INFECTION WITH COLIFORM BACILLI AS A CAUSE OF RHEUMATOID ARTHRITIS AND CHRONIC RHEUMATISM: ITS DIAGNOSIS AND ITS TREATMENT BY AUTOGENOUS VACCINES.

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CONTENTS.

I. INTRODUCTION.

Although research is still proceeding into the pathological conditions which form the subject of this communication, it is thought advisable to place on record the results obtained up to the present. It was hoped to submit the report of a completed investigation, but each step that has been taken in the experimental production of the diseases concerned, has opened up so many new avenues for research, that this paper must be looked on as only a preliminary report, to be followed by others as material for such accumulates.

It is necessary first of all to attempt to define certain of the terms used in this paper. The designation "coliform bacilli" is applied to organisms isolated from human faeces which belong to the colon-typhoid group of bacilli. In regard to diagnostic terms, those defined by Stockman[1] in relation to rheumatism and arthritis have been mostly followed and are detailed here.

"Acute rheumatism" is a specific fever, the result of a general infection, showing both constitutional and local symptoms, the latter affecting especially the white fibrous tissue of the joints, muscles, heart and other organs.

[1] Stockman. *Rheumatism and Arthritis.*

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"Chronic rheumatism" is a condition of chronic inflammation of the white fibrous tissue of fasciae, aponeuroses, sheaths of muscles and nerves, ligaments, tendons, periosteum or subcutaneous tissue, leading to pain, aching, stiffness and other symptoms in the affected part, and is the result of preceding general inflammation, or of local inflammation or injuries.

"Rheumatoid arthritis" is a very chronic disease affecting chiefly the fibrous tissues of the locomotory system, especially involving the joints, causing pain, deformities and crippling, and being non-suppurative and, until recently, unamenable to any specific treatment.

"Osteo-arthritis" is a very chronic disease involving primarily the bone and cartilage, and only secondarily the fibrous tissue of joints, and characterised by marked deformities of the joints resulting from simultaneous bone absorption and bone production.

The first patient treated by the method which will be described in a later section, was sent to the Medical Research Laboratory, Nairobi, by Dr Gilks, now Principal Medical Officer of the Colony, but at that time Resident Surgical Officer at Nairobi Hospital, with a view to determining whether vaccine treatment was possible or advisable for the rheumatoid arthritis from which the patient was suffering. The usual sites of septic absorption were examined and eliminated one by one, until only the intestines remained. It was then that the idea of recovering a number of organisms from the faeces and testing their agglutinability by the patient's serum, suggested itself. Several lactose-fermenting bacilli were isolated on MacConkey's lactose bile-salt neutral-red agar and subcultured on to agar slopes. The purity of these cultures was then tested, and when this was assured, the agglutination reactions of the bacilli with the patient's serum were examined. One organism was agglutinated markedly by a 1 in 100 dilution of the serum, and from it the vaccine was prepared. The initial dose was made very small, under one million bacilli, as it was not known what the reaction might be. Succeeding doses were put up so that each was twice as strong as its immediate predecessor in the series, and were administered at weekly intervals. The first few doses produced no effect, but as the strength of the injections gradually increased, more and more reaction was seen, and was followed by distinct clinical improvement, shown by lessened pain, increased freedom of movement of the affected joints, and the cessation of the progressive character of the disease. Since this patient was seen, more than one hundred cases of disease attributable to infection with coliform bacilli, have been or are being treated, and a significant fact is that most of the new patients have been sent by former sufferers cured or improved by our procedure. The first case treated by our vaccines has now been under observation for five years, and during that time the improvement affected has been maintained.

As time has gone on and brought to the Laboratory a large number of sick persons seeking relief for their various complaints, the conditions in which agglutinable coliform bacilli may be isolated from the faeces have been

found not to be confined to rheumatoid arthritis as was believed at first, but to include cases of chronic rheumatism and other allied diseases, almost all being apparently amenable to vaccine treatment.

In the present paper it is our intention first to describe briefly the technique employed in the examination of patients for infection by coliform organisms and in the preparation of the vaccine, then to give an account of the clinical effects of the treatment, next to discuss shortly experimental facts discovered in the laboratory, and finally to attempt to formulate a working hypothesis to explain our results on the basis of our present knowledge.

II. TECHNIQUE EMPLOYED IN DIAGNOSIS AND IN THE PREPARATION OF AUTOGENOUS VACCINES OF COLIFORM BACILLI.

When a patient complains of symptoms, or on examination shows signs suggesting the presence of an infection with an organism of the coli group, a sample of his blood is withdrawn from a vein to provide serum for the agglutination test and he is instructed to send to the Laboratory as early as possible a sample of his faeces. When the latter arrives, a small loopful of it is inoculated into each of two tubes containing bile-salt peptone water (sodium taurocholate 0·5 per cent.: peptone 1·0 per cent.). These tubes are incubated at 37° C. for 24 hours, and then a loopful from each is spread over the surface of neutral-red bile-salt lactose peptone agar in Petri dishes in such a manner as to secure a large proportion of isolated colonies, the number of plates required for each loopful to obtain this result depending on the technical skill of the operator. These plates are incubated at 37° C. for 24 hours, and then two or three representatives of each variety of colony seen are picked off and inoculated into tubes of nutrient broth, which after incubation for 24 hours at 37° C. form the cultures to be tested for agglutination with the patient's serum. Four methods of conducting this test have been employed at various times. The original one used was that described by Broughton-Alcock[1]. This did not prove very satisfactory in practice, probably owing to the delay experienced in the reaction with coliform bacilli. The microscopic hanging-drop method using a platinum loop to make the dilutions, was then adopted, but was found to give rise to anomalous results which can reasonably be attributed to the inaccuracy of the method of dilution used in this test, a surmise supported by the discovery that when dilution of the serum, and the mixing of the diluted serum with the broth culture are carried out in bulk by means of a calibrated dropping pipette, and the hanging-drop taken from the resulting mixture, the findings conform very closely to those obtained with Neisser's or Dreyer's technique. The methods at present in use are those described by Neisser[2] and by Dreyer[3]. It is probable that Neisser's method is theoretically open to objection, but as in a long series of tests, the results

[1] Broughton-Alcock. *Journ. Royal Army Med. Corps*, xxx. 424.
[2] *Med. Res. Council* (1920). Special Report Ser. No. 51. p. 111.
[3] *Ibid.* No. 51, p. 128.

obtained with it have been comparable with those given by Dreyer's method at serum dilutions considered significant of infection, from the practical point of view it does not seem to be a matter of great importance which method is used. Of course when the end-titre of a serum for a particular bacillus is in question, it is essential to employ Dreyer's technique, and here it must be emphasised that when this method is used, the bacillary emulsions must be grown in veal broth, and must be very thin, that the flocculation obtained is much finer than with typhoid cultures, and that the agglutination tubes must be incubated for 5 hours at 50–55° C. As in all our cases a titre of 1 in 90 has been found to be constantly present in the patient's blood for the coliform organisms considered responsible for the infection, this dilution of serum has been adopted as the lowest significant in the test, the usual controls of course being set up.

The cultures in broth of those organisms which show agglutination at the significant titre with the patient's serum, are again plated out on neutral-red bile-salt lactose peptone agar to test for purity. If the cultures are pure, an isolated colony from each is picked off and inoculated on to an agar slope, but if the cultures are mixed, each variety of colony that appears, must be re-tested for agglutination with the patient's serum, and those which show agglutination, re-examined for purity. When pure cultures of the agglutinating bacilli are obtained, a 24 hours old growth of each variety on an agar slope is emulsified in a small quantity of a freshly prepared 0·5 per cent. solution of carbolic acid in normal saline. The emulsions so obtained are mixed in amounts directly proportional to the agglutinability of each variety of bacillus in a sterile bottle containing sterile glass beads, and then shaken in a mechanical shaker for an hour or more until the bacteria are uniformly distributed throughout the liquid. The emulsion is allowed to stand for 48 hours, after which a loopful is subcultured on to an agar slope to test it for sterility. As soon as the vaccine is sterile, it is standardised by the method described by Brown and Kirwan[1] to contain 0·1 milligramme of dried bacilli per cubic centimetre, and placed in a small sterile bottle which is then closed by a sterile rubber cap, and sealed by a gelatin solution. The method of dosage recommended at present is an initial injection subcutaneously of 0·1 c.c. (equivalent to 0·01 mgm. of dried bacilli), subsequent doses being usually increased by 25 per cent. until a maximum of 2·0 c.c. (equivalent to 0·20 mgm. dried bacilli) is reached. The vaccine is administered at intervals varying with the amount of reaction shown to each dose by the patient, a period of four clear days after all reaction from the previous injection has disappeared, being ordinarily sufficient.

[1] Brown and Kirwan (1914–15). *Indian Journ. Med. Res.* II. 763.

III. THE CLINICAL RESULTS OF TREATMENT BY AUTOGENOUS VACCINES OF COLIFORM BACILLI.

In Table I will be found a list of all cases treated by our vaccines of which information as to the original clinical condition and the final result is available. At the foot of this table will be found a summary of the results, not only of the cases detailed, but also of all other cases treated by us, these latter being found grouped under three headings, "Result unknown," "Cases insufficiently treated," and "Cases still under treatment."

Table I.

Case No.	Diagnosis	Result
1	Rheumatoid Arthritis	Greatly improved
2	Rheumatoid Arthritis	Improved
3	Rheumatoid Arthritis	Greatly improved
4	Rheumatoid Arthritis	Cured
5	Rheumatoid Arthritis	Greatly improved
6	Rheumatoid Arthritis	Greatly improved
8	Chronic Rheumatism	Cured
9	Chronic Rheumatism	Cured
11	Rheumatoid Arthritis	Cured
12	Rheumatoid Arthritis	Greatly improved
14	Chronic Rheumatism	Cured
15	Acute Rheumatism	Slightly improved
16	Chronic Rheumatism	Cured
17	Osteo-arthritis	No improvement
20	Osteo-arthritis	Slightly improved
21	Rheumatoid Arthritis	Cured
24	Chronic Rheumatism	Cured
25	(?) Gonorrhoeal Rheumatism	Improved
27	Rheumatoid Arthritis	Greatly improved
28	Chronic Rheumatism	Cured
29	Chronic Rheumatism and Sciatica	Cured
31	Osteo-arthritis	Not improved
32	Infected gunshot wound of leg	Cured
34	Chronic Rheumatism	Cured
36	Rheumatoid Arthritis and Sciatica	Greatly improved
37	Arthritis	Not improved
38	Rheumatoid Arthritis	Cured
39	Chronic Dyspepsia	Cured
40	Chronic Rheumatism	Improved
41	Rheumatoid Arthritis	Greatly improved
42	Chronic Rheumatism	Greatly improved
44	Membranous Colitis	Cured
45	Chronic Rheumatism	Cured
46	Bacilluria, (*B. coli*)	Cured
47	Bacilluria, (*B. coli*)	Cured
48	Rheumatoid Arthritis	Greatly improved
49	Chronic Rheumatism and Neuritis	Cured
50	Chronic Rheumatism and Sciatica	Improved
51	Chronic Rheumatism and Sciatica	Cured
52	Bacilluria, (*B. coli*)	Cured
53	Rheumatoid Arthritis	Greatly improved
54	Chronic Rheumatism	Cured
55	Sciatica	Greatly improved
56	Rheumatoid Arthritis	Cured
57	Chronic Rheumatism and Neuralgia	Cured
58	Bacilluria, (*B. coli*)	Cured
60	Rheumatoid Arthritis	Greatly improved
61	Pyrexia	Improved
62	Colitis and Chronic Rheumatism	Cured
63	Acute Rheumatism	Cured
65	Chronic Rheumatism	Greatly improved
66	Chronic Rheumatism and Bacilluria, (*B. coli*)	Cured
67	Chronic Rheumatism	No improvement
68	Colitis	Cured
69	Chronic Rheumatism and Bacilluria, (*B. coli*)	Greatly improved
70	Rheumatoid Arthritis	Cured
71	Rheumatoid Arthritis	Greatly improved
72	(?) Gonorrhoeal Rheumatism	Improved
73	Chronic Rheumatism and Bacilluria, (*B. coli*)	Greatly improved
74	Chronic Rheumatism	Cured
75	Lumbago and Sciatica	Greatly improved
77	Lumbago	Cured
78	Rheumatoid Arthritis	Not improved
79	Chronic Rheumatism	Cured
80	Chronic Rheumatism	Cured
83	Lumbago	Cured
85	Rheumatoid Arthritis	Cured
86	Colitis	Not improved
87	Colitis and Cystitis	Greatly improved
88	Chronic Rheumatism	Greatly improved

SUMMARY. (*Results of all cases treated up to June*, 1924.)

Disease	Total cases treated	Results of treatment: Cured	Greatly improved	Improved	Slightly Improved	Not improved	Result unknown	Insufficiently treated	Under treatment
Abscess	1	—	—	—	—	—	—	—	1
Arthritis	1	—	—	—	—	1	—	—	—
Bacilluria, (*B. coli*)	7	4	—	—	—	—	—	—	3
Colitis	4	2	1	—	—	1	—	—	—
Constipation, Chronic	1	—	—	—	—	—	—	1	—
Dyspepsia, Chronic	1	1	—	—	—	—	—	—	—
Infected gunshot wound of leg	1	1	—	—	—	—	—	—	—
Osteo-arthritis	4	—	—	—	1	2	—	1	—
Pyrexia	1	—	—	1	—	—	—	—	—
Rheumatism, Acute	4	1	—	—	1	—	—	—	2
Rheumatism, Chronic	47	20	7	2	—	1	1	6	10
Rheumatism, (?) Gonorrhoeal	2	—	—	2	—	—	—	—	—
Rheumatoid Arthritis	22	7	12	1	—	1	—	1	—
Diagnosis unknown	15	—	—	—	—	—	9	2	4
Total	111	36	20	6	2	6	10	11	20

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A short explanation must here be given as to what is meant by certain of the headings used in the table.

"Cured" means a complete return to health of the previously affected parts as far as can be determined by clinical examination.

"Greatly improved" means that the patient's condition has become as normal clinically as was permitted by the permanent pathological changes present when treatment was commenced.

"Improved" means that there has been complete cessation of the progressive character of the disease, and marked amelioration of the physical signs and symptoms.

"Slightly improved" means that there has been some definite though not great improvement in the patient's condition.

"Not improved" means that the vaccine has produced no apparent change in the progressive character of the disease.

"Result unknown" means that the practitioner in charge of the case has failed to supply information as to the results of the vaccine.

"Cases insufficiently treated" are those in which the patient through carelessness or through dissatisfaction at the failure of the vaccine to cure in a few doses, has only received two or three injections at irregular intervals. Certain patients who failed to come for their vaccines when these were ready, are included in this class.

The diagnoses used in the table conform to those set out in the introduction to this paper.

It is proposed in this section to give a detailed description of a few cases treated by autogenous coliform vaccines which either demonstrate most clearly the results commonly obtained, or are important as illustrating points of significance in the elucidation of the pathogenic properties of coliform bacilli.

The following three cases have been selected as typical of the results of autogenous coliform vaccines in rheumatoid arthritis.

Case 1. Male. Adult. Came under observation in August 1919. His history was that rheumatism began in 1913 in the shoulders, wrists and ankles. He was treated with salicylates, potassium iodide, liniment and radiant heat. Slight local improvement resulted, but the general condition remained unchanged. Endocarditis developed at the end of 1913, and the patient was in bed for three months, and off duty for six months. He was advised to come to East Africa in 1917, at which time he had considerable pain in, but little fixation of, his joints. After his arrival in Kenya Colony, the pain became worse, and the joints, especially those of the left shoulder and the right wrist, became more or less fixed. In 1918 the patient came under the care of Dr Gilks for the first time. The left shoulder-joint was then markedly limited in movement, and much creaking was observable on attempting to move it. The shoulder muscles were very wasted. The right wrist joint was swollen, the swelling involving the carpal joints as well, while there was considerable bony deformity and a great thickening of the periarticular fibrous tissue. Movement was practically absent. The interphalangeal joints of the fingers showed spindle-shaped swelling with much restriction of movement, though bony deformity was absent. Other joints were affected less markedly, though in most there was considerable pain, especially in the ankles. In August 1919 this patient was seen at the Laboratory. There was no evidence of pyorrhoea, nor was there a sign of any other source of septic absorption, except that suggested by the chronic constipation complained of by the patient. His faeces were examined, and from them *Bacillus coli communis* and *B. paragrünthali* were isolated and found to be agglutinated by the serum of the patient in a dilution of 1 in 100. A vaccine was prepared from these two organisms. The patient improved greatly as the result of treatment with

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this vaccine. His general health became better, he put on fat, his appetite returned, the mental depression disappeared, and the muscles recovered much of their tone. The movements of the left shoulder-joint increased so that the left hand could touch the right ear over the top of the head. The movements of the right wrist joint did not improve except slightly, but the finger joints became normal in shape and movement. The disability which remained was very largely due to permanent pathological changes in the joints present when treatment was commenced and which vaccine therapy could not be expected to remove. At the present time the patient is able to play tennis and ride a motor-cycle. Slight recurrences of pain have occurred on two occasions subsequent to the first vaccine treatment (in December 1921 and December 1922). Both were successfully treated by vaccines prepared from fresh agglutinable coliform bacilli isolated from the patient's faeces. There has been no relapse in the clinical improvement shown in the affected joints although it is now five years since the first vaccine was given.

Case 5. Female. Adult. Came under observation in May 1920. The patient was 53 years of age. She had had rheumatic fever 12 years previously, and had been subject to twinges of rheumatism all her life. In June 1915 she developed pain, stiffness and swelling in the right knee, and had to walk with a stick. She had marked pyorrhoea at the time. She returned to England, and was treated at Buxton. All her teeth were removed, and the rheumatism improved. On the return voyage, the weather was very bad, and the left knee became affected. The disease spread to her right hand, then to both ankles and later to other joints. The patient gradually became more crippled, and in May 1920 she could only shuffle about with a stick. Her back was bowed, and the stoop on walking was very marked. Both knees were very swollen owing chiefly to thickening of the periarticular tissues and of the synovial membrane, the fluid present in them not being great in quantity. The right ankle was swollen from the same causes. Both hands were swollen and showed ulnar flexion, more marked in the right one, while the fingers of this hand were flexed and ankylosed. Anti-rheumatic treatment with salicylates, iodides and radiant heat had no effect. From this patient's faeces in May 1920 a coliform bacillus was isolated which was agglutinated by the patient's serum in high dilution. A vaccine was therefore prepared from this organism, and steady improvement followed treatment with it. Pain ceased almost immediately, and at the conclusion of the course the patient could walk two miles with ease, and the stick was discarded. She now could carry out her full school duties. The back became straight, and there was no stoop on walking. Some slight swelling of the knee persisted, and flexion of that joint only improved to three-quarters of normal, though extension was full. The deformity of the right hand remained, but the use of it was almost completely regained, and the ulnar flexion became less pronounced. The left hand still showed enlargement of the metacarpo-phalangeal joints of the index and middle fingers. The patient's general health greatly improved, although the housing and other environmental conditions of her life remained unchanged. Since treatment ceased four years ago, the improvement has been maintained, indeed some of the deformities have become less marked, while there has been no recurrence of pain or disability.

Case 70. Female. Adult. Came under observation in July 1923. There was a family history of lumbago, chiefly amongst the patient's brothers and sisters. Since she was 18 years old, she had had acute attacks of lumbago at intervals of about three years, while between attacks she had had stiffness and limitation of movement of the joints of the back. In 1918, she had neuritis in the right arm causing much pain, and great wasting of the muscles. A short time prior to coming to the Laboratory, the patient noticed that the interphalangeal joints of the right hand had become thickened and painful, and were subject to exacerbations during which the affected joints became reddened and swollen. The patient was unable without considerable difficulty to open her hands after gripping an object. She complained of chronic constipation, and of loss of appetite and sleeplessness. On examination the affected joints were found to be enlarged due to thickening of the

periarticular tissues, and on passive movement they gave slight crepitation, while the patient complained of creaking sensations. Slight ulnar flexion was observable in the right hand. *B. pseudocoloides* A. was isolated from the patient's faeces, and was found to be agglutinated by the patient's serum in a dilution of 1 in 300. A vaccine was prepared from it, and after the third dose the patient's appetite and sleeplessness improved. After ten injections she could do everything with her right hand without pain or disability, except that after exerting much force, the third or even all the fingers sometimes became locked, and had to be forced open. The local condition in the hand was steadily improving while the patient's general health was very much better. In March 1924 she came to report that she had never felt so well in her life, and that the right hand had completely recovered, being free from any pain or disability and having no tendency to locking of the fingers after closing the hand forcibly. The periarticular thickening had entirely disappeared, and the hand looked normal. The patient remarked that the improvement due to the vaccine continued for some considerable time after the injections ceased.

The following two cases are reported as examples of patients who have had several relapses. Fortunately such cases are uncommon.

Case 12. Female. Adult. Came under observation in November 1920. The patient had a history of increasing pain for three years in the hands and feet, which was so great that, coupled with the disability attendant on stiffness of the joints of the hands, it rendered her work on her farm impossible of execution. On examination the hands were almost completely crippled, and could not be closed. The interphalangeal joints of the hands showed spindle-shaped swellings due to thickening of the periarticular tissues, while the metacarpo-phalangeal joints were enlarged from the same cause. There was marked limitation of movement in all the affected joints, and ulnar flexion of both hands. A coliform bacillus was isolated from the patient's faeces, which was agglutinated by her serum in high dilution, and from it a vaccine was prepared. After treatment with this vaccine, the patient remained free from pain for ten months. The periarticular thickening of the affected joints diminished, so that the deformity occasioned by this, and the disability due to limitation of movement almost completely disappeared. In December 1921 there was a slight recurrence of pain and stiffness in the fingers. The faeces were again examined, and a coliform bacillus Type IV (see Table II) isolated which was agglutinated by the patient's serum in a dilution of 1 in 100. A vaccine prepared from this bacillus led to disappearance of pain and stiffness for eight months. In September 1922 there was another slight recurrence of the same symptoms, but these disappeared completely after a vaccine prepared from *B. pseudocoloides* A, isolated from the patient's faeces and agglutinated by her blood serum in a dilution of 1 in 200, had been administered. In July 1923 the patient asked for another vaccine to be made to take with her to England for fear a relapse should occur during her absence from Kenya Colony. A coliform bacillus Type IV was again isolated from her faeces, and found to be agglutinated by her blood serum in a dilution of 1 in 100. The vaccine was made but no occasion arose for its use. While in England the diagnosis of the disease as rheumatoid arthritis was confirmed, and as X-ray examination of her teeth showed these to be normal, she was persuaded to have her enlarged tonsils removed. The patient stated on her return to Kenya in May 1924 that her condition had been steadily improving since the last vaccine administration, and that the tonsillectomy had made no appreciable difference. At her request her faeces were once more examined, and a *Bacillus pseudocoli* isolated therefrom which was agglutinated by a 1 in 250 dilution of the patient's serum. A vaccine was made up from this organism, but no further information regarding this patient has since come to hand.

Case 27. Female. Adult. Came under observation in August 1921. The patient had a history of attacks of rheumatism for the past 12 years affecting almost all the joints of the body. When these attacks occurred, the patient became a complete cripple, and was

unable to move any of the joints affected. The rheumatism was markedly worse when the rainy season began, and more especially so, when the patient was nursing a baby. The small joints of the hands had only been affected for the preceding three months. Clinically during an attack there was swelling of the affected joint, and fixation of the latter by involuntary muscular contraction, while even the slightest passive movement caused intense pain. Ordinarily the attack would confine itself to one joint for a few days, then pass on to another, leaving the original joint with the periarticular tissues more or less thickened. Rarely was there an interval when no joint was affected. There was a long history of chronic constipation. On examination of the patient during a quiescent interval, there was found to be a thickening of the periarticular tissues of the ankle joints, which were swollen and painful on movement. There was a considerable dropping of the arch of the foot on both sides, the result seemingly of these rheumatic attacks. The wrists showed the same periarticular tissue thickening as the ankles, though not to so great a degree. The interphalangeal articulations of the index and middle fingers of the right hand, and of the index, middle and third fingers of the left hand showed spindle-shaped swellings, due mainly to thickening of the periarticular fibrous tissue. The other joints of the body showed no gross change detectable by clinical examination. From the patient's faeces was isolated a coliform bacillus Type XVIII which was strongly agglutinated by the patient's serum when diluted 1 in 100. A vaccine was made from this bacillus, and administered to the patient. The result was most satisfactory, as all symptoms disappeared for seven months, and all the clinical signs of the former pathological condition practically cleared up. At the end of March 1922, however, there was a slight recurrence of pain in one arm, an event coinciding with the onset of the long rainy season. *B. pseudocoli* was then isolated from the patient's faeces, and this was agglutinated by the blood serum in a dilution of over 1 in 300. A vaccine was prepared from this organism, and its administration resulted in complete disappearance of the symptoms which did not recur till June 1923 in spite of the fact that the patient while on vacation in England, experienced a very cold and inclement winter. In January 1923 the patient became pregnant, and in June of that year following some damp and cold weather at the end of the rainy season, she suffered some slight twinges of pain in the arm. The patient's faeces were examined again, and three bacilli, *B. pseudocoli*, *B. metacoloides* and a coliform bacillus Type VII, were isolated, and were agglutinated by a dilution of 1 in 100 of the patient's serum. A vaccine was made up from these three organisms, but not then administered in view of the pregnancy and the disappearance of the rheumatic symptoms. A month after the baby was born, the patient who was suckling the infant, began to suffer severely again from rheumatism, and then remembered that after each of her two previous confinements, she had had bad attacks of this disease while feeding the babies. The vaccine prepared in June of this year was now given with good effect, but although the pain almost completely disappeared, the swelling of the interphalangeal articulations remained, and was accompanied by considerable stiffness. For six weeks after the completion of this vaccine course, the patient, who was still breast-feeding the baby, was much better, but after this period, her rheumatic symptoms gradually got worse, more especially so in the feet. A further examination of the faeces was advised in June 1924 and *B. pseudocoli*, and an unidentified coliform bacillus were isolated, and found to be agglutinated by the patient's serum in a dilution of 1 in 100. A vaccine was prepared from these bacilli and administered to the patient, and a report received in October 1924 states that she has completely recovered, and now shows no clinical symptoms or signs whatever.

This case is of interest as showing both the persistence of a coliform organism, and its association with a variety of other allied coliform bacilli on different occasions as pathogenic agents, and also from the correlation of the rheumatic attacks and lactation. It seems probable that the rheumatic symptoms complained of in June 1923 are explicable by the coincidence of pregnancy with inclement climatic conditions. The existence of each sepa-

rately, apparently induced no attack of rheumatism, as witness the absence of the disease in England, and again after June 1923 when the weather had improved, though the pregnancy continued. The strain of lactation however broke down the patient's resistance, and permitted a re-infection with one of the original organisms in association with a new coliform bacillus.

The four cases reported below are described because they illustrate points of importance in the elucidation of the pathology of the group of diseases dealt with in this paper.

Case 32. Male. Adult. Came under observation in October 1921. During the late war he received some shrapnel wounds in the back, and a severe gunshot wound of the right thigh causing comminution of the middle third of the femur. The patient was in hospital in England for two years, but left finally with a sound femur albeit a stiff knee, the surgeons fearing to attempt the breaking down of adhesions in the joint lest the healed sinuses from the gunshot wound should become inflamed again. Amongst other treatment during his stay in hospital, he received a stock coli vaccine, as the pus from the wound was largely the result of an infection with coliform organisms. This vaccine apparently produced little effect. The patient came to Kenya Colony at the end of 1919, but a few days after arrival had the misfortune to catch his foot in an open drain, and in falling heavily on the ground, to break the previously injured femur close to the site of the original comminution. Although the fracture was not compound, the tissues around the old wound became inflamed, and some of the former sinuses began once more to discharge pus, which occasionally contained tiny sequestra of bone. The inflammation had periods of subsidence or quiescence, and periods of recrudescence, this state of affairs persisting for about 12 months. During this time several fragments of bone were removed at operation, and at the end of it, the physical signs pointed to an abscess situated close to the bone. Strange to say, the fracture, in spite of the inflammation, had reunited. The abscess was opened by an exploratory incision, and the pus, which contained no bony sequestrum, evacuated. This pus on bacteriological examination only showed *Staphylococcus albus*. Unfortunately these surgical measures did not give the measure of relief expected, nor did a staphylococcal vaccine, and the patient's condition rapidly became worse as shown by the appearance of a slight quantity of albumin in the urine, coupled with a decided loss of weight and with progressive weakness. It was then suspected that the patient might be still suffering from the original coliform infection acquired when he was wounded, although the pus from the discharging sinuses only showed an infection with *Staphylococcus albus*. Recourse had therefore to be had to the measures adopted by us for the preparation of vaccines for coliform infections. The patient's faeces and urine were examined; the latter was sterile, but the former yielded *B. pseudocoli* which was agglutinated by the patient's serum in a dilution of 1 in 250. From this organism a vaccine was prepared, the administration of which caused immediate improvement in the patient's condition. The appetite returned, and weight increased; the sinuses healed up, and the albuminuria disappeared. This improvement has been maintained without a sign of relapse up to the present, and the patient plays tennis regularly, and golf occasionally.

This case suggests that the original coli infection had never actually died out, while from the high agglutination titre it might be concluded that the coliform bacillus isolated from the faeces was identical with that causing the original infection. If this was so, the failure of the stock coli vaccine is understandable, and the case supports the view that if a patient has in his blood a certain concentration of agglutinins for a coliform bacillus isolated from his faeces, he is suffering from an infection with, or from the absorption of toxins elaborated by that coliform bacillus.

Case 37. Male. Adult. Came under observation in December 1921. He was a man aged about 40, who had led a very active life up to a few years previously. He had had gonorrhoea when a youth, but had never suffered from a relapse. There was no history of syphilis, either congenital or acquired. The illness for which he sought relief, began in 1919 with pain in the knees. It was thought by his medical adviser to be due to the original attack of gonorrhoea, and it was suspected that the prostate, which was enlarged and hard, was the seat of a latent infection with gonococci. Prostatic massage was therefore instituted, but was followed immediately by synovitis of both knee-joints, at first only slightly painful and remaining but a few days, then disappearing to reappear a week later. A definite periodicity in the incidence and subsidence of the synovitis of the knees became established, which was unaffected by any treatment. The patient then returned to England, where he was treated by many doctors and by many different methods, including the administration of gonococcal vaccine, with very little, if any, improvement in the disease. Business necessitated his return to Kenya Colony. On arrival there it was noted that the knees were swollen for three days, and more or less normal for three days, that there was a definite rise of temperature associated with the synovitis, and that there was also a coincident synovitis of both ankle-joints, and pain in the right shoulder. At the time of the exacerbation of pain and swelling of the knees, there was a definite tendency to increased frequency of micturition. These were the symptoms when the patient first came under our observation. The physical signs present in an attack were extreme distension of the knee-joints, the skin over which seemed to be on the point of bursting, accompanied by tenderness so great that the patient was confined to bed, slight synovitis and tenderness of both ankle-joints, and slight creaking in the right shoulder-joint on passive movement. The temperature rose to 100–101° F. The urine passed during an attack was thick and cloudy. During the quiescent period no pathological changes could be made out by clinical examination in the ankles or shoulder, but there was a decided thickening of the synovial membrane of the knee-joints, though very little of the periarticular fibrous tissues, and none of the bony structures of the joints. There was a certain amount of lateral movement in both knee-joints due undoubtedly to the overstretching of the lateral ligaments of those articulations. The prostate was still enlarged and hard. The patient's general condition was fair, though lack of exercise had rendered him fat and flabby, and the constant attacks of pain had induced a condition of neurasthenia. The Wassermann reaction was negative on three occasions. The temperature curve, the periodicity of the attacks, and the increased frequency of micturition suggested a bacterial infection, but no obvious focus for this could be found, as there was no pyorrhoea, and both the synovial fluid from the knees and the secretion from the prostate were sterile. Gonococcal vaccines had been tried recently with no effect whatever. The faeces and urine of the patient were therefore examined by the technique described in the preceding section, and three bacilli, *B. pseudocoloides* A, *B. pseudocoli* and *B. coli communis* were obtained from the faeces, and *B. metacoloides* from the urine, all of which were agglutinated by a 1 in 150 dilution of the patient's blood serum. A vaccine was prepared from these bacilli, and its use was followed by complete disappearance of the frequency of micturition, a diminution in the pain in the knees, and a slight delay in the onset of the synovitis of the left knee. Unfortunately this last result had the effect of rendering the presence of synovitis in one or other knee practically constant, and can be traced in all probability to the too frequent administration of the vaccine, and the production of too violent reactions in a patient who was apparently hypersensitive. Later on it became obvious that the initial improvement produced by the vaccine had not been maintained, and a further investigation of the patient's secretions and excretions was advised in May 1922. The prostatic fluid was again sterile, but the faeces yielded *B. pseudo coli*, and three coliform bacilli Types II, V and VII, and the urine on one occasion *B. pseudocoli* and a coliform bacillus Type XXI, and on another occasion, a month later, during an attack of increased frequency of micturition, *B. coli communis*. All these bacilli

were agglutinated by the patient's serum in a dilution of 1 in 200. A vaccine was prepared from these organisms, and the patient who meanwhile had decided to return to England, took it with him. No further report on this patient has yet been received.

This case is of great interest in spite of the failure of the vaccine. The symptoms all pointed to a bacterial infection of some kind. Our investigations indicated that the intestinal tract was the most probable source of the bacteria, this view receiving support from the finding of the same agglutinable coliform bacilli in the urine as in the faeces. Possibly had the gonococcal vaccine been combined with the coliform one, more favourable results would have been seen. A very important fact to be noted in this case is the finding of atypical coliform bacilli in the urine, and also the variation of type of the agglutinable coliform bacilli isolated from the faeces even within a comparatively short period. An explanation for this latter fact might be that variation in cultural reactions in the case of the coliform bacilli does not necessarily imply a fundamental difference in species, or else that a bacillus against which the body has acquired immunity, can change its antigenic properties, this being accompanied by a change in cultural reactions in certain cases, or else that when the body has become protected against one bacillus, a very closely allied species of bacteria may acquire pathogenic properties and carry on the disease.

Case 40. Male. Adult. Came under observation in February 1922. The patient had suffered for some years with a fistula-in-ano, but some time prior to its development, there had been pain and stiffness of the vertebral column, more marked in the cervical and thoracic regions, stiffness but no pain in the small joints of the fingers of both hands, and discomfort in the head almost amounting to headache. Patient had suffered from several attacks of malaria since he had been in Africa. At the time the patient first came under observation, these complaints were confined to the stiffness in the fingers, the stiffness and pain in the neck, and a very considerable degree of malaise. Soon after this, the fistula-in-ano gave rise to an ischio-rectal abscess, and coincidently there was a distinct improvement in the rheumatic symptoms in the hands. Later however the pain and stiffness reappeared, and the patient requested vaccine treatment. At this time the fistula-in-ano had not completely healed. The patient's faeces were examined, and *B. coli communis* isolated, while at the same time the secretion of the fistula yielded a coliform bacillus Type V, both these organisms being agglutinated by the patient's blood serum in a dilution of 1 in 600, other bacilli from the same sources not being agglutinated even in a dilution of 1 in 50. A vaccine was prepared from these two agglutinable bacilli, and its administration led to very considerable improvement, though not to a complete cure. A further examination of the faeces was therefore made, and a coliform bacillus Type VII, *B. coli communis*, and *B. paragrünthali* were isolated, and were agglutinated by the patient's serum in dilutions of 1 in 200, 1 in 400 and 1 in 800 respectively. A vaccine was prepared from these three bacilli, and its employment was followed by healing of the fistula-in-ano, and the complete disappearance of the rheumatic symptoms for some months. In September 1923 there was a slight recurrence of pain and stiffness in the cervical and thoracic regions of the vertebral column. The faeces on examination yielded *B. coli communis* and a *B. paragrünthali*, both of which were agglutinated strongly by a 1 in 100 dilution of the patient's serum, higher dilutions not being tested. A third vaccine was prepared from these bacilli, but apparently from the report received from the patient, its use had little or no effect on the symptoms. The hands recovered completely after the first batch of vaccine was given.

The points which this case emphasise are, firstly that in some instances the patient's blood serum agglutinates these coliform bacilli in very high dilutions, and secondly that where a vaccine does not cause a complete cure, it is often due to the failure to isolate all the coliform bacilli responsible for the infection, though here it must not be forgotten that, more especially in cases where the vaccine first of all causes marked improvement and later fails to maintain it, it is always possible that a new infection with a closely allied species of coliform bacillus may have supplanted the bacillus against whose action the body is now protected, or that by changing its antigenic properties, the originally infecting bacillus may have managed to evade the new defences set up in the body. A possible portal of entry for the coliform bacilli causing the original symptoms was the fistula-in-ano, which may have been in existence some considerable time before it caused symptoms. It is interesting to note in this patient the disappearance of the rheumatic symptoms when the ischio-rectal abscess appeared, a phenomenon which may perhaps be attributed to autoinoculation by the bacteria or bacterial toxins in the contents of the abscess.

Case 46. Male. Adult. Came under observation in May 1922. This patient was first seen in 1917 when he complained of pain in the lumbar region, loss of weight, and "thick" and very offensive urine. There was then slight albuminuria, but the urine contained no casts. His symptoms were relieved by the exhibition of alkalies. Shortly afterwards the patient left for England, and on arrival there his former symptoms recurred, and as he was undoubtedly suffering from an infection of the urine by a coliform organism, he was given a stock coli vaccine which gave great relief; indeed there was no recurrence of the disease till the spring of 1922. In May 1922 he was complaining of pain in the right loin over the kidney, dysuria and greatly increased frequency of micturition. Drugs this time brought no relief. On examination the right kidney was enlarged and tender, and the urine, when passed, was very cloudy. From the urine *B. coli communis* was isolated, and was agglutinated by the patient's blood serum in a dilution of over 1 in 100. A vaccine was prepared from this bacillus, and its use resulted in a complete cure. There was no recurrence of the disease till the spring of 1924, when all the previous symptoms began to return, and these had become as bad as ever by the time the patient sought relief in June 1924. On this occasion it was discovered that the patient's wife had been suffering from a coli infection of the urine for 12 years, that is to say her infection existed before that of her husband. An investigation of the organisms isolated from the urines of both patients was undertaken, but no report as to the results obtained had been received up to the time of writing.

This case is of interest in that both husband and wife were sufferers from an infection with coliform bacilli. As another instance of this family incidence, it may be noted that Case 73 is the mother of Case 58.

IV. AGGLUTINATION REACTIONS FOR AUTOGENOUS COLIFORM BACILLI SHOWN BY "NORMAL" PERSONS.

Eight patients suffering from arthritis, when examined by the technique described in Section II, did not show in their faeces coliform bacilli which were agglutinable by their sera. Further examination and enquiry revealed that in four instances the disease was gonorrhoeal in origin, and in a fifth case

was due to tuberculosis. Considering the number of cases seen, however, the percentage of positive agglutination results has been so high as to raise a doubt in our minds as to whether the agglutination on which our technique was based, was specific or not. To determine this question it was decided to examine a series of "normal" cases. When the task was begun, it was found to be extremely difficult to obtain "normal" cases, as nearly every person examined claimed to have or to have had "rheumatism" in some form or another. Fifty-four patients who were in hospital for treatment of non-intestinal diseases, were found clinically free from any evidence of an infection with a coliform bacillus, and the faeces of these persons were investigated in the manner described in the second section of this paper. In only two instances were coliform bacilli found which agglutinated with a 1 in 90 dilution of the patient's serum, and in one of these the agglutination was so poor that had the faeces been under examination with a view to vaccine treatment, the result would have been discarded as unreliable. Unfortunately there was no opportunity of examining these two patients again as they left hospital before the tests were completed.

Dudgeon, Wordley and Bawtree[1] have stated that out of 66 "normal" individuals examined for agglutinins for a typical *B. coli*, only five showed any in a serum dilution of 1 in 50, using Dreyer's method. In a later paper[2] the same authors report that in a further 104 "normal" persons believed free from a coli infection, sera from 22 cases only gave a positive agglutination reaction at a dilution of 1 in 50, and two of these on further investigation showed evidence of a probable coli infection. The difficulty mentioned above of obtaining "normal" patients undoubtedly free from a coliform infection, was also referred to by these authors. Although these investigators only tested the sera of their "normal" cases against examples of typical *B. coli communis*, their results support very strongly the findings obtained by us in Kenya, more especially so as in our experience a considerable number of the coliform bacilli isolated from patients suffering from coli infections, conform in cultural reactions at least with the type *B. coli communis*. It seems fair to believe therefore that in the blood of "normal" persons, agglutinins for coliform bacilli do not exist except in inappreciable amounts, so that when they are detectable in 1 in 100 dilutions of the blood serum, they may be looked on as pathological and as evidence of a coli infection. The only objection which can be raised to this conclusion is that, as will be shown in a later section, coli agglutinins produced artificially are specific for the bacillus used as antigen, and therefore it may be argued that experiments conducted on "normal" cases with stock coli antigens are of no value. This objection however only holds good for the experiments of Dudgeon, Wordley and Bawtree, as the tests made by us were carried out with the full technique employed by us in making up autogenous vaccines of coliform bacilli and described in

[1] Dudgeon, Wordley and Bawtree (1921). *Journ. Hygiene*, xx. 149.
[2] Dudgeon, Wordley and Bawtree (1922–3). *Journ. Hygiene*, xxi. 178.

the second section of this communication. It may be pointed out here, as it will be again later, that the very specificity of coli agglutinins is evidence in favour of their being pathological when they are found in a patient's blood.

V. AGGLUTINATION REACTIONS FOR AUTOGENOUS COLIFORM BACILLI SHOWN BY "INFECTED" PERSONS.

As has been explained in a preceding section the standard demanded by us before we recognise that a person is suffering from an infection with one or more coliform bacilli, is the ability of that person's blood serum to agglutinate such coliform bacillus or bacilli in a dilution of 1 in 90. It is not uncommon however to find that the blood serum in these cases causes agglutination of coliform bacilli in dilutions several times greater than 1 in 90. The highest titre obtained has been one of 1 in 800, but titres of 1 in 160 and 1 in 320 have been comparatively common. Dudgeon, Wordley and Bawtree[1] found a titre of 1 in 2000 in one patient who was the victim of a coli infection. It is also not unusual in our experience to find that agglutinins are present in a patient's serum for two and sometimes three different coliform bacilli isolated from his faeces, though ordinarily not in identical amounts. A practical question then arises as to whether we are going to consider all these bacilli as infecting agents, or to conclude that the lower titre agglutinins are examples of secondary agglutinins produced in response to infections with the coliform bacillus, the agglutinin titre for which is highest. In view of the great specificity of agglutinin formation for coliform bacilli produced experimentally in rabbits, the second alternative would seem to be an improbable explanation, and we must then believe that these are cases of double infection with coliform bacilli, possibly at different periods, thus partially accounting for the differences seen in titre. This question is of the greatest importance in view of the fact that we have to set an artificial boundary between agglutinin titres which can be taken as evidence of infection with coliform bacilli and those which cannot, and it is essential that this boundary should not be too high for fear of excluding true cases of infection.

In regard to cross-agglutination between the sera of, and the coliform bacilli isolated from, different patients no results worth recording have been obtained up to the present. It has only been possible to apply this test in a few instances, and in only two cases was any evidence of cross-agglutination found. In all other cases the serum from one patient failed to cause agglutination of coliform bacilli from any of the other patients, even though by cultural reactions some of these bacilli were identical with those obtained from the patient whose serum was being tested. As will be described in a later section, experimentally produced agglutinins for coliform bacilli are apparently specific for the bacillus used as antigen during the immunising process, so cross-agglutination is probably the exception rather than the rule.

[1] Dudgeon, Wordley and Bawtree (1922–3). *Journ. Hygiene*, XXI. 178.

VI. THE ISOLATION OF COLIFORM BACILLI FROM THE BLOOD OF PATIENTS SUFFERING FROM COLI INFECTIONS.

In seven of our cases, cultures of the blood of the patient were made in lactose bile-salt broth, in ordinary broth, and on blood-agar with negative results. In one case also a culture was made from the synovial fluid of the knee-joint, again with negative results.

VII. THE PATHOGENICITY FOR RABBITS OF COLIFORM BACILLI ISOLATED FROM "INFECTED" PERSONS.

Two of the organisms isolated from a case of chronic rheumatism, Bacillus 681 L and Bacillus 681 N, were tested for their pathogenic action on rabbits. A live bouillon culture of Bacillus 681 L (a true *B. coli communis* by cultural reactions) was inoculated into the synovial cavity of the femoro-tibial articulation of Rabbit 59. There was a marked local reaction, heat and tumefaction of the joint resulting, associated with loss of function. Besides the synovitis, there was also considerable swelling of the periarticular tissues of the joint, although precautions were taken to ensure that the culture was injected into the synovial cavity only, and by the use of a very fine needle that leakage was prevented along the needle track after withdrawal. There was practically no alteration in the animal's temperature or weight, while, apart from its obvious disinclination to use the affected limb, the rabbit never appeared indisposed. A control injection of sterile bouillon was made into the corresponding joint of the opposite side of the animal, but no change other than a very slight synovitis causing no disability, was seen, and the leg was used quite freely. The same experiment was made on Rabbit 58, using this time Bacillus 681 N (a coliform Bacillus Type V), with similar results. A living culture of this same Bacillus 681 N was also injected intravenously into Rabbit 60 without causing any apparent indisposition, or any change in weight or temperature. It may also be recorded here that six rabbits used for the production of immune sera were inoculated intravenously on three occasions at weekly intervals with live cultures of six different types of coliform bacilli with no apparent ill-effect other than the production of a small abscess where leakage of the culture occurred from the vein into the surrounding subcutaneous tissue. Rabbit 29 was used as a control throughout. It is noteworthy that no suppuration occurred in either the joints or the periarticular tissues, and that the disability produced by the injection of the live cultures into joint cavities gradually disappeared, and the joints became clinically normal again.

The experiments recorded here cannot be considered to reproduce in the rabbits the state of constant absorption of bacillary toxins which we believe occurs in patients suffering from coli infections, and indeed they were only made as a preliminary step to form a guide as to the best experimental course to pursue in the investigation. Unfortunately a serious epidemic among the laboratory animals caused so great a shortage of rabbits that we were forced

to abandon the experiments for the time being, though it is hoped that they will be continued as soon as the supply of animals permits. From the few results recorded above, no conclusions of any value can be legitimately drawn.

VIII. THE CLASSIFICATION OF THE COLIFORM BACILLI ISOLATED FROM PATIENTS SUFFERING FROM COLI INFECTIONS.

A. CLASSIFICATION BY CULTURAL REACTIONS.

The cultural reactions of most of the coliform organisms isolated from patients in the course of the investigations described in this paper are given in Table II as far as they are known. In a few instances the cultures were lost before it was possible to examine them. Unless the reactions shown by a bacillus were typical of an already recognised and named member of the coli-typhoid group, they were re-tested, and in some cases where the variation from a known bacillus was small, this was done as many as four times.

Table II.

NOTE. A = Acid formed. G = Gas formed. sG = Slight gas formation. C = Clot formed. T = General turbidity. P = Pellicle formed. Alk. = Alkali formed. + = Positive. 0 = Negative. ± = May be positive or negative. ? = Not examined. * = Name suggested for this organism.

Culture Group No.	No. of times isolated	No. of patients in which found	*Cultural reactions found*															Remarks
			Lactose	Mannite	Glucose	Saccharose	Dulcite	Salicin	Adonite	Sorbite	Inosite	Litmus milk	Indol production	Voges-Proskauer reaction	Gelatin	Bouillon	Motility	
I	49	39	AG	AG	AG	AG	AG	AG	0	AG	0	A→AC	+	0	0	TP	+	*B. pseudocoli*
II	5	5	AG	AG	AG	AG	AG	AG	0	AG	AG	A→AC	±	+	0	TP	0	
III	1	1	AG	AG	AG	AG	AG	AG	AG	AG	AG	A→AC	+	+	0	T	+	
XXV	1	1	AG	AG	AG	AG	AG	AG	AG	AG	0	A→AC	+	+	0	T	+	
IV	19	17	AG	AG	AG	AG	AG	0	0	AG	0	A→AC	±	0	0	TP	±	*B. nairobiensis**
V	13	12	AG	AG	AG	AG	0	0	0	AG	0	A→AC	±	0	0	T	+	*B. kenyaensis**
VI	15	15	AG	AG	AG	AG	0	AG	0	AG	0	A→AC	+	0	0	T	+	*B. pseudocoloides* A.
VII	3	3	AG	AG	AG	AG	0	AG	0	AG	0	A→AC	+	+	0	T	+	
VIII	1	1	AG	AG	AG	AG	0	AG	AG	AG	AG	A→AC	+	0	0	T	0	
IX	5	5	AG	AG	AG	AG	0	AG	AG	AG	0	A→AC	±	±	0	T	±	
X	4	4	AG	AG	AG	AG	0	AG	0	AG	AG	A→AC	±	+	0	T	±	
XI	4	4	AG	AG	AG	0	0	0	AG	AG	0	A→AC	+	0	0	T	+	*B. acidi lactici*
XII	15	13	AG	AG	AG	0	0	AG	0	AG	0	A→AC	+	0	0	TP	+	*B. paragrünthali*
XIII	16	16	AG	AG	AG	0	0	AG	AG	AG	0	A→AC	+	0	0	T	+	*B. ukambaensis**
XIV	36	32	AG	AG	AG	0	AG	AG	0	AG	0	A→AC	+	0	0	T	+	*B. coli communis*
XV	10	10	AG	AG	AG	0	AG	0	0	AG	0	A→AC	+	0	0	T	+	*B. metacoloides*
XVI	3	3	AG	AG	AG	0	AG	0	0	AG	AG	A→AC	+	0	0	T	+	
XXVI	1	1	AsG	AG	AG	0	AG	AG	AG	0	0	A→AC	+	0	0	T	+	
XXVII	2	2	AG	AG	AG	0	0	0	0	AG	0	A→AC	+	0	0	T	+	
XXVIII	1	1	AG	AG	AG	0	AG	AG	0	0	AG	A→AC	+	0	0	T	+	
XVIII	2	2	0	A	A	A	0	A	A	0	A	Alk.	±	0	0	T	+	It is doubtful where these bacilli should be classified. As all were agglutinated by the patient's serum, they may be variants of coliform bacilli which have temporarily lost their power of fermentation
XIX	1	1	0	A	A	0	0	A	0	A	0	Alk.	+	0	0	T	0	
XX	1	1	0	0	0	0	0	0	0	0	0	Alk.	0	0	0	T	+	
XXI	2	2	0 (or A)	A	A	0	A	0	0	A	0	A→AC	+	0	0	T	0	
XXII	1	1	A	A	A	A	AG	A	0	AG	0	A→AC	+	0	0	T	+	
XXIII	1	1	0	A	A	A	0	A	0	0	A	Alk.	±	0	0	T	+	
XXIV	1	1	AG	0	AG	0	0	AG	0	?	0	A→AC	+	0	0	T	0	
XXIX	1	1	0	AG	AG	0	AG	0	0	?	?	A→AC	0	0	0	T	+	
XXX	1	1	0	0	AsG	0	0	0	0	?	?	Alk.	+	0	0	T	+	

A word is first necessary in regard to the cultural reactions chosen as a basis for identification. The classification of the group of coliform organisms is still in a state of chaos, more especially so when it is founded on the action of the bacilli on various carbohydrates and proteins. Papers are constantly

being published suggesting new chemical reactions as a basis for grouping these bacteria, but most of these have not so far received the general recognition of bacteriologists. MacConkey[1] suggested that lactose primarily, and saccharose and dulcite secondarily, should be used to differentiate various classes of coliform bacilli. Another author has advised an amendment by the replacement of dulcite by salicin as leading to a separation into groups which are better correlated with their natural sources than those obtained by MacConkey's suggestion. A new step in the separation of the coliform organisms into classes was taken by Clark and Lubs when they introduced the gas ratio obtained during fermentation by the different bacilli as a basis for differentiation. The same authors in a later paper[2] found that acid production, as shown by the methyl-red test, is correlated closely with the gas ratio if media of definite composition are used, and thus a simple test, applicable in any laboratory, is given to the bacteriologist. Levine[3] has shown that the methyl-red test and the Vosges-Proskauer reaction follow each other very closely, so that it is really unnecessary to apply both when studying the biology of a coliform bacillus. Incidentally it may be noted that Levine states in the same paper that coliform bacilli giving a positive Vosges-Proskauer reaction are rare in faeces, and that amongst the bacilli isolated from our patients with coli infections, under 10 per cent. have given a positive carbinol test. Mackie[4] believes that inosite is a substance, the fermentation or non-fermentation of which has considerable claims to being taken as a basis for differentiation. In choosing the cultural reactions, therefore, to which the coliform bacilli isolated from our cases were to be submitted, due consideration had to be given to the suggestions of these and other authors, and as far as was possible with the materials at command, all suggested reactions, with the exception of the estimation of the gas ratio and the methyl-red test, which were omitted as the Vosges-Proskauer reaction was included, were employed in our examination. The results have led to the conclusion that as yet no sound classification based on cultural characteristics has been evolved for the coliform bacilli. Certainly, as will be seen from Table II which records the cultural reactions of most of the bacilli isolated by us, a very considerable number fall into fairly large and well-represented groups, some of which agree with coliform bacilli already named, and some of which on the other hand have not received a designation, at least so far as the literature which is available to us shows. For one or two of these unnamed groups containing a number of our coliform bacilli provisional names have been suggested. On looking at Table II, one cannot but be struck by the number of groups represented by only one or two bacilli, which vary from already described and named types in one or two reactions. As previously stated such bacilli were

[1] MacConkey (1905). *Journ. Hygiene*, v. 333.
[2] Clark and Lubs (1915). *Journ. Infec. Dis.* XVII. 160.
[3] Levine (1916). *Journ. Infec. Dis.* XVIII. 358.
[4] Mackie. *Applied Bacteriol.* p. 209.

re-tested as regards cultural reactions several times, but maintained the differences found at their initial examination. They must therefore be looked on as true variations from known species, but inasmuch as the variations shown are not limited to one particular reaction, but involve each of the cultural reactions used when the variants are taken as a whole, it is obvious that no reaction at present employed in the classification of coliform bacilli can as yet be considered entirely satisfactory. We have made attempts to produce variants of certain bacilli which do not ferment saccharose, mannite or lactose by growing such bacilli in media containing one or other of those sugars, and subculturing them daily. Success has been obtained with comparative ease in the case of lactose; indeed it is a common incident in our experience for a lactose-fermenting bacillus after cultivation for some time on agar, to show a non-lactose fermenting variant, which after a few subcultures in a medium containing lactose, regains completely its power of fermenting the sugar. As regards saccharose we have been successful in one instance in converting a non-fermenting bacillus into a fermenting one after three months' daily subculture. This experiment is being repeated to obtain confirmation of the result. In the case of mannite, a bacillus which produced acid but no gas in media containing this alcohol, failed to produce gas-forming variants after daily subcultivation for four months. An interesting fact observed by us is that in the case of a few of our coliform bacilli, the original cultural reactions have been lost after artificial cultivation for some time.

The question of the existence of haemolytic properties amongst the coliform bacilli isolated by us has not yet been investigated, though this will be done at an early date.

B. Classification by Serological Reactions.

1. *Production from Rabbits of Immune Sera for Coliform Bacilli.*

In order to carry out serological tests on coliform bacilli, it was necessary to produce immune sera. Six bacilli typical of the main cultural groups of coliform bacilli isolated in the course of these investigations were selected, and 24 hours old peptone broth cultures prepared. Half a cubic centimetre of the living culture was inoculated into the marginal vein of a rabbit's ear, a second injection of a similar amount was given a week later, and a third injection of double the quantity administered after the lapse of another week. Eight days after this last injection the rabbit was bled, and the serum removed after the blood had clotted. When the sera so obtained were tested for agglutinative capacity against the bacillus used as antigen, titres ranging from 1 in 16,000 to 1 in 32,000 or higher were obtained.

No ill effects from the intravenous injection of these living cultures into the rabbits were seen, if one excepts the occasional small abscess which sometimes followed a leakage of the culture from the vein into the surrounding subcutaneous tissue.

2. *Agglutination Reaction Experiments.*

The method used in these investigations into the agglutination reactions of coliform bacilli was that described by Dreyer[1], but modified by incubating the tests in a water-bath at 55° C. for a period of five hours instead of four. It was found most convenient to read the results immediately after the tubes were removed from the water-bath, and again after standing overnight, a period of approximately 16 hours elapsing between the two readings. The necessary dilutions were made by the use of Donald's dropping pipettes. It is necessary to lay stress on two important details of Dreyer's technique: the cultures must be made in veal broth, and the emulsions of the bacilli used in the test must be very thin. Unless these two instructions are strictly carried out, either the agglutination may fail completely, or false agglutination may occur.

Using then this slightly modified technique, the dilutions of the immune rabbit sera, obtained as described above, which produced standard agglutination (taken as the highest dilution showing distinct flocculation without precipitation) of the homologous bacillus, were found to be 1 in 16,000, 1 in 32,000 or 1 in 64,000 according to the serum examined. As a preliminary to attempting the serological grouping of the various coliform bacilli, and to determining the amount of cross-agglutination that was obtainable with artificially-produced agglutinating sera, a selection of eight to ten other bacilli was made from each of the cultural groups to which the bacilli used for immunising the rabbits, as described in Section VIII B. 1, belonged. In each group the bacilli selected had the same cultural reactions, and were tested in one batch for agglutination with the serum prepared against the representative bacillus of the group, which was also included in the test. The results obtained were, for our purpose, extremely disappointing. In the case of each batch, although the titre for the representative bacillus reached that originally found, the highest titre for other bacilli of the group was at the most 1 in 3000, and in most instances was under 1 in 2000. When cross-agglutination between the groups was tested, titres up to 1 in 3000 were obtained against heterologous bacilli. Although the technique of the test was modified in various ways in the attempt to find some method whereby each group of bacilli could be differentiated, no change made any alteration to the results obtained previously. The possibility of this low agglutination titre being a general reaction separating off the coliform bacilli causing coli infections from others, was not lost sight of, but when two coliform organisms which were isolated by the technique described in Section II, but were not agglutinated by the patient's serum, were tested with one of the immune rabbit sera, and gave approximately the same agglutination titre as agglutinable coliform bacilli, the conclusion was reluctantly reached that the sera produced by the inoculation of rabbits with coliform bacilli were specific for

[1] *Med. Res. Council* (1920). Special Report Ser. No. 51, p. 128.

the bacilli used in the immunising process, and that no group-agglutinins could be produced, so that classification of the coliform bacilli by agglutination reactions was impossible. A similar conclusion was reached by Mackie[1] and other observers.

The protocol of one of the agglutination tests is given in Table III, and as this is representative of the results obtained with all the immune sera, it is unnecessary to publish the remainder.

Table III.

Agglutination Tests.

Culture number	Serum 682 F. Dilutions											Serum control
	1 : 2,000	1 : 3,000	1 : 4,000	1 : 5,000	1 : 6,000	1 : 8,000	1 : 10,000	1 : 12,000	1 : 14,000	1 : 16,000	1 : 24,000	1 : 32,000	
682 F	*T*	*T*	*T*	*T*	*T*	*T*	*T* −	*T* −	*S* +	*S* +	*S* +	*S* −	0
315 C	*S* +	*S* −	*S* −	tr.	0	0	0	0	0	0	0	0	0
445	*S* +	tr.	0	0	0	0	0	0	0	0	0	0	0
471 A	tr.	tr.	0	0	0	0	0	0	0	0	0	0	0
577	tr.	0	0	0	0	0	0	0	0	0	0	0	0
585	0	0	0	0	0	0	0	0	0	0	0	0	0
586	tr.	0	0	0	0	0	0	0	0	0	0	0	0
600 A	0	0	tr.	0	0	0	0	0	0	0	0	0	0
693 A	0	0	0	0	0	0	0	0	0	0	0	0	0
764 G	*S* −	tr.	tr.	0	0	0	0	0	0	0	0	0	0
768 K	tr.	0	0	0	0	0	0	0	0	0	0	0	0

NOTE.—(1) The notation used to indicate the degree of agglutination is that of Dreyer. *T* = total agglutination. *S* = standard agglutination. tr. = trace of agglutination.
(2) Bacillus 682 F was that used as antigen in the production of immune serum 682 F. Bacilli 764 G and 768 K were non-agglutinable coliform bacilli. The other bacilli all belong to the same group as Bacillus 682 F.

3. *Complement Fixation Reaction Experiments.*

When the agglutination tests with immune sera failed to afford a basis for the serological grouping of the coliform bacilli, the complement fixation reaction suggested itself as a possible means of achieving the same aim, especially as Mackie[2] indicated that he had been able to obtain a certain amount of group reaction among the members of the coli family by it. The first essential was to develop a technique, as from investigation of the literature available, every worker on the reaction with bacterial antigens seemed to have devised a method for himself, a serious difficulty in the way of obtaining comparable results. In the Nairobi Laboratory, the method employed in the Wassermann reaction is that designated Method IV in *Report* No. 14 of the Medical Research Council. As this has yielded most satisfactory results with a minimum of trouble in our hands, essentially the same method was applied in the study of complement fixation amongst coliform bacilli. The antigen employed was an emulsion of the bacillus under investigation, and this was standardised by the opacity method to contain 4000 million bacilli per c.c.

[1] Mackie. *Applied Bacteriol.* p. 203. [2] *Ibid.* p. 206.

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After titration of the complement in the same way as for the Wassermann reaction, the anticomplementary power of the antigen was tested by making varying dilutions of the standardised bacterial emulsion and finding that one which just failed to deviate a minimal haemolytic dose of complement. The antigen was used in the test proper in half the strength obtained by this titration. The haemolytic system employed was the same as in the Wassermann reaction, namely, a mixture containing 5 M.H.D. anti-sheep cell amboceptor and a 3 per cent. suspension of sheep red blood corpuscles. In the test proper, varying dilutions of the serum under examination ranging from 1 in 10, 1 in 20, 1 in 30, etc. up to 1 in 80, 1 in 90, 1 in 100, were put up with each bacterial antigen, the complement being used in two strengths, 3 M.H.D. and 15 M.H.D. The quantities of diluted serum, diluted antigen, and diluted complement were all equal, and can be designated as one volume. After the mixing of the reagents in the tubes, the latter were allowed to stand for half-an-hour at room temperature, and were then incubated in a water-bath at 37° C. for another half hour. At the end of this time, one volume of the haemolytic system was added, and the tubes, after shaking, again incubated in the water-bath at 37° C. for half-an-hour, after which the results were read.

Unfortunately this test again failed to demonstrate any grouping amongst the coliform bacilli examined. The homologous bacillus in the case of each serum caused a considerable amount of complement fixation, a 1 in 60 dilution of the serum giving almost complete fixation of 3 M.H.D. of complement, and a 1 in 20 dilution a similar degree of fixation of 15 M.H.D. of complement. When bacilli belonging to the same group according to cultural reaction, were tested, a 1 in 10 dilution of the immune serum only gave a very slight fixation with 15 M.H.D. of complement, and failed to fix more than half 3 M.H.D. of complement as judged by the amount of haemolysis. Bacilli which belonged to other coli groups by cultural reactions, gave approximately the same amount of complement fixation as bacilli of the same cultural group, and when bacilli which gave no agglutination with patients' sera, and belonged culturally to groups outside those found in our tests, were examined, they behaved in the same way as the non-homologous bacilli. A repetition of the tests confirmed the original findings. As this method of testing complement fixation differed somewhat from that employed by Mackie[1], the same antigens and antisera were re-tested by a technique founded on the somewhat meagre description given by him in his paper, and completed by reference to the description of Browning's method of performing the Wassermann reaction given in *Report* No. 14 of the Medical Research Council, which seemed to be the basis on which Mackie's method rested. Even by this change of procedure we failed to get any semblance of grouping, which was the more surprising as we were dealing with many bacilli which belonged to the division of non-inosite-fermenting indol-producing gas-forming coliform organisms which, according to Mackie, fall into one group in complement fixation. Several

[1] Mackie. *Applied Bacteriol.* p. 206.

modifications of technique were made both in the direction of increased delicacy and in the opposite one of greater coarseness, but by no method was there any indication of grouping, and we had reluctantly to come to the conclusion that as with the agglutination test, so with the complement fixation test, the antigenic properties of the coliform bacilli were too specific to allow of a basis for grouping being found. The use of defatted coliform bacilli as antigens for the production of immune sera is under investigation, and it may prove to be the solution of the difficulty of obtaining group reactions.

A protocol of one of the complement fixation tests is appended in Table IV as an example of the results found by us. Those obtained with other immune sera and other coliform bacilli were identical, so that it is unnecessary to publish all.

Table IV.

Complement Fixation Tests.

Dosage of complement	Culture No.	Serum 682 F. Dilutions 1 : 10	1 : 20	1 : 30	1 : 40	1 : 50	1 : 60	1 : 70	1 : 80	1 : 90	1 : 100	Serum control without antigen
3 M.H.D.	682 F	0	0	0	0	+	+	++	++	++	+++	++++
	315 C	++	++	+++	+++	++++	++++	++++	++++	++++	++++	++++
	445	++	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++
	471 A	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	577	++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++
	585	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	586	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	600 A	++	++	+++	+++	++++	++++	++++	++++	++++	++++	++++
	693 A	++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++
	764 G	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	768 K	++	++	+++	+++	+++	++++	++++	++++	++++	++++	++++
5 M.H.D.	682 F	0	+	++	++	++	+++	+++	++++	++++	++++	++++
	315 C	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	445	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	471 A	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	577	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	585	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	586	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	600 A	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	693 A	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	764 G	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	768 K	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

NOTE. (1) The degree of haemolysis is expressed by the number of crosses. 0 = No haemolysis. ++++ = Complete haemolysis.

(2) Bacillus 682 F was that used as antigen in the production of serum 682 F. Bacilli 764 G and 768 K were non-agglutinable coliform bacilli. The other bacilli all belonged to the same group as Bacillus 682 F.

4. *Precipitin and Bacteriolytic Reaction Experiments.*

As agglutination and complement fixation tests have failed to give a serological basis of classification of the coliform bacilli, the precipitin and bacteriolytic reactions are in the process of being examined for the purpose. So far no results are to hand, but it is hoped to furnish a report on them at a later date. The experience of Dudgeon, Wordley and Bawtree[1] with precipitin tests applied to coli strains isolated from the urine would suggest however that these are as specific as those of complement fixation.

[1] Dudgeon, Wordley and Bawtree (1922–3). *Journ Hygiene*, XXI. 192.

5. *Saturation Experiments.*

Strictly speaking our immune sera should have been subjected to desaturation experiments, but we felt that owing to the specificity found in our antisera for their respective antigens, and the almost complete absence of interaction with heterologous antigens, it was unnecessary to carry out these tests, at any rate while so many and more hopeful lines of examination remained open to us.

From the results reported above on the experimental investigation into the classification of coliform bacilli isolated from our patients, it can only be concluded that up to the present, serological reactions have failed to help us, indeed they have increased our difficulties by suggesting that antigenic differences exist even amongst bacilli which fall into the same group according to cultural reactions. On the other hand, the very specificity of the agglutinins in an artificial immune serum for the homologous bacillus, strongly supports our view that the agglutinins for coliform organisms which were found in the blood of our patients were not accidental, but the result of infection.

IX. DISCUSSION.

For long the subject of toxic absorption from the bowel has been discussed in medicine, and the results of vaccine treatment recorded above support the views of those who hold that this absorption is responsible for much disease, and at the same time afford an explanation of the cases of failure which have occurred, with remedies directed against toxic absorption generally, and not against a specific infective agent.

It will have been obvious from all that has been said in the preceding sections that the clinical use of our vaccines is based on a theory that if a patient has in his blood a certain concentration of agglutinins for a coliform bacillus isolated from his faeces, he is suffering from an infection with, or from the absorption of toxins elaborated by, that coliform bacillus. In this section it is our object to set out the facts noted clinically or experimentally, which are or are not in accordance with this theory.

In the first place we have to consider the results of the examination of "normal" persons for agglutinins for coliform bacilli. It is usually held that the presence of agglutinins in the blood in any quantity is the result of an attempt on the part of the body to produce immunity to the organism agglutinated or to one of its near allies. It is however recognised that agglutinins for certain bacteria may be present normally in the blood of certain individuals, in some cases in comparatively high concentrations. In the case of the coliform bacilli we are met with an initial difficulty in that it is often almost impossible to be certain whether a person is free from a coli infection or not, as so many persons who believe themselves healthy and only occasionally suffer twinges of "rheumatism," present on examination the symptoms of an infection with one or other member of the coli group. Such cases must be carefully eliminated

from any series of "normal" persons who are being examined for the presence of "natural" agglutinins for coliform bacilli. As stated above, 54 apparently "normal" cases have been examined by us, and so far as a careful clinical examination revealed, none of them showed any evidence of a coli infection. Of this series the blood serum of only two individuals had an agglutination titre of 1 in 90 for coliform bacilli isolated from their faeces. This result is supported by the work of Dudgeon, Wordley and Bawtree who report that in one series[1] of 66 "normal" cases, the serum of five only agglutinated a stock *B. coli communis* in a dilution of 1 in 50 (Dreyer's method), while in another series[2] of 104 cases, the serum of 22 only had an agglutinin titre of 1 in 50 for *B. coli* antigens, two of these patients later being found to have a probable coli infection. These results contrast markedly with those obtained in the cases set out in Table I, for the serum of all these showed the presence of agglutinins for coliform bacilli isolated from their faeces in a dilution of at least 1 in 90, and in many instances in dilutions varying from 1 in 160 to 1 in 800. It is important to note also that, whereas Dudgeon, Wordley and Bawtree have only tested their sera for agglutination with typical strains of *B. coli communis*, an organism always present in the faeces, many of our patients have shown agglutination in high dilutions for atypical coliform organisms, a very significant fact taking into consideration the marked specificity of agglutinins for members of the coli group recorded in the preceding section, and the dictum of Robertson[3] that "Aberrant types of *B. coli communis* are somewhat common, especially in the intestine, and there are very good grounds for believing that they are in nearly every instance in which they occur exercising a pathogenic action."

Certainly it is very noticeable that when plated out on neutral-red bile-salt lactose peptone agar, the faeces of patients showing agglutinins for coliform bacilli in their blood, usually give a much larger variety of colonies than those of persons who do not possess such agglutinins.

The somewhat meagre evidence afforded by injections of live cultures of coliform bacilli into joints, if anything, supports the belief that the coliform bacilli isolated from our cases have a selective pathogenic action, since inflammatory changes without suppuration were produced mainly in the periarticular tissues, and only to a small extent in the synovial membrane, and soon subsided, leaving a clinically normal joint. The experiments in this direction so far are too few in number to justify definite conclusions, and must be repeated and extended to form the subject of another report at a later date.

So far the evidence we have put forward has been based on results obtained by laboratory methods rather than on clinical facts observed in our patients, and we must now pass on to this new line of evidence, though first making a slight digression in order to clarify our ideas. The summary which is found

[1] Dudgeon, Wordley and Bawtree (1921). *Journ. Hygiene*, xx. 149.
[2] Dudgeon, Wordley and Bawtree (1922–3). *Journ. Hygiene*, xxi. 178
[3] Robertson. *Therapeutic Immunization*, p. 143.

at the foot of Table I is liable to give the impression that we have been treating widely different clinical conditions with our vaccines, but although the familiar labels for diseases may suggest this, a careful consideration of the typical symptoms exhibited in those diseases will show that a toxaemia might well explain them all, the variations exhibited from patient to patient which lead to the emphasis of different symptoms and physical signs and so to a different diagnostic label, being the result of idiosyncrasy on the part of the different tissues, or possibly the effect of local injury. In support of this latter suggestion we may quote the work of Brewer[1] who showed that if the lumbar region of an animal is bruised and living cultures of bacteria are injected into the vein of its ear, an acute surgical kidney will develop, whereas when no trauma has occurred, the kidneys remain healthy. Now the general symptoms of a coli toxaemia have been recognised fairly widely for a long time, general malaise and "rheumatic" pains being two of the most prominent ones. If then we find an agglutinin for a coliform bacillus in the blood of a patient exhibiting these symptoms, we are justified in suggesting that there is probably a causal connection between that coliform bacillus, and the disease. The belief that the symptoms we are referring to are probably the result of a coli infection, receives support from an incident recorded by Savage[2] who quotes it from a statement of the Vaughans. These latter investigators

worked with large masses of *B. coli* grown for two weeks in agar in metal tanks. At the end of that time the crude bacterial substance obtained by extracting this mass with absolute alcohol and ether was powdered finely in an agate mortar for certain special investigations. They state "the person who did the pulverizing was often quite seriously poisoned during the process unless he took the precaution of wearing a mask which hindered the inhalation of the powder." Apart from symptoms due to direct irritation on the mucous membrane the chief symptoms were a feeling of depression and malaise and a chilly sensation. Occasionally a decided chill would be experienced, but no temperature readings were taken. Nausea and even vomiting were occasionally noted. After a period of discomfort varying from six to ten hours, during which the patient often complained of dull pains in the various joints, recovery would rapidly and completely take place.

It is inevitable that when a disease is the result of an attack on the connective tissues by bacterial toxins, the symptoms should be varied. Indeed, if the literature which has arisen on the subject of rheumatism is read, we cannot but be struck by the great variety of symptoms shown by diseases described by different authors under the same name, but careful consideration of each case shows that there is a marked resemblance between the general symptoms and the local symptoms, if due regard be paid to the severity of the infection and to the sites attacked, and allowance made for variations of resistance shown to bacterial toxins by different individuals.

To return now to clinical facts observed in our patients, attention must be drawn to the cases recorded in an earlier section of this paper. In Case 32,

[1] Brewer. *Surg. Gynaec. Obst.* II. 485; *Journ. Amer. Med. Assoc.* LVII. 179.
[2] Savage. *Food Poisoning and Food Infection*, p. 98.

we have an illustration of the effect of a vaccine made from an atypical coliform bacillus on a patient suffering from inflammation of an old gunshot wound of the leg, which was known to have been infected originally with a coliform organism. Here we know that an infection of the patient's tissues with a coliform bacillus had taken place through a gunshot wound five years before, that the wound after healing was liable to recurrent attacks of acute inflammation, that the patient's blood when examined five years after the original infection contained an agglutinin for an atypical coliform bacillus present in the patient's faeces, and finally that the inflammatory condition in the leg completely subsided under treatment with a vaccine prepared from this atypical coliform bacillus. These facts seem to point to the conclusion that the agglutinins found in the patient's blood were not "natural," but were a direct result of infection with a coliform bacillus at the time of the original wound, and that their persistence for so long a period as five years showed that an infection was still present in the patient's tissues. In Case 38 we see that from the faeces and urine of a patient, the same atypical coliform bacillus was isolated, showing a definite infection of the tissues of the patient, whilst agglutinins for this bacillus were found in the patient's blood serum. This is of importance as evidence that an atypical coliform bacillus in the faeces can produce an infection, which leads to the formation of agglutinins in the blood.

A still more interesting fact is the change of type of the coliform bacillus in those cases in which a relapse has occurred, which is explicable on the ground that where the body has been immunised against a coliform bacillus, this is able to produce variants which, owing to the strictly specific antibodies apparently produced with this group of organisms, are able to carry on the disease.

Finally, in setting out the evidence in favour of our theory, we cannot neglect that afforded by the results of vaccine treatment based on our ideas. A success rate as high as our figures show is strong evidence of the value of the vaccines, and it is difficult to explain it on any grounds other than that we have contrived to incorporate in them the causative organism of the disease. It is hardly conceivable that the effects of the vaccines are due to non-specific protein administration, for if they were, a stock vaccine should produce the same results as an autogenous one whereas we know by experience that it does not. In the early days of the vaccines, realising that so many different and often unrecognised factors enter into the cure of a disease, we hesitated somewhat in rushing to claim a high curative value for our method of treatment, but now that over 100 patients have been or are under observation, a total of 56 cases (out of 70 whose treatment has been completed) in whom a cure or great improvement has been wrought, justifies us in believing that our theory regarding agglutinins for coliform bacilli is correct, and that treatment based on that theory offers a very great hope of cure or alleviation in diseases in which such agglutinins are found in the patient's

blood. A less reliable but still a significant indication of the value of the vaccines is afforded by the number of cases sent to us by former patients.

This completes the evidence we have to offer in support of our theory regarding the presence of agglutinins for coliform bacilli in a patient's blood. Against our ideas it may be urged that a suspiciously large proportion of our patients have given positive agglutination results, but in opposition to this it must be stated that a number of persons whose cases are not recorded here, have been examined with negative results in spite of their showing signs indicative of a coli infection. Further indeed we may say that in view of the fact that all our patients approach us for the relief of a definite group of symptoms, it would be strange if we did not have a large proportion of positive results, unless our theory were wrong.

Another objection which can be raised against the matter contained in this paper is that we are basing our conclusions on the results obtained from a very small number of patients, who show a large variety of symptoms. Naturally in a country like Kenya which has a very small and very scattered white population, it is not to be expected that we should be able to show the numbers that could be obtained in England, and allowing for this a record of 70 completed cases is not to be despised as evidence, especially as many of the patients have been under observation for three or more years after treatment ended. As to the symptoms being so varied, this point has been dealt with somewhat at length above.

It must be granted as an objection to our theory that blood culture has so far given negative results, but it must also be remembered that it has only been possible to carry it out in a comparatively small number of cases, none of these being in the acute stage. It is also quite possible, indeed very probable, that in these patients we are dealing rather with a toxaemia than a bacteriaemia, especially as Dudgeon, Wordley and Bawtree[1] have shown that the injection of filtered broth cultures of coliform bacilli can give rise to agglutinin production in rabbits.

A further objection, which could be based on our experimental results, is that the injection of live cultures of coliform bacilli intravenously in rabbits gave rise to no other ill-effects than the formation of local abscesses. (It will be noted that strangely enough the leakage of live cultures of coliform bacilli into the subcutaneous tissue of a rabbit's ear causes the formation of a local abscess, whereas the direct injection of such cultures into a joint causes no suppuration.) This is true, but in these animals only three injections were given and these at weekly intervals, whereas the probable condition for the production of disease by coliform bacilli is the constant slow absorption of the bacterial toxins, coupled with some injury such as results from a strain to the connective tissues of a part. Brewer's work[2] already quoted, supports this view. It may not even be necessary to have an injury in the case of a

[1] Dudgeon, Wordley and Bawtree (1922–3). *Journ. Hygiene*, XXI. 184.

[2] Brewer. *Surg. Gynaec. Obst.* II. 485; *Journ. Amer. Med. Assoc.* LVII. 179.

coli infection, but merely a state in which the normal tissue changes of a part have become unhealthy owing to a general lowering of vitality.

Weighing up now the evidence that has been set out, it can only be concluded that the balance of it is in favour of our contention that the presence of agglutinins for a coliform bacillus in the blood is evidence of a pathological condition resulting from the infection of certain of the tissues of the body with that bacillus, and that vaccines made from this presumed causative organism exercise a marked curative effect in these cases. We do not presume to say that vaccines afford the only means of cure, nor even that they are the best means, but we do believe that they have consistently given better results than any other method of treatment so far reported. At the present moment when we are hearing of the cure of sprue cases by the administration of parathyroid extract[1], we have felt it necessary to investigate our cases, more particularly those which have shown recurrences, with a view to determining the amount of combined and free calcium present in their blood serum, and we are also testing the effects of parathyroid extract alone and in combination with the coli vaccine. If parathyroid extract is all that is necessary, we should expect that the agglutinin content of the blood would disappear under its exhibition. This, however, is matter for a later report, and is only mentioned to indicate the lines along which we are now working. Should parathyroid extract prove to be the only essential curative agent, the initial steps of our technique for the preparation of the vaccine will still be necessary to show the presence or absence of agglutinins.

A few words are necessary to indicate some of our views on certain aspects of the pathology of the infection. Fortunately or unfortunately no post-mortem examinations have been possible on any of our patients as none have died, and most unfortunately owing to the necessity of preserving our laboratory animals, we have not been able to spare a rabbit to see the pathological effects of an experimental coliform infection. Under these conditions, naturally, we are unable to describe any pathological histology, and we are entirely in the dark as to whether the tissue changes seen in the various cases are identical or not.

Since it seems likely that we are dealing with a toxaemia, or at most a bacterial infection producing its pathogenic effects by its toxins, it is probable that similar symptoms can be brought about by other bacterial toxins, so that our results are not adverse to the theory that pyorrhoea is a cause of rheumatoid arthritis, but rather support and extend that theory, suggesting indeed the application of our technique to the organisms isolated from the mouth to see if agglutinins are also formed in the blood for any of them.

The question of the site of origin of the bacteriaemia or toxaemia is a difficult one to decide with our present scanty knowledge. Undoubtedly chronic constipation is a very marked feature in the symptomology of the diseases we have treated, but whether as cause or effect has been impossible

[1] Scott (1924). *Brit. Med. Journ.* II. 305 (contains references as well to his former papers).

to determine. The experiments of Thiele and Embleton who painted *B. coli* on the glans penis or anterior portion of the urethra of a rabbit, and recovered them later from the iliac and renal lymph glands, and eventually from the urine, suggests that coliform bacilli have no difficulty in penetrating mucous membrane and in reaching the blood stream. This may take place more readily in the unhealthy condition of the bowel induced by chronic constipation, or when an injured mucous membrane is left following diseases such as dysentery and colitis, though, as has been mentioned above, no proof exists at present of a bacteriaemia other than the finding of the same atypical coliform bacillus in both urine and faeces, and agglutinins for that bacillus in the patient's blood. The difficulty of postulating the penetration of the mucous membrane of the bowel by *B. coli* in chronic constipation and its apparent absence in health might be met with by an application of Besredka's local vaccination theory of typhoid immunity, and this might explain the cases which relapsed in our series, these being extremely difficult to account for on a theory of absorption of toxin from the bowel. However, as can be seen from the above statements, we have no solid facts on which to form a hypothesis as yet, and until they can be produced, a discussion of the possible pathology of the disease is of but little practical use.

Finally in this section some reference must be made to our attempts to classify the coliform bacilli isolated by us. The results of our investigation have been to show that the coliform bacilli obtained from our patients cannot be grouped by any of the cultural reactions which have been suggested from time to time, without the production of a large number of groups containing only one to three representatives, all of which are probably variants of the larger groups. As these variations are not limited to one reaction, but involve all the reactions used when the variants are considered as a whole, it is obvious that it is impossible to say where variation begins and ends. It is stated that certain cultural reactions are stable for particular types of bacilli, but our experience would suggest that this may be so on artificial media but not for bacteria living in the body. Experiments indeed are proceeding to see whether it is possible to produce variants of coliform bacilli by altering the diet of the animal. As regards the types of coliform bacilli that we have isolated in the examination of our patients, it may be surmised from consideration of the number of times certain of them have been found in association and agglutinated by the same patient's serum, that the following groups in Table II are really identical, representing merely accidental variants from one another. These are Groups I, II, IV, V, VI, VII, IX and XIII; Groups XV and XXVII; Groups XII and XVII. It is possible also that Groups I, XII, XIV and XV are also really identical, and if this is proved later to be so, then the present division of coliform bacilli would not be justified, and it would probably be found that this group of organisms could be divided into two or three sections, in each of which the variants from type would be innumerable. If some such discovery is not made, it seems probable that the multiplication of species

in the group of coliform bacilli will go on until the possible number of combinations afforded by the cultural reactions which can be applied, is exhausted.

An interesting problem is opened up by the existence of these variants. Several of our patients who suffered from relapses during the course of their treatment, when re-tested for the presence of agglutinable coliform bacilli in their faeces, yielded a different organism culturally from that originally isolated, though usually the differences were only shown in one or two reactions. A possible explanation of this phenomenon is that variants of the original infecting bacillus have been produced which having different antigenic factors, escape the action of the immune substances produced by the body against the original bacillus as a result of the administration of vaccine. Knowing the marked specificity of the serological reactions of the coliform bacilli as seen *in vitro*, this explanation must be considered a possible, if not indeed a probable one. In connection with this, should the experiments in hand at present for the production of variants from type coliform bacilli be successful, it will be necessary to test these artificially produced variants as regards their antigenic similarity to or dissimilarity from the parent organism. If one does not admit the feasibility of this antigenic change, another possible explanation of the alteration of type of the infecting bacillus in the relapses of our patients, is that these variants were present in the patient's faeces from the start, and that when the body became protected against the original infecting bacillus, these variants got the opportunity of entering the tissues by the same portal as the first invading organism and, becoming pathogenic, caused a recurrence of symptoms. This explanation does not sound so convincing as the other as it is difficult to see why the variants should not have entered with the original infecting bacillus. A third explanation may just be indicated, namely that these variant bacilli arose on account of a change in the constitution of the faeces, and not as a result of the attempt of the original infecting bacillus to defeat the immune-bodies produced in response to the administration of the vaccine.

As regards serological classification of the coliform bacilli, we have to report that with agglutination and complement fixation tests, the immune sera proved so specific that no group reaction could be obtained. Possibly the use of defatted antigens may supply the necessary key to the solution of this difficulty.

X. SUMMARY.

A technique is described for the preparation of a vaccine from coliform bacilli isolated from the faeces of a patient and agglutinated by his blood serum.

The encouraging results obtained in the treatment of a number of patients suffering from rheumatoid arthritis, chronic rheumatism, and allied diseases with an autogenous vaccine prepared by this technique, are reported.

The absence of agglutinins for coliform bacilli from the blood of healthy persons is established, while it is shown that such agglutinins are present in high concentration in the group of diseases under consideration.

A report is made on the result of attempts to classify the coliform bacilli by cultural and serological reactions.

The facts reported are discussed, and the proposition adduced therefrom that if a patient has in his blood a certain concentration of agglutinins for a coliform bacillus isolated from his faeces, he is suffering from an infection with, or from the absorption of toxins elaborated by, that coliform bacillus, and a vaccine prepared from this organism will probably have a marked curative effect.

Suggestions are made as to the mechanism of infection with coliform bacilli, and as to the significance of variants in that group of organisms found in the faeces.

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