably in terms of years before toxin fixation is complete, and death of the cell ensues.

What exactly determines the fixation of toxin in some nerve cells and not in others has yet to be elucidated, but it is in harmony with previously conceived views that inherent susceptibility in highly specialised cortical and basal ganglion cells must play an important part. Consistent with this it has been shown by a number of observers, Wassermann, Futaki, and Metchnikoff, that the combining power of the

emulsified brain of an animal is directly proportional to the susceptibility of that animal to tetanus toxin. Other factors however that may predispose are trauma, and psychological wear and tear resulting from environmental conditions, especially of those cells that are concerned in the will, intellect, and control of the emotions. The operation of this process on nerve cells is probably closely parallel to the well-known lowering effect on the resistance of the somatic tissues of cold and fatigue, except that mental stress and strain, if prolonged, is ultimately harmful to the somatic side of the economy, and may predispose to the invasion and action of special bacterial agencies and the establishment of a pathological state. The last mentioned relationship is very frequently cited as being solely causal to the poor physical state of incipient and fully established mental state. This view cannot however be accepted as such on the facts which have been presented.

In place of this widely held conception one possible aspect of neurotoxic action has yet to be considered. The vitality of all living tissue is maintained by trophic nerve function, complete loss of this trophic influence resulting in wasting of the tissues, whether it be the involvement of a group of muscles or a secreting gland. From this extreme all degrees of tissue disfunction can be cited. In severe

neurotoxaemia it is probable that the whole economy is more or less saturated, and while definite morbid changes through selective action may be going on in nerve centres within the brain, it is not unreasonable to suppose that the whole nervous system is likewise being affected but to a much lesser degree. If this is so, then it follows that almost every tissue and function of the body is materially altered, the change being as a rule depletion of vital activity. This condition which seems to exist I propose to term "neurotoxic atrophic effect". Generalised as this process may be, absence of its effects is sometimes evident in a certain tissue, organ, or system, which escapes owing to an inherently greater resistance than the rest of the economy. Only in this way does it seem possible to explain the wide differences of disordered somatic tissue function seen in mental cases. In my clinical study of patients I have been much impressed with the general condition of debility, sub-intoxication, and lack of recuperative power which so many cases show. Its physical expression in terms of the cardinal signs of inflammation are notably absent, and their tissue response to pyogenic and other infections remarkably different from that seen in general practice. Yet from a bacteriological aspect their gums, tonsils, etc., carry as much if not more pathogenic species of streptococci and other bacteria as do those normal patients suffering from some acute septic condition causing serious illness. That this

sepsis without reaction, or as it is sometimes called unresolved sepsis, is dependent upon a greatly deficient vital immunity reaction of the tissues, is shown by the fact that many organisms, especially those that are endotoxic in type, do not as a rule exercise the pathogenic action until their toxic products are released by leucocytic and other ferments such as opsonin and bacteriolysin. The absence or diminished power of tissue resistance must account for the degree of relatively superficial oral sepsis that is so common in mental patients. Many of the bacteria are partially or wholly saprophytic, but tend to become less so the deeper they work into the tissues where free drainage of their toxic products cannot occur. Hence bacteria in apical foci, periodontal membranes, the accessory sinuses, and those that travel via the lymphatic paths, must be regarded as pathogenic even if relatively and intermittently mildly toxic. The high incidence of faecal and other types of streptococci in the intestine is I believe partly accounted for on these grounds. Exactly what neurotoxic relationship they have has not yet been determined, but there is good reason to suspect that in certain phases of mental disorder their toxic effects are of considerable importance. With virulent bacteria however this lack of response may see the reverse of what has just been stated, the absence of defence resulting in overwhelming toxaemia and death. This paradox may in some measure account for the great liability of the chronically insane,

especially adolescents, to succumb to intercurrent infections. If any other hypothesis can be formulated that will better explain these special physical features so characteristic of mental disorders as a whole, I would be glad to accept it.

X. THE INCIDENCE OF DIPHTHERIA IN MENTAL HOSPITALS AND OTHER STATE INSTITUTIONS.

Through the kindness of Dr M.J.McGrath I have been able to obtain information from replies to a Questionnaire which his sub-committee on infectious diseases sent out to mental hospitals. The enquiry covered typhoid, dysentery, and diphtheria, and the incidence of carriers, but it is necessary here to refer only to the question of diphtheria. From 71 mental hospitals the returns are briefly as follows;-

1. Sixty-six hospitals report no diphtheria or carriers amongst patients. In the majority this freedom would seem to cover several years, and in some the investigations go back as long as ten years.
2. One hospital reports one suspected case as being a carrier but this was not confirmed.
3. Three hospitals report one carrier each, and one institution, two carriers in which one was associated with sinusitis.

From one mental hospital whose staff live out I am informed that the number of cases of diphtheria does not vary from that amongst the ordinary population; further confirmation of this is however required.

Dr W.N.East, medical inspector of prisons, has kindly supplied me with relative information amongst prisoners and inmates of Borstal Institutions for 1929, their number being 10,861. Only one case occurred and

this was regarded as being a carrier. With regard to prison staff of whom most live outside, information is not available. The basis for comparison is unfortunately not the same in both groups as regards time, the incidence for the mental hospitals covering many years while for the other group only a period of twelve months is available. However, it will be seen that the occurrence of diphtheria is apparently the same for both over a period of one year. The conditions of isolation from the general community, and the fact that, should cases occur, they are quickly noticed and segregated, are generally taken as being the main reasonsfor the absence of epidemics. In examining the question more closely however, and comparing the conditions of these two segregated sections of the community, it must be pointed out that, in the case of mental patients at least, their isolation is only partial and that they come into fairly close association with those living outside. The nursing staff in the course of their duties are in daily contact with patients much in the same way that the staff in fever hospitals are, where the greatest care is taken that diphtheria does not spread into wards allotted to other diseases. Carriers amongst both patients and staff are responsible for the outbreaks that do occur in fever wards. Further, the weekly influx of visitors to mental hospitals must bring into contact with patients the rather younger elements of the population.

Added to this is the fact that, owing to circumstances in some hospitals at least, patients live even more closely together than is the rule in general hospitals and orphanages where epidemics are known to occur from time to time. In regard to prisons, the conditions of isolation are much more severe; warders replace the nursing staff and their contact with prisoners must be much less intimate. The inmates are also relatively little in contact with one another. Further, visitors are not allowed except under special circumstances, and even then regulations do not permit them to come into actual contact with the prisoners. With Borstal Institutions perhaps less rigorous conditions prevail, the inmates probably being collected into large groups in the course of occupations.

While it is allowed that a closer investigation is required in order to make a strict comparison between the conditions of each section to determine the opportunities of infection, I think it is probable that mental patients will be found, taking all the circumstances into consideration, to live in conditions which involve the greater risk of diphtheria infection. Nevertheless it is a most remarkable fact how exceedingly rare acute diphtheria is, those found with infection being carriers. That between 40% - 50% of mental patients are carriers of anaerobic Klebs-Loffler and closely allied species has been shown and in this connection it is to be remembered that the tonsils are only a /

minor focus, and in addition is the intestinal tract bearing the same infection in a much greater degree. When this is appreciated it is reasonable to infer that most patients have a long-acquired immunity which is sufficient to eliminate the incidence of acute faucial diphtheria. This does not however prevent them from becoming carriers of perhaps virulent diphtheria bacilli contracted from outside. Significant as I believe these facts to be in establishing the relationship of diphtheria and allied infections as one of the causes of insanity, it cannot be overlooked that the conditions of relative isolation, though less important, must necessarily be taken into consideration.

In connection with acute diphtheria and carriers in the community, it is perhaps hardly necessary to mention that they are bound to be regarded in a new light in view of the facts presented. Numerous problems would appear to arise; what relationship has diphtheria to the pathogenesis of mental disorder? Is its acute epidemic form a more fertile source of subsequent anaerobic residual infection with its possible sequelae, or are carriers,who are less frequently noticed, more prone to become generally infected and become candidates for mental disorder? These are only two of the many questions requiring an answer. From the point of view of public health, the need for co-operation in this matter refers especially to those whose responsibility it is to pronounce a case free

from infection or a contact to be non-carrier. It would seem essential to adopt anaerobic methods in these instances for patients in whom the recognised bacteriological tests prove negative.

One other aspect remains to be mentioned. If Science can prove, as I believe it will be able to do, that diphtheria and allied infections can be linked with the somatic pathogenesis of mental disorder, two definite advances in psychiatry will have been made. In the first place early recognition of the underlying toxic etiological factors will be possible long before cases have advanced to the stage of complete breakdown of the brain and nervous system. In addition to the medical treatment of such cases, there would be involved the study and correction of inherent and acquired abnormal psychic tendencies, and the ordering of the environment as far as possible to meet the needs of each case. Thus would our treatment endeavour to become preventive rather than curative.

Secondly, it is probable that if the lay mind were to associate the well-known physical entity acute diphtheria in a more subtle and chronic form as being one of the causes for mental illness, much of the mystery and dread that still surrounds insanity would gradually be dissipated. Further, it would give the psychiatrist and medical practitioner alike a better opportunity of teaching the need for early attendance as in or out patients at psychiatric cliniques where necessary treatment and advice would be given.

XI. THE METHOD OF DIAGNOSTIC SURVEY, ITS INFLUENCE ON TREATMENT, MEDICAL AND PSYCHOTHERAPEUTIC.

Those whose work lies in the domain of the study of the mind and who tend to disassociate it from the functioning of the rest of the body may question the value of this research in its application to the treatment of mental disorders. This attitude of mind towards the physical aspects of mental illness has, in part, naturally arisen on account of the predominance, in so many patients, of a purely psychic symptomatology in the clinical picture, which effectively masks the subtle but nevertheless fundamental somatic disease processes that underly insanity. One well founded explanation for this characteristic can, I believe, be sought in the mechanism of abnormal reflex arc functioning, which not only effectively conceals the appreciation of underlying disease from the consciousness, but diverts the stimuli to the higher centres, the effects of which will vary according to the potential psychic stability of the individual. Once these aberrant paths are established, it can be seen that the more severe the underlying morbid process becomes, the greater will be the masking of its physical expression. The psychological effect of this mechanism will be more appreciated perhaps by the psychiatrist than by those whose work lies in other fields, and its importance realised. In

lacking a true physical symptomatology, mental disorder requires, above all other maladies, the application of scientific medicine to come to its aid. The truth of this has been amply borne out by the problems with which I have been faced for the past three years. The degree of serious and almost wholesale physical disorder in some mental patients is, to put it mildly, nothing short of appalling when revealed by the method of diagnostic survey. Ordinary clinical methods, no matter how minutely and carefully conducted, leave the physician with little or no clue to the real nature of the physical disorders present, and therefore he is robbed of his power to treat what is in so many a serious if not dangerous illness of an acute or chronic type.

Diagnosis by the methods I have employed has opened up a vast and fascinating field of therapeutics, the application of which taxes the skill of both physician and nursing staff alike. Nevertheless, the results that have been obtained have, I believe, more than justified the time and patience which are necessary if many of our patients are to be restored to physical and mental health. No panacea is claimed; on the other hand, I think that, without exaggeration, no greater task confronts scientific medicine than in mental disorders, so fundamental and elusive are the problems involved. As our knowledge progresses it becomes evident that more and more will the purely psychological

aspects of insanity yield the first claim upon the patient to scientific research in respect of early treatment. It thus becomes necessary to ask, what place will psychotherapy have in the domain of psychiatry? I hold that the comparative limitations of its value as an instrument of cure per se will ultimately be realised and that its future field of service will be enormously enhanced if only those who practise it will seek fuller co-operation with the scientist and physician. The need for the skilled psychologist will always remain but his place in the scheme of rational treatment will be altered. This adjustment will, I believe, be advantageous to both patient and psychotherapist alike, whose task it will be to re-habilitate the normal functioning of the mind where illness has left its train of mental weakness and abnormality. Notable though the results of pure psychotherapy have been in certain cases, the future holds out infinitely greater possibilities of success if such treatment is postponed until convalescence and not adopted in those cases, so ill in mind and body, that the co-operation which is so essential cannot, or will not, be given.

One other aspect of psychotherapy remains to be referred to, that of the important problem of child-psychology which is now receiving the attention of so many. In this field psychology will perhaps find its greatest service to humanity, not only by direct care of the patient, but in the guidance of those who are

responsible for the environmental influences which modify or aggravate the inherent psychic tendencies in each individual. Fundamentally important as these aspects are in the early recognition and treatment of mental disorder, it must not be forgotten in the searching of the soil for the seeds that may germinate insanity, that amongst them are often those belonging to the somatic side of the economy, which, if left undiscovered, will later be reaped as an absence of response in many who, with earlier medical care, might have been saved being a burden to the community.

XII. CONCLUSIONS.

1. The adoption of strict anaerobiasis and special conditions of nutriment have been shown to be essential to the carrying out of this research. It follows from this that, if anaerobic cultural technique is omitted in the bacteriology of insanity, the greater and most important part of the flora is inevitably missed.
2. The fact that anaerobic Klebs-Loffler species exist in the tonsils and intestinal tract has been established, and in association with them are numerous closely allied bacteria belonging to the anaerobic diphtheroid group.
3. Strictly anaerobic species of leptothrix bacteria have been found which are probably closely related and similar in toxic action to the anaerobic diphtheroids. As a group they are more clearly defined, and have aerobic species which usually show strongly facultative anaerobic habits.
4. Both groups by ordinary methods of animal experiment are non-virulent to guinea-pigs, but may prove mildly toxic, and would appear to have an affinity for the lower alimentary canal.
5. Systematic methods of bacteriological examination have clearly demonstrated the importance of the colon as the main focus of infection for the strictly anaerobic species of bacteria, and that it is

possible to trace them to their source in the minor foci of infection.

6. The incidence of these organisms has been carefully compared in controls and those suffering from definite mental disorder, borderland, or neurotoxic states. The results show conclusively how infinitely greater is their occurrence in insanity and allied states, a fact which, it is claimed, must have an etiological significance of the greatest importance.
7. Of the two sub-groups the neurotoxic cases are regarded as being potentially much more liable to severe and lasting mental breakdown than those belonging to the psycho-neurosis and neurasthenias.
8. From the study and comparison between the action of known neurotoxic bacteria and the anaerobes in question, there would seem little doubt that the latter have a similar but necessarily modified toxic action to the former.
9. That in this process of modification special more specifically acting neurotoxins are formed which exercise a more selective action on the central nervous system.
10. Endotoxic action is regarded as being an important additional factor in the production of neurotoxaemia.
11. That neurotoxic action, in addition to effecting slow degenerative changes in the nerve tissues of the brain, has marked local effects, especially on Aurbach's nerve plexus and the autonomic system.

12. That the condition of tonic hardening of the colon results in perverted function of the reflex arcs centred in the cord and can give rise to disturbances of cerebral function; further, that tonic hardening is productive of the later and more extensive morbid changes in the lower alimentary canal, and of establishing the conditions of severe toxaemia that lead to permanent mental disorder.
13. Absorption of neurotoxin from the alimentary canal or elsewhere may reach the brain by two possible routes, one by the systemic circulation, the other via the nerve trunks and spinal cord.
14. That the almost negligible incidence of diphtheria and carriers in mental hospital patients is mainly due to the fact that they are already infected with anaerobic Klebs-Loffler and closely allied organisms and thus have an acquired immunity.

XIII SUMMARY OF DEMONSTRATION SLIDES AND CASES, ILLUSTRATING THE TYPES OF ANAEROBIC KLEBS-LOFFLER, ANAEROBIC DIPHTHEROID, LEPTOTHRIX INFECTIONS.

Case 1. Female, aged 42. acute mania, three cyclic attacks, markedly toxic; recovered.

Intestinal Flora;- Three types of anaerobic diphtheroid; one type anaerobic leptothrix; total, 4½+. Aerobic streptococcal flora very marked during manic phases.

Five slides stained either by Gram or Neisser demonstrating the microscopical characteristics of No. 1 anaerobic diphtheroid. Sugar reactions, those of Klebs-Loffler except for fermentation of mannite. (Strain 9, Table 6). This sugar broth proved toxic to guinea-pig. Four slides show the characteristics of the anaerobic leptothrix, sugar reactions given (Strain 2, Table 7).

Case 2. Female, aged 20, adolescent insanity, onset of illness at 18. On admission confused, deluded, impulsive type, physical condition very poor, septic; physical and mental state much improved, able to live at home.

Intestinal Flora;- Three types of anaerobic diphtheroid; one type anaerobic leptothrix; total infection, 5½+.

Nine slides demonstrating the characteristics of No. 1 anaerobic diphtheroid (Strain 12, Table 6). Sugar reactions those of Klebs-Loffler, filamentous branching forms seen in broth. Anaerobic diphtheroid No. 2 (Strain 1, Table 6), with different type of primary colony. Sugar reactions given, filamentous branching forms seen in broth.

Case 3. Male, aged 20, dementia praecox, onset of illness at 16. On admission confused, suspicious, impulsive, and childish, habits faulty. Physical condition poor, very toxic. Under treatment.

Intestinal Flora;- Four types of anaerobic diphtheroid; total, 6+.

Series of ten slides demonstrating anaerobic diphtheroids Nos. 1,2, and 3, each with different colony and microscopical characteristics. No. 1 anaerobic diphtheroid (Strain 20, Table 6) gave sugar reactions of Klebs-Loffler. No. 2 (Strain 14, Table 6) likewise gave same reactions except for trace acid in saccharose. No. 4 (Strain 21, Table 6) is not demonstrated but gave Klebs-Loffler reactions.

Case 4. Female, aged 18, recurrent confusional, very acute type, onset of illness at 16. Physical condition very poor. Recovered.

Intestinal Flora;- Anaerobic diphtheroid two types; leptothrix one type; total, 4½+.

Six slides illustrate the possibility of tracing diphtheroid infection from the tonsils to the intestine. Anaerobic intestinal leptothrix seen in smears from tonsillar debris. This case is a good example of a residual minor focus with a severely infected major focus.

Case 5. Male, aged 33, recurrent confusional with delusions of persecution and auditory hallucinations, underdeveloped mentality. Physical condition, outwardly fairly good. Admitted after second attack during which temperature reached 101°F. Under treatment.

Intestinal Flora;- Anaerobic diphtheroid two types; anaerobic leptothrix one type; total infection, 4+.

Seven slides show aerobic type of leptothrix non-facultative anaerobe, anaerobic diphtheroid No. 1 (Strain 15, Table 6) gave sugar reactions of Klebs-Loffler. Anaerobic leptothrix (Strain 5, Table 7) sugar reactions given.

Case 6. Male, aged 27, dementia praecox, paranoid type, confused, excited, and deluded, religious and persecutory ideas, unstable mentality, rather a failure in life. Physical condition unsatisfactory. Under treatment.

Intestinal Flora;- Anaerobic diphtheroid three types; total of 5+. Anaerobic leptothrix 2+.

A series of ten slides are given showing the microscopical characteristics of the above flora. Anaerobic diphtheroid No. 1 (Strain 16, Table 6) gives sugar reactions of Klebs-Loffler.

Case 6A. Five slides are also given illustrating an atypical aerobic diphtheroid in the intestine with strongly facultative anaerobic habit. Non-metachromatic, probably solid, type; later small granules developed in anaerobic sugar broth. Sugar reactions (Strain 17, Table 6) of Klebs-Loffler bacillus; filamentous granules and non-granular branching forms present in sugar broth.

Case 7. Male, aged 44, melancholia, severe, nine months duration, delusions of suspicion and persecution, at one time homicidal. Physical condition very toxic. Recovered.

Intestinal Flora;- Anaerobic diphtheroid two types, $3\frac{1}{2}$+. Anaerobic leptothrix two types, $3\frac{1}{2}$+.

Five slides demonstrating the characteristics of the two types of anaerobic leptothrix. one metachromatic, the other non-granular. No. 1 leptothrix (Strain 7, Table 7) sugar reactions given.

Case 8. Male, aged 29, recurrent cyclical confusional attacks, duration of illness nearly two years, very toxic during acute phases. Recovered from attacks, out on trial.

Intestinal Flora;- Anaerobic diphtheroid two types, 4+. Anaerobic leptothrix 3+.

Four slides show the microscopical characteristics of the anaerobic leptothrix (Strain 6, Table 7) and sugar reactions. This case is another example of residual tonsillar infection which was traced to the intestine.

Demonstration Set No. 1.

Sixteen slides illustrate the microscopical characteristics by Gram and Neisser stain of eight of the classified anaerobic groups given in Table 5.

Demonstration Set No. 2.

A series of slides demonstrate the great contrast between the aerobic and anaerobic intestinal flora in mental patients.

Demonstration Set No. 3.

These show the presence of diphtheroids and leptothrix-like organisms lying in the cheesey debris extracted from the tonsillar crypts in two cases; one is from demonstration case 4, the other from a case of melancholia. One slide illustrates the same bacterial flora in pus from around a molar tooth in an agitated melancholic.

Demonstration Set No. 4.

These show an anaerobic diphtheroid isolated from the cerebro-spinal fluid in a female patient suffering from general paralysis of the insane of the expansive, grandiose type. Good remission followed vaccine treatment. Tryparsamide given later. Wassermann reaction remained ++.

XIV.. SUMMARY.

SECTION 1. Introduction. This deals with the circumstances of the past six years leading up to the present stage of this research. The experience gained by the valuable results of systematic biochemical, haematological and bacteriological examination in many chronic physical disorders and in some mental and borderland patients, both as a means of research, and a starting point for rational treatment, lead me to apply this scheme of diagnostic survey to all admissions to Wantage House. During the period of research amongst control cases, it was striking how very similar in one respect were the underlying physical disorders of the mental patients, the great difference being that from very few could a definite physical symptomatology be ascertained. The contrast between their bacteriological infections, especially in the intestine, was however very marked, the latter having special types of anaerobic infection which were in every respect similar to those found in well-established mental cases investigated at the Scottish Asylums' Laboratory. This fact influenced me in extending the bacteriological side of the research and the application of strictly anaerobic methods to all possible foci of infection in order to discover if these anaerobic species of bacteria had any etiological significance in insanity and allied conditions.

SECTION II. Historical Review. This deals with the researches upon the bacteriology, toxicology, and haematology of the psychosis and allied disorders, from 1875 - 1929. It contains forty-four references which are summarised in a bibliography at the end of this thesis.

SECTION III. Classification of case groups and foci of infection studied in each, with table of cases on whom a diagnostic survey has been carried out. The total number of cases dealt with in this research is 450 and for convenience they are divided into four groups. Groups 1, 2, and 3 have a total of 189 cases which are sub-divided into 145 cases of established mental disorder, 22 borderland cases, and a similar number which are classified as neurotoxic. The balance of 261 cases are controls, The mental cases are further sub-divided according to their psychological symptomatology,and fall into seven sections. The main features of each group are outlined and special mention made of group 3 which, on account of their co-operation, capacity to respond to treatment, and appreciation of their condition, makes them particularly valuable for clinical and scientific observation. Further, it is this group of cases whom, if treated early, respond well, but who, on the other hand, if disregarded, become amongst the most severe and often hopeless cases of mental disorder. Over a thousand bacteriological examinations have been carried out amongst the four case groups and of these in all but

124, anaerobic as well as aerobic methods have been employed. Out of the 450 cases, 209 have been examined by the method of diagnostic survey; of these 154 belong to the mental and allied case groups.

SECTION IV. Bacteriological technique. In this section a brief outline is given of the aerobic cultural methods, these having run parallel with the anaerobic technique. The composition and preparation of media, and a description of the special methods employed for ensuring strict anaerobiasis are given; also the method of sheep-bleeding and preparation of sterile serum and haemoglobin so essential to the study of anaerobic bacteria. The spectroscopic characteristics of the aerobic and anaerobic media are also mentioned. Methods of obtaining material to be cultured are described and the need for special care with intestinal samples if good results are to be expected. The examination of the cerebro-spinal fluid for evidence of growths of delicate anaerobes is also given, the technique employed yielding a percentage of positive results not obtained by other methods. The special uses of the different anaerobic media in the study of the anaerobic diphtheroid and leptothrix group, and the staining methods employed are also outlined.

SECTION V. The anaerobic diphtheroid and leptothrix group. Their cultural morphology and microscopical characteristics are described in detail and a classification table given which, though necessarily tentative, serves to indicate the main groups into which

the anaerobic diphtheroid bacteria fall. Thus it has been necessary to divide the 312 strains into six cultural, and eleven microscopical sub-groups, a table being given to show their incidence and relationship to one another. The anaerobic leptothrix group is dealt with in the same way and is seen to be much more compact, only three sub-groups being at present necessary. The relationship of the anaerobic diphtheroid and leptothrix groups is also discussed.

SECTION VI. Biochemical reactions of anaerobic diphtheroid and leptothrix groups. Twenty-one anaerobic diphtheroid strains and one facultative anaerobe tested in 5 - 9 different carbohydrates are described, and given in detail in tabular form. The significance of the results are discussed in relation to the most authoritative views on the subject. Out of the 21 anaerobic strains, 7 give reactions of the Klebs-Loffler bacillus, the one obligatory anaerobic strain also proving similar. Some of the remaining strains gave reactions which leave it a matter of opinion as to their being Klebs-Loffler, others however are definitely relegated to the diphtheroid group. Of the anaerobic leptothrix group the sugar reactions of 11 strains are given and tabulated. The results show tham to be varied in their fermentation power, no definite grouping as yet being possible. Glucose, dextrin, and laevulose are most frequently and strongly fermented by these bacteria. Their possible relationship to leptothrix Buccalis is also discussed.

SECTION VII. Animal experiments. This section deals with the results of a limited number of experiments with guinea-pigs on which tests for virulence have been carried out. Eleven different strains of anaerobic diphtheroid, two giving Klebs-Loffler sugar reactions, were inoculated by the sub-cutaneous or intra-peritoneal route. Nine strains proved non-virulent, one gave rise to marked toxic symptoms, while the last invaded the colon and was recovered in the stool of the animal post-mortem. The condition of cultural growth in relation to the production of toxicity, and the difference between virulence and toxic power are discussed. In connection with the former the feeding experiments on rats and mice carried out by Ford Robertson are cited as being striking proof of the capacity of the afore-mentioned bacteria in this direction. The present concensus of opinion that most of the diphtheroid group are non-pathogenic can be refuted on the evidence of the present research, and various points are mentioned supporting this claim. The action of four strains of anaerobic leptothrix on animals is also given, as well as the results of past experiments having a bearing on the present work.

SECTION VIII. Analysis of the aerobic and anaerobic bacteriological flora of the minor and major foci. In this section ten tables are given to illustrate the incidence of the numerous types of bacteria both aerobic and anaerobic that have been found in the various foci of infection in the control and mental groups.

The method of assessment of the numbers of bacterial species is given and the reason for adopting the plus and plus-over-minus system up to the maximum of 8+ in any one culture, discussed. The terms minor and major foci are explained, the former including all regions except the large and small intestine. The minor foci constituting oral sepsis are discussed in relation to their importance as starting points for dissemination of infection throughout the body, especially in the formation of the major lower alimentary focus. Evidence is given of the significance and existence of lymphatic spread, reference being made to researches bearing upon the presence of oral sepsis as it affects gastric function and later the colon. Foci of infection other than the mouth and intestine that may become important pathologically are discussed, and the findings given of the incidence of anaerobic infection of the cerebro-spinal fluid, accessory sinuses, and uterus. The possible tracking of anaerobic diphtheroid and leptothrix infection from tonsillar and dental foci to the intestine, is cited in several cases, and the importance of realising that the latter forms the ideal nidus for these bacteria is emphasised. The question of how these infections originate and their relationship to the development and onset of mental illness is discussed, and the influence of susceptibility, age, and other relevant factors being mentioned. Analysis of the

tables definitely establishes how infintely greater is the incidence of anaerobic diphtheroid and leptothrix infection in the mental and two allied case groups as compared with the controls. The diagnosis of the physical disorders in those controls having moderately severe and severe infections is given, these showing a fairly large proportion suffering from gastro-intestinal trouble and debility. The occurrence of intestinal streptococcal infection in the mental groups is also discussed and related to the incidence in physical disorders. Comparison between the three sub-divisions of the psychogenic group is made, and the fact that the neurotoxic group 3 cases bear as much anaerobic infection as do group 1 stands out clearly. The inherent psychological instability of the psychasthenic and neurasthenic patient in contrast to the much greater resistance and driving force of the neurotoxic cases is suggested as being a reason for the difference of capacity to withstand neurotoxic action. It is emphasised however, that in the latter this very fact may be their undoing and that breakdown to them, if it comes, is infinitely more serious. The need for the proper recognition and early medical treatment of such cases is stressed.

SECTION IX. Neurotoxins and neurotoxic action. In this section the subject of neurotoxins receives consideration, the composition and action of the specific nerve poison of B. tetanus being compared with

that of B. diphtheriae. The action of the two toxins is described in experimental animals and later correlated with those produced by the anaerobes. The parallel development of toxicity and subsequent modification of B. diphtheriae and B. tetanus in cultures and in the body is discussed in relation to our present knowledge of the subject, and from this conclusions are drawn regarding neurotoxic action as observed in animals and in man. Views as to the formation of special neurotoxins by the anaerobic Klebs-Loffler and diphtheroid group are put forward, and the fact that they are similar except in degree to those produced by the B. diphtheriae is shown; further, it is claimed as being probable that they form even more specific neurotoxins which partly account for their selective action in mental disorder. Evidence of endotoxic action is given and regarded as an important additional factor, the exact neurotoxic role of which requires further investigation. Reference is made to the production of immunity and reasons given for probable deficiency in this respect. Correlation of established facts concerning our knowledge of the morbid changes occurring in the organs and central nervous system in insanity, has made it possible to formulate several hypotheses of the manner in which neurointoxication affects the economy in causing local and general changes which ultimately result in the dysfunction and gradual degeneration of nerve cells belonging to the higher grades of

psychic function. These practical and partly theoretical considerations are based primarily upon the evidence of local changes, and later upon those of a more general character. The condition of tonic hardening of the colon,and the abnormal reflex arc mechanism described by Wilson is given,and his views confirmed from the writer's own observations on a hundred mental cases. It is claimed that this local lesion is a specifically neurotoxic one affecting Aurbach's plexus. The high incidence of severe constipation is correlated with tonic hardening, and the importance of the two in establishing extensive morbid changes leading to chronicity is discussed. Evidence of this is cited in the X-ray work of others and from the writer's own investigations in the subject. Another local effect of neurotoxic action is that upon the autonomic system which seldom escapes from the symptomatology of mental disorder. The views of Trotter on the insulation of the nervous system, and the importance of the maintainance of its integrity stressed if nerve function is to remain normal. Based on this theory, a new conception of the relationship of the somatic tissues to the nervous system is put forward in distinction to the effects of the usual meaning of the term environment upon the reactions of the central nervous system. The special protective structures of the brain are outlined in connection with the barrier action against systemic poisons, and the known facts of altered permeability in various

mental disorders mentioned. Experimental evidence of bacterial toxins, amongst them diphtheria, in lowering permeability is cited. Morbid changes in the brain in mental disease are referred to, and the experimental and histological observations of three investigators given, having a bearing upon the process of nerve cell degeneration in man as being suggestive of neurotoxic action. The factors predisposing to neurotoxin fixation are discussed and correlated with recognised factors affecting the somatic side of the economy. A hypothesis based on the trophic function of nerves is put forward as being the expression of a generalised but less specific effect of neurotoxic action, resulting in the lowering of the vitality of the whole economy. This condition, which is termed neurotoxic atrophic effect, explains, the writer believes, much that is peculiar to the physical aspects of so many of the insane. Various instances are given to bear out this theory.

SECTION X. The incidence of diphtheria in mental hospitals and other state institutions. Evidence relating to the absence of acute diphtheria in mental hospitals is given and compared with similar information regarding prisons and Borstal Institutions. The conditions of isolation of the inmates are contrasted, and the fact mentioned that the former are much more exposed to contact with those outside. The fact that carriers are occasionally found is in agreement with the finding that, in the writer's experience, between

40% and 50% are found to have either anaerobic diphtheroid or Klebs-Loffler types in the throat. This, and the knowledge that the majority have the same infection in the intestine, results in a long acquired immunity which is sufficient to account for the absence of acute diphtheria. It is however admitted that relative isolation has a bearing upon this freedom. The pathogenesis of diphtheria and carriers of the organism in relation to the development of mental disorder is discussed, and its probable influence upon Public Health work mentioned. The results of establishing the relationship of diphtheria and allied infections to mental disorders is mentioned as ulimately leading to the recognition of the latent stage of the disease, and also to the association in the lay ming of the dread word, insanity, with a well-known acute physical disease. This, it is hoped, will assist in effecting early diagnosis and treatment on medical and psychotherapeutic lines.

SECTION XI. The diagnostic survey, its influence on treatment, medical and psychotherapeutic. In this section reference is made to the psychological aspects of mental disorder as they would appear to be affected by the method of diagnostic survey with its great scope for instituting medical treatment based on rational lines. The great importance of psychotherapy is stressed but it is mentioned that its value can be enhanced if those who practise it will seek fuller co-operation with the scientist and

physician. The writer maintains that the place of psychotherapy in the scheme of treatment will be changed to the time of convalescence after the weakening effects of serious physical illness, this being to the advantage of both patient and psychotherapist alike, The task of mental rehabilitation and the correction of abnormal psychic processes would appear to be more rational at this stage than labouring with patients who cannot, or will not, give their co-operation on account of the underlying physical disease. The absence of true physical symptomatology in insanity is made the justification for the adoption of the diagnostic survey as a means of diagnosis and treatment. The writer refers to his experiences and results in this direction as justifying the amount of time and patience required. Reference is also made to child-psychology and the importance of this field of psychotherapy, the writer however maintaining that the somatic or physical aspects must not be overlooked if failures are to be avoided.

SECTION XII. Conclusions.

SECTION XIII. Summary of demonstration slides and cases illustrating the types of anaerobic Klebs-Loffler bacillus, anaerobic diphtheroid and leptothrix infections.

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