On the curative effect of carbonic acid gas or other forms of carbon in cholera, for different forms of fever, and other diseases / by C.J. Lewis.

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OR OTHER FORMS OF CARBON

IN CHOLERA, FOR DIFFERENT FORMS OF FEVER, AND OTHER DISEASES.

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PARKIN PRIZE ESSAY, 1902.

[*From the Laboratory of the Royal College of Physicians of Edinburgh.*]

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EDINBURGH:
WILLIAM GREEN & SONS.
18 AND 20 ST GILES STREET.
1903.

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ON THE CURATIVE EFFECT OF CARBONIC ACID GAS

OR OTHER FORMS OF CARBON

IN CHOLERA, FOR DIFFERENT KINDS OF FEVER, AND OTHER DISEASES.

So long ago as 1831 Dr John Parkin had become satisfied as to the efficacy of carbon, in one or other of its forms, in the treatment of cholera; and when in that year epidemic cholera made its appearance in London, Dr Parkin advocated the claims of these remedies, and made representations to the General Board of Health concerning the matter. A few years later he published his opinions in a volume entitled *The Antidotal Treatment of the Epidemic Cholera.*

Dr Parkin was born in 1801, and died at Brighton in 1886 at the ripe age of eighty-five years, notwithstanding the possession of a congenital affliction not usually associated with longevity and ability for such arduous work as treating cholera epidemics. He studied at St Bartholomew's Hospital, and subsequently became a Fellow of the Royal College of Physicians of Edinburgh and a Fellow of the Royal College of Surgeons of England. In addition, and mainly on account of his work on cholera, he was a Corresponding Fellow of the Royal Academies of Madrid, Barcelona, and Cadiz. His opportunities for the study of cholera comprised experience of the disease in very different parts of the world, ranging from India to Spain, England, and the West Indies. Having had the opportunity of witnessing a serious epidemic of cholera in Spain, he subsequently investigated the disease in the West Indies, and on a recurrence of cholera in the latter quarter, he was appointed as Medical Inspector for Her Majesty's Government, and made further acquaintance with the epidemiology of the disease. At another time he was employed in the service of the Honourable East India Company and visited Calcutta, where he studied the phenomena of cholera as manifested under conditions of soil, climate, and racial susceptibility entirely different from his European and West Indian experience. Dr Parkin was convinced that cholera and other epidemic diseases were associated with atmospheric conditions secondary to volcanic disturbances; and further, that in carbonic acid gas he possessed a specific antidote capable of controlling and curing the manifestations of the disease in the individual victim. To the former of these convictions detailed reference is here unnecessary, since it has formed the subject of previous essays, and is not included in our subject, the prescribed scope of which is the beneficial therapeutic influence of which Dr Parkin was the exponent.

Dr Parkin's recommendation of carbon was communicated in 1831, not only to the General Board of Health, but also to local authorities and to

medical practitioners in places visited by the epidemic cholera. In 1832 evidence slowly accumulated in favour of the use of carbon. In a letter to the *Lancet* about this time Radcliffe refers to two serious cases of cholera successfully treated with effervescing saline draughts containing excess of carbonate of ammonia with other ingredients. Though other remedies were also used in the same cases, the writer attributes the benefit to these alkaline effervescing draughts, and Parkin regards the cases as instances of the good effect of carbonic acid gas. It is to be noted that alkalinity of the prescribed medicine is regarded by Parkin as less important than the capacity for evolution of carbonic acid gas.

In 1834 epidemic cholera broke out in Spain, and, attracted by the opportunity of testing his remedies, Dr Parkin visited Spain and obtained a trial of carbonic acid gas by physicians in various places, notably Barcelona, Mataro, and Xeres. Their evidence was in favour of the remedies. Dr Ardevol of Barcelona testified that carbonic acid gas is "a specific remedy for the cure of the Asiatic Cholera in its first and second periods," and that "the efficacy of this medicine is most visible at the commencement of collapse."

Dr Pascual of Mataro says that "a patient who took carbonic acid, before the period of asphyxia, generally recovered speedily and securely."

Dr Wilson of Xeres used carbon in the form of charcoal, and gave it by the mouth or by enema. Of it he says that "in all stages of the disease it is a most beneficial adjuvant, and, anterior to collapse and in the stage of reaction, most eminently curative."

Dr Sauch of Barcelona is quoted by Parkin as having written to him in the following terms:—"I attended a great many patients in a state of confirmed collapse, whose recovery was entirely due to the administration of carbonic acid gas."

The Royal Academy of Medicine and Surgery in Barcelona appointed a Commission (by order of the Supreme Board of Medicine and Surgery in Spain) to inquire into the plan of treatment which had produced the best effects in cases of epidemic cholera in those towns within their district which had been attacked by the disease. This Commission strongly commended Parkin's treatment with carbonic acid gas as "the most efficacious and direct of all the plans that have been tried," and as producing "wonderful effects." The report of the Commission sealed with authoritative approval the method of treatment instituted on Dr Parkin's recommendation.

During the English visitation of 1849 Parkin's treatment was tried in London in both St Bartholomew's Hospital and in King's College Hospital, with the result, as stated by him, that fatality under this treatment was only half that when other methods were adopted. The case mortality, however, was high, and this in Parkin's opinion was in part due to the imperfect manner in which the treatment was carried out. The physicians of St Bartholomew's Hospital published no account of their trial of the remedy, and a Committee of the Royal College of Physicians of London, which was appointed to inquire into methods of treatment adopted during the 1849 epidemic, made no reference to it. The King's College Hospital results were, however, published in the *Lancet* along with a *résumé* of the methods of treatment adopted in the various Metropolitan hospitals, in most of which no trial appears to have been given to the carbonic acid gas treatment. At St Bartholomew's Hospital reliance was placed on a preliminary emetic dose of zinc sulphate, and, amongst other remedies used later in the case, effervescing solution of citrate of soda was given. Parkin prints a table,

prepared by himself, showing that of 358 cases there submitted to other modes of treatment 48 per cent. died, and of 120 cases in which he considered carbonic acid gas to have been employed only 22 per cent. died. The hospital authorities do not appear to have issued any classification of cases or methods of treatment, and cannot be taken as responsible for these figures. At King's College Hospital, on the other hand, a table was issued showing the mortality under different methods of treatment, and including all the thirty cases admitted. Ammonia, wine, and ice, in addition to a combination of vegetable astringents with effervescing saline mixture, formed the method of treatment adopted in most of the cases, and with most success. Even by this method the mortality reached something like 50 per cent., and other methods were wholly unsuccessful. Among individual practitioners who tried the remedy, Lewis mentions, in the *Lancet*, that he prescribed an effervescing mixture after each motion and to relieve vomiting. The mixture contained bicarbonate of soda, prepared chalk, carbonate of ammonia, and opium, and was combined with small doses of tartaric acid. Lewis does not attribute so much good to the effervescence as to the excess of alkali; he is particular, however, to order it effervescing, and testifies that it is the only safe and certain method of arresting cholera as met with in England. Parkin looks upon the good effects of such a mixture as entirely due to the carbonic acid gas derivable from the alkaline carbonates. Niddrie testifies that he was "fully convinced of the great value of calomel and carbonic acid gas constantly and perseveringly administered at short intervals," and adds that, although useful, they are "as powerless as other means unless thrown constantly into the stomach."

In 1850 cholera commenced in Jamaica, and Parkin was enabled to study the disease in its tropical form, and to investigate there the effects of carbonic acid gas. The prevalent type of disease was extremely severe—so much so, that in several villages practically all the cases were fatal. Nevertheless, in those cases submitted to treatment with carbonic acid gas the case mortality only reached 11 per cent. Dr Parkin's experience included several hundreds of cases, so that here one cannot allege that the figures were calculated on a small number of cases, nor that a few isolated recoveries sufficed to show a large percentage of good results. The death-rate from cholera in the whole of Jamaica at this time reached the high point of 10 per 1000 living, and in individual districts as many as one-third of the whole population succumbed. Moreover, the duration of the attack in fatal cases was extremely short, never exceeding twenty-four hours if the case were untreated. Indeed, even in those cases that in spite of treatment died, the duration only exceeded twenty-four hours in 35 per cent. Of 746 cases treated with carbonic acid gas, 84 were fatal, thus showing a case mortality of 11·26 per cent., as compared with a fatality of 70 to 80 per cent. amongst cases in which this remedy was not used.

Dr Parkin embodied his views with regard to the virtues of carbon and carbonic acid gas in numerous writings, of which the most important was his book issued under the title of *The Antidotal Treatment of the Epidemic Cholera.* This book passed through four editions (the last of which was issued less than twenty years ago), and was translated into more than one Continental language. A pamphlet published in 1836, *On the Efficacy of Carbonic Acid Gas in the Diseases of Tropical Climates, with Directions for the Treatment of the Acute and Chronic Stages of Dysentery,* shows that the author advocated the use of this remedial agent, not only in cholera but in a large number of other diseases. Amongst these may be mentioned ague, dysentery, continued fever and malarial affections

generally, including both remittent and intermittent fevers. This list is further enlarged in his book on *The Antidotal Treatment of Disease*, to which a later reference will be made.

Parkin's view was that these diseases were all due to some gaseous or other substance generated in the bowels of the earth, extricated from the surface, diffused throughout the surrounding atmosphere, absorbed by the lungs, and thus introduced into the circulating blood. He arrived at the conclusion that carbon is not only highly beneficial in attacks of epidemic cholera, but also possesses specific antidotal properties, as there is no way of accounting for the result excepting on the supposition that it neutralises the poison productive of the disease. In his experience of the carbonic acid gas treatment of cholera, Parkin says the result is invariably helpful except late in the stage of collapse. In those cases with symptoms only of deranged stomach, two or three doses of carbonic acid gas produce relief of symptoms, dissipate the nausea, remove the giddiness and faintness, and soothe the burning pain in the epigastrium. In cases in the stage of diarrhœa, which is frequently the precursor of severe cholera, the morbid process is arrested by the carbonic acid gas, but at a somewhat longer period than in the earlier cases. When the disease has reached the evacuant stage, characterised by vomiting and rice water stools, the gas relieves the irritability of the stomach, and arrests the vomiting and the bowel relaxation. But it is in the commencement of the collapse stage that clinical observation reveals most improvement. Five or six doses of the gas then begin to check the vomiting, relieve the thirst, heat, and burning pain, soothe the spasms, arrest the diarrhœa, and remove the depression. Continued administration frequently ends in recovery, and it is remarkable that in cases in which carbonic acid gas was taken early there was no consecutive fever such as is not uncommon in the disease. When the case has reached the stage of confirmed collapse the gas is not uniformly successful, though some cases show improvement. Its failure at this stage is attributed to limited absorption from the stomach, and to the suspension of the circulation. In his explanations as to the mode of action of carbonic acid gas, Parkin supposes the gas to unite with the poison of the disease and form an innocuous substance, analogous to the formation of a salt by a base and an acid. The action of the solid carbon in the form of charcoal is explained in two ways—first, by its affinity for gases, and its power of absorption of putrefactive gaseous emanations such as might possibly result from decomposition of the elements of disease; second, by the contained carbonic acid gas which recently-prepared charcoal has imbibed during the combustion of the wood. Charcoal is stated to be useful in the early stages of cholera, though carbonic acid gas is preferable; and it is admitted that when the case has reached the stage of collapse charcoal is useless.

Having considered the effects described by Parkin as produced by the remedies, it is now necessary to show by what methods the drugs were administered in order to obtain their therapeutic aid. Solid carbon was used in the form of wood charcoal. Parkin expressly disclaims any virtue for animal charcoal. The wood charcoal should be freshly prepared, and may be obtained from white beech or any wood free from resin, or may even be prepared from corks. It is given by the mouth or rectum; in the former case a tablespoonful every two hours is the usual dose. For an enema two or three tablespoonfuls are suspended in any convenient fluid by means of the white of an egg, and the enema is used at frequent intervals.

The efficiency of the charcoal, in whichever way it is used, is much

increased if its use is preceded by a few doses of carbonic acid gas in the methods now to be described. Parkin evidently had much greater faith in carbonic acid gas than in any other form of carbon. The gas may be absorbed by various channels; it may be swallowed or inspired by the patient, or may be injected into the rectum by the physician. The alimentary tract more often than the respiratory system formed the portal of reception. As a rule, the carbonic acid gas was given in the form of an effervescing draught prepared from bicarbonate of soda or of potash, with either citric or tartaric acid. Thirty grains of the alkali are powdered and thoroughly mixed in a wineglassful of water with a dessertspoonful of simple syrup; to this there is then added twenty grains of the acid, dissolved in half a wineglassful of water. The draught must be taken at once before effervescence has subsided, and the object of adding the syrup is to cause the gas to be given off more slowly and not lost before the draught can be taken. For the acid ingredient may be substituted two tablespoonfuls of lime-juice, and in this case the syrup may be omitted. This dose is repeated four or five times at very short intervals, in severe cases a quarter or half an hour only being allowed to elapse between the doses. Instead of the saline effervescing draught, soda or seltzer water may be used; but these are not so good, since less carbonic acid gas is obtainable from them. Carbonic acid gas may be given by injection into the rectum. In this case the gas itself may be collected in a bladder fitted with a pipe and stop-cock, and may be passed directly into the bowel. Another method used by Parkin was to place solutions of acid and alkali, one in each half of a constricted bladder, and to bring them in contact by loosening the constriction after a pipe connected with the bladder was in position in the rectum.

Inspiration of carbonic acid gas found its chief métier in the pyrexial stage of intermittent fever, though occasionally also used in cholera. The gas was administered from a bladder fitted with a stop-cock and mouth-piece. Air was admitted at the same time by the nostrils, and expiration permitted only through the nostrils.

Other remedies used for cholera and fevers, and believed by Parkin to owe any remedial virtue they possess to the amount of carbon in their chemical composition, are naphtha in use in the Caucasus, olive-oil in use in Spain, and creosote in use in Archangel. Naphtha is strongly recommended by Andreoski for cases of choleraic diarrhœa; indeed, he calls it an infallible remedy in doses of four to eight drops. Parkin advances no personal experience of it, but, as it contains over 80 per cent. of carbon, he looks upon this as the source of its virtue, and explains its failure in severe cases of cholera by the arrest of assimilation and absorption which then characterises the disease. Similarly, in the case of creosote and olive-oil he attributes to the carbon present any good effects observed from these remedies. The alkaline carbonates were frequently prescribed in cases of cholera, and Parkin regards these as effective in proportion to the acidity of the stomach, and to the amount of carbonic acid gas evolved from them by such acidity. He places these compounds far below the direct administration of the gas itself.

So far reference has only been made to cholera and malarial diseases, but later Parkin extended his list, and included most of the communicable diseases amongst those in which carbon was believed to be a sovereign remedy. Parkin advances the view that all epidemic diseases are due to one and the same cause, viz., an atmospheric poison extricated from the earth (more especially during volcanic action) and absorbed by the lungs of the

sufferers. This theory is fully discussed by him in his book on *Epidemiology, or the Remote Cause of Epidemic Diseases.*

With this theory we are not now concerned, but reference must be made to its corollary. Since all these diseases were regarded as similarly produced, they were all also regarded as curable by one and the same means. In a book on the *Antidotal Treatment of Disease* Parkin set forth his view that carbonic acid gas is a true antidote and specific for one and all of the following diseases:—cholera, malarial fever and ague, yellow fever, typhus fever, typhoid fever, puerperal fever, smallpox, scarlatina, measles, and the exanthemata generally. The clinical evidence as to benefit derived from the use of carbonic acid gas and of other forms of carbon in many of these diseases is trivial; only with regard to cholera and malarial fevers does Parkin detail any large number of cases. Malarial fever or ague was treated by Parkin with carbonic acid gas, not only in the hospitals of Alicante and Madrid, but also in a hospital in Rome, and with uniform success. The gas was given with most effect prior to the onset of the cold stage, some four or five doses being taken during the two hours preceding the expected paroxysm. The attack was usually prevented, and repetition of the procedure on succeeding days generally resulted in curing the patient. Parkin concludes that "carbonic acid gas is an antidote for the poison termed malaria, and a specific for ague."

Consideration of the writings of Parkin explains, therefore, the subject set for this essay. In his view these remedies cured cholera, the different forms of malarial fever (including remittent and intermittent fevers, and also simple continued fever), and such 'other diseases' as yellow fever and other forms of communicable disease mentioned in the above list. Having thus set forth his views, held at a time when scientific knowledge of epidemic diseases was limited and modern bacteriological discoveries unknown, it is necessary to compare these views with our present beliefs, and to see how far they are confirmed or negatived by recent advances of science, and how far they have received since publication the support of writers and teachers of medical science.

Bacteriology lends no support to the view that epidemic diseases in general are due to only one virus. The fact that in many of these diseases a specific bacterial organism has already been isolated, enforces judgment by analogy that all are associated with similar causes, each having its own special microbe, and that in the infectious diseases in which as yet no special organism has been found, there will ultimately be discovered a bacterial cause. The conditions under which such organisms live in nature, and more particularly the conditions regulating their epidemic spread in man, are as yet not fully understood: one may not therefore say that the atmospheric conditions outlined by Parkin have nothing to do with such organisms. It is possible that atmospheric conditions, induced by volcanic or other agencies, may have some influence on the cycles of life and virulence observed amongst such organisms. Conclusive proof to this effect may or may not be forthcoming in the future. Meanwhile we may premise that the various epidemic diseases are associated with different organisms, and that no amount of polymorphism, such as we know to be characteristic of many bacteria, could disguise the fact that such bacteria as, for example, the Vibrio choleræ and the Bacillus anthracis are entirely different organisms, and not one and the same organism in another shape.

So far, then, bacteriology does not support Parkin's views on the unity

of the etiology of epidemic diseases. It does not, however, disprove, in regard to their epidemiology, the possibility of dependence on atmospheric compositions of which the precise nature is unknown. Atmospheric conditions might be such as to favour virulent development of one kind of organism at one time, and of another variety at another time. Next we shall consider the attitude of the medical profession to the opinions set forth by Dr Parkin.

With regard both to the claims of carbonic acid gas as a specific and antidote of wonderful power in the treatment of cholera and other diseases, and also as to the beneficial action of other forms of carbon such as charcoal, it must be said that Dr Parkin's belief was not, and is not, shared by many of his professional brethren, notwithstanding the support he received at the hands of Spanish physicians and West Indian doctors.

Ritson mentions the exemption from cholera of porters and labourers engaged in discharging and storing charcoal cargoes at Malta, and he says the same fact was noticed at Palermo and other Mediterranean ports where charcoal is used for fuel. The inhalation of particles of dust of the charcoal was considered to be the cause of this exemption. At Marsala in Sicily there happened to be little cholera compared to its prevalence in surrounding districts, and this was thought to be due to carbonic acid gas given off from wine casks. So also the neighbourhood of mineral springs was sometimes said to serve as a protection against cholera. While Parkin explained this by reference to the carbonic acid gas given off at the springs, modern views of water-borne cholera would suggest that any good effect was rather due to the water-supply being derived from a pure source, and being less liable to contamination than ordinary sources. London physicians were not impressed with the value of the remedy, and in many recent textbooks of medicine and of therapeutics carbonic acid gas is not mentioned as of use in cholera and epidemic diseases.

A notable exception is found in Ziemssen's *Cyclopædia*, where Lebert recommends in the treatment of cholera ice-cold carbonic acid water or effervescent powders (4 parts bicarbonate of soda and 3 parts tartaric acid) in a few teaspoonfuls of water, or bicarbonate of soda and one or two teaspoonfuls of lemon-juice. He says: "Of course these mixtures are to be taken at the moment of effervescence," and confirms Parkin and the Spanish physicians as to the relief of burning thirst, of constant nausea, and of frequent vomiting. Lebert also corroborates their statements as to patients once commenced with these remedies continually calling for them afterwards, so great was the good effect.

On the other hand, Rumpf, in the *Twentieth Century Practice of Medicine*, says, with regard to cholera nostras, that carbonated waters increase vomiting, and he therefore prefers ice water. He makes no other reference to the use of carbonic acid gas as treatment. The same author, writing on the treatment of Asiatic cholera, says: "I do not recommend the use of water rich in carbonic acid gas"; and again, "My decided impression is that carbonic acid acts unfavourably on the vomiting."

Osler does not mention carbonic acid gas in the treatment of cholera, and Lauder Brunton does not include it in his *Index of Diseases and Remedies* as one of the remedies of use in cholera, nor does he allude to this use of it in his account of the uses of carbonic acid gas.

Packard (Hare's *System of Practical Therapeutics*) mentions carbonated effervescing waters as remedies for the vomiting in cholera morbus, but, in dealing with epidemic cholera, does not mention carbonic acid gas or regard it or the above waters in any way as a specific.

Macleod (Allbutt's *System of Medicine*) gives no support to Parkin, since he states that there is no specific drug which will cure Asiatic cholera; and he does not even refer to carbonic acid gas, though he recommends iced soda water for the relief of thirst.

Macnamara (Quain's *Dictionary of Medicine*) does not recommend or even consider the use of carbonic acid gas in cholera, nor does Bristowe, although he states that saline effervescents are useful in the stage of reaction by relieving sickness and promoting diuresis. The latter author would probably have held the view that Parkin's cases of recovery from cholera by the use of carbonic acid gas were really cases of that form of simple diarrhœa not uncommonly present in times when cholera is epidemic. On this point he says that "if the case be one of simple diarrhœa, it will not run on to cholera under any form of treatment"; and "if the case be one of commencing cholera, there is no ground for believing it can be cut short." Doubtless many of Parkin's contemporaries must have taken such a view of his results, otherwise one can hardly explain the general want of belief in the efficacy of his method of treatment and the absence of any attempt on a large scale to test its value during a cholera epidemic in this country. Fagge says that Bristowe stands alone in the above-quoted opinion as to cases of diarrhœa prevalent when cholera is epidemic, and that most writers think that such cases, unless checked, may end in cholera. Fagge does not mention Parkin, but quotes Lebert (already referred to), and cites the experience of the disease at the London Hospital in 1866 when a 'saline lemonade' was employed with apparent advantage. He does not, however, say anything of the virtues of carbonic acid gas *per se* as a curative agent in cholera.

Manson, writing in Gibson's *Textbook of Medicine*, mentions the use of iced effervescing drinks for the relief of thirst, but says nothing of the carbonic acid gas treatment, or of any special benefit from the effervescing remedies.

Widal, who is responsible for the article on cholera in Charcot's *Traité de Médicine*, does not specially mention carbonic acid gas, and denies that naphthaline is of any use in the treatment.

Neither Johnston in Pepper's *System of Practical Medicine*, Davidson in the *Encyclopædia Medica*, nor Strümpell in his *Textbook of Medicine*, refers to the use of carbonic acid gas. Pepper, in his *Textbook of Medicine*, recommends carbonic acid water; but this is, of course, a very different thing to recommending Parkin's treatment.

Further citation of textbooks would be wearisome: it is sufficient to say that in none other that I have consulted have I found reference made to carbonic acid gas as an antidote to cholera.

This unanimity of neglect to refer to Parkin and his published views on the antidotal treatment of cholera by means of carbonic acid gas evidences a general agreement of belief that Parkin had overestimated the value of the remedy, and attributed to it powers which in reality it did not possess. Of recent clinical evidence on the subject there appears to be remarkably little, and I regret that I cannot personally add anything to the clinical aspect of the question, not having had an opportunity of treating cholera with carbonic acid gas. It appeared to me, however, that a research into the effects of carbonic acid gas and other forms of carbon upon the specific organisms of cholera and some other diseases might furnish data contributing to the elucidation of the question. One might even go further and suggest that this is, in the light of the bacteriological advances of recent years, one of the most valuable methods of estimating the power of the

remedies as antidotes to diseases such as cholera. Should it appear that carbonic acid gas, or carbon in any of its forms, were highly inimical to special organisms, the treatment recommended by Dr Parkin might claim some scientific basis; whereas, on the other hand, if laboratory experiment showed that such pathogenetic organisms, and particularly the Vibrio choleræ, were not injuriously affected by these agents, then any clinical good which the remedies might be capable of producing could hardly be regarded as the result of a specific and an antidote, and these remedies should be classed as merely adjuvant.

I propose to consider, as examples of the diseases in which Parkin considered carbonic acid gas a specific remedy, cholera, typhoid fever, and diphtheria. Since his day definite micro-organisms have been discovered as invariably associated with these diseases. After describing these organisms and their cultural characteristics, reference will be made to any previous experiments as to the effect of carbon in its various forms upon these and other organisms. Next, I propose to submit an account of my own experiments and of the results of my research. Finally, I hope to consider the whole subject in the light of its clinical and experimental bearings, and to arrive at certain conclusions.

BACTERIOLOGY OF CHOLERA.

The Vibrio Choleræ, the specific organism of cholera, was discovered by Koch in 1884 in the excreta of cholera patients and in the intestinal contents of recent cadavera. The organisms are slightly curved rods, hence their common name of 'comma bacilli.' The union of these curved organisms into a chain gives rise to the appearance of long spirilla or corkscrew forms, and these are most commonly present in cultures of slow development. Their occurrence has caused the organism to be also known as the 'Spirillum Asiaticæ Choleræ.' Each organism possesses a single terminal flagellum, and has a very high degree of motility. The comma bacillus is aerobic, but also facultative anaerobic, though it grows best in ample air-supply. It possesses the power of liquefying gelatine, and does not grow well at very high or very low temperatures. It is generally regarded as a non-sporing form of bacillus. It may be stained by such ordinary stains as fuchsin and methylene blue, and is decolorised by Gram's process.

In artificial cultures it presents the following appearances and reactions:—

In gelatine stab it shows, after a day or two of incubation, a funnel-shaped liquefaction of the jelly. There is usually a central mass of growth at the bottom of the funnel. This consists of agglomerated bacilli, and is often curled or bent in shape.

In gelatine plate small white colonies appear in the depths of the gelatine, growing towards the surface and liquefying in the form of a funnel, so that by the second or third day the gelatine appears to be perforated by holes, and later it becomes entirely liquefied. The appearance of the plate in the earlier stages has gained for it the comparison implied in the term 'silver sand.'

On agar the vibrio grows as a moist, shining, whitish layer; but on *blood-serum* it rapidly liquefies the medium.

In broth the comma bacillus grows aerobically with extreme rapidity, causing very slight cloudiness throughout the medium, and the formation

of a very distinct pellicle on the surface. The pellicle is a wrinkled membranous layer composed of spirilla and comma bacilli, and it is evident at 37° C. even in twenty-four hours. The cholera-red reaction is easily obtainable in broth cultures, and consists in the development of a pink or red colour on the addition of a few drops of strong sulphuric acid. The reaction is due to the formation of indol and of a nitrite in the peptone broth; the sulphuric acid liberates nitrous acid by acting on the nitrite, and the nitrous acid with indol gives the pink colour known as the nitroso-indol reaction. Many other organisms produce indol, but few coincidently produce nitrite, so that the reaction with sulphuric acid alone is a valuable test for the cholera spirillum. The source of the indol is the albumin peptone present in the broth, and the nitrite is believed to be derived from the reduction of nitrates also present in the broth.

Milk forms an admirable medium, and, if it be coloured with litmus, slight acid production may be observed to accompany the growth of the organism. The original Vibrio choleræ described by Koch did not coagulate milk, and most cultures do not do so; but Flexner states that recently cultures obtained from cholera patients have at times caused coagulation. In contradiction to the views of Hesse, who claimed that uncooked milk destroyed the Vibrio choleræ in twelve hours, Basenau says that up to the point of coagulation these organisms increase in number, and that even after the milk becomes acid they are present in an active state. The difference of opinion is doubtless due to the other organisms present, and is dependent upon the particular varieties in the milk.

Metschnikoff has shown that, while sarcina and torula favour the development of comma bacilli, the Bacillus pyocyaneus prevents its growth. The same observer holds that the bacteria of the digestive tract influence the susceptibility of man to the cholera organism. It seems probable that the Bacillus coli communis favours the growth of Vibrio choleræ in certain media, though the same organism has the very opposite effect on Bacillus typhosus. Wiener states that the Bacillus coli helps the growth of the cholera organism in gelatine plates, and that animals inoculated with both organisms die more quickly than when inoculated with cholera alone. Animals are not very susceptible to the virus of cholera in the ordinary way by the mouth or subcutaneous injection. It is, however, pathogenic to guinea-pigs when introduced into the intestine by pharyngeal catheter after neutralisation of the stomach contents with carbonate of soda, and after the injection of opium in large doses to check peristalsis. Also it is pathogenic to rabbits and mice when injected in large quantities into the abdominal cavity. Lately Wiener has succeeded in producing general infection of young cats by administration of cholera vibrios by the mouth, and this notwithstanding the acid reaction of the gastric contents.

In sterilised river or well water the organism multiplies to some extent and preserves its vitality for several months; but if the same water be unsterilised, the vibrio only lives a few days, usually not more than a week. The organism is easily killed by heat, a few minutes at 60° C., or higher, sufficing to kill it. It is also but feebly resistant to chemical disinfectants. Desiccation quickly destroys the organism if the drying be thorough, but in the moist condition the bacillus may retain its vitality for considerable periods. Since the experiments to be recounted deal with the viability of the cholera bacillus in carbonic acid gas and in charcoal, it may be noted here that Dunham has fixed its viability under a variety of conditions as follows:—On agar, 1–2 years according to the temperature; in fæcal matter, 6 days, and urine, 5 days (stale urine and sewage, only 24

hours); river water, 6 days (dirty water, 48 hours); textile fabrics, 2–12 days according to the moistening used; and on fruits and vegetables, a few days.

BACTERIOLOGY OF TYPHOID FEVER.

The Bacillus Typhosus, the specific organism of typhoid fever, was discovered by Eberth in 1881 in the spleen and diseased intestinal glands of typhoid cadavera. Later it was isolated from the same sources and fully described by Gaffky. It also occurs in the dejecta, and occasionally in the urine, of typhoid patients, and in a certain percentage of cases may be present in the blood. It has also been found in post-typhoidal abscesses.

The bacillus is a rod with rounded ends, and is provided on all sides with very numerous flagella, which are long, spiral, and slender, and which confer upon the organism a high degree of motility. It moves with a rapid serpentine action. It stains with aniline dyes, and is decolorised by Gram's process. It grows best in the presence of ample oxygen, but, while distinctly aerobic by preference, it is also facultative anaerobic, and it does not form spores. It does not liquefy gelatine nor coagulate milk, though in the latter medium it produces a slight acid reaction. In broth it causes uniform turbidity, and does not rapidly produce indol. In solid media its growth is very slow, and in plate preparations its colonies are irregularly circular in outline and bluish-white in colour. Under the low power of the microscope the colonies present a granular, yellowish-brown appearance.

In stab cultures an opaque, greyish-white, finely nodular line of colonies is formed. It produces no gas in gelatine, or even in glucose gelatine, cultures.

In addition, it gives a valuable test by agglutination of the bacilli, when a fresh culture is tested with the blood-serum of a typhoid patient (Widal reaction).

The viability of the typhoid bacillus is not very great. Its recovery from admixture with other organisms is extremely difficult, and is seldom possible after a few days' interval. Thus its isolation from suspected river or well water, or from sewage and fæces, is by no means easy.

BACTERIOLOGY OF DIPHTHERIA.

The Bacillus Diphtheriæ, the specific organism of diphtheria, was discovered by Klebs in 1883 in the membranous exudation present in diphtheritic throats. It was demonstrated specially in the deeper portions of the false membrane; the outer layers yielded few of these bacilli, though liable to contain streptococci, micrococci, and some bacilli of different kinds. Löffler in 1884 succeeded in isolating the B. diphtheriæ in pure culture, and proved its possession of pathogenic properties. The organism is frequently known, therefore, as the Klebs-Löffler bacillus. It produces a soluble toxin, which was recognised by Roux and Yersin; and this toxin, apart from the presence of the living organism, was shown by these observers to be capable of producing death or paralyses when injected into animals.

The bacillus is a rod with rounded ends, and may be either straight or curved. It is liable to vary greatly in size, shape, and appearance in different conditions of cultivation; indeed, its polymorphism is one of the most notable characteristics of this bacillus. Certain portions of the rod,

especially the ends, are often larger, more refractile, and stain more deeply than the general substance, while in other portions of the rod vacuoles may appear to be present. The bacillus is aerobic and also facultative anaerobic, but when grown under anaerobic conditions, its development is restrained. It is non-motile, and stains with aqueous solutions of various dyes without being decolorised by Gram's method. Owing to the differentiation of its protoplasm, it may be subjected to contrast staining by the methods of Neisser or of Crouch. This aids greatly in its recognition under the microscope. It is not regarded as a spore-bearing bacillus, though Babes considers that it may be so under certain conditions. Its optimum temperature is about 35° C., and though capable of growth upon ordinary media, such as gelatine, it flourishes best on blood-serum, on glycerinated serum, or on glycerinated agar. In broth at 37° C. the organism produces general cloudiness, accompanied, it may be, by a slight pellicle and some sediment. Continued growth produces some acidity of the medium (more pronounced if sugar be present), followed after some days by alkalinity. Gelatine is not a very satisfactory medium owing to the low temperature at which it must be incubated, a temperature at which the organism does not grow luxuriantly. The organism does not liquefy gelatine, and forms in it very small white colonies often composed of bacilli of anomalous forms. On blood-serum the B. diphtheriæ grows as a homogeneous grey or whitish smear, having a moist appearance, or as isolated raised colonies of similar appearance. It does not liquefy the serum. In milk the organism grows well without producing coagulation. The organism possesses considerable vitality, and may retain its existence for many weeks or even months. Thus it has been recovered from dried silk threads or toys after four to five months, and from dried false membranes after eighteen months. There are several organisms which, from their resemblance to the diphtheria bacillus, are termed pseudo-diphtheria bacilli, and which are mainly distinguished from it by staining reactions, and by their deficiency in power of acid production in broth. Of these Schabad has very fully described the characters.

The diphtheria bacillus is not very resistant to heat, and, in fact, is destroyed by moderate exposures to temperatures above 58° or 60° C.

BACILLUS COLI COMMUNIS.

This organism was described by Emmerich and by Weisser, but is generally known as the bacillus of Escherich, since this latter observer more fully investigated it. It was included in my experiments, but as its characters are well known, and as it is generally non-pathogenic, a full description of it is here unnecessary. The variety which was used conformed to the main characters of its group—that is to say, it was a short motile bacillus which did not liquefy gelatine, which produced gas in gelatine and glucose gelatine cultures, which coagulated milk and produced in this medium a strong acid reaction, and which rapidly produced indol in peptone broth.

These descriptions of the organisms with which my experiments were chiefly performed are here given, in order that in describing the experiments themselves it may be possible to term the appearance and growth of the organisms as 'normal,' meaning thereby the possession of the above characteristics. It will thus be possible to avoid detailing in each

experiment the series of cultures which were made on each occasion to determine whether these characters were maintained unaltered under the conditions of experiment. Also it will be unnecessary to describe in detail the appearances of the control cultures which provided verification of the possession by the organisms of their standard cultural characteristics at the commencement of the research.

PREVIOUS RESEARCH.

Experiments have been made as to the effect of carbonic anhydride and carbonic oxide gases on Koch's comma bacillus. Of these we will first consider Frankland's experiments, in which gelatine plate cultivations were submitted to an atmosphere of these gases. The plates, resting on glass stages one above another, were placed in a flat porcelain dish and covered with a glass bell-jar. Mercury was poured into the dish as a seal, and sterilised water poured on the top of the mercury. The weight of the bell-jar caused it to sink into the mercury, which thus acted as the effective seal. A piece of sterilised rubber tubing was passed into the chamber, and a current of gas was introduced through it. The excess of gas and air was allowed to escape at the edge of the bell-jar through the mercury and water. After filling the chamber, the tubing was removed and the dish with its contents set at a certain temperature of incubation. This was usually 20° C. Control plates were at the same time incubated in a damp chamber with ordinary air but at the same temperature. The number of colonies in the control and experiment plates were counted and compared after a certain number of days. The carbonic acid gas was prepared in Kipp's apparatus by the action of dilute hydrochloric acid upon marble. It was purified by passing it first through a saturated solution of carbonate of soda, and then through a plug of sterilised cotton wool. The carbonic oxide gas was prepared from potassium ferrocyanide and strong sulphuric acid. It was purified by passing it through a saturated solution of caustic soda, and then through a small tower filled with slaked lime, and finally through a plug of sterilised cotton wool.

The results of Frankland's experiments were as follows:—Koch's comma bacillus showed no growth in the plates in carbonic acid gas even after eight days, and further, when these plates were left for three more days in air they still showed no colonies. Carbonic oxide did not so completely prevent growth, though there were not nearly so many colonies in it as in air. Only a few of the spirilla were developed, and subsequent growth in air was relatively small. Frankland concludes (1) that carbonic acid gas arrests the growth of spirilla; (2) that carbonic acid gas is unfit for use as a method of anaerobic culture, and is more deleterious than other gases, *e.g.*, hydrogen, for that purpose; (3) that there is a great variation in the power of resistance possessed by individual organisms in ordinary cultivations, and that conditions which exert a rapidly destructive influence on the majority of the microbes leave the more hardy individuals of the same culture unaffected.

It is necessary to point out, however, that the first-given of these conclusions should be restricted to the medium used and to the temperature of incubation. While perfectly true for a gelatine medium at 20° C., it does not follow that the same results would have been obtained at blood-heat and on agar or in broth. There is no doubt that Vibrio choleræ, like most pathogenetic microbes, develops in artificial media better at 37° C.

than at 20° C. It is possible that Frankland would have found some growth in carbonic acid gas at the optimum temperature. Further, it must be noted that there is no statement as to the actual amount of carbonic acid gas present. The experiment was conducted in a bell-jar full of air, into which the gas was passed; the air would contain a large percentage of the gas, but it is impossible to say exactly how much. With such an apparatus and such a seal it is not possible to exhaust the air so as to form a partial vacuum, and then to fill that vacuum with carbonic acid gas. This method, with a volumetric estimate of the percentage of carbonic acid present, and with an anaerobic control culture, would give an accurate result of the effect of carbonic acid gas.

Fraenkel also conducted experiments by submitting various organisms in a medium of gelatine peptone to the action of a current of carbonic acid gas. Amongst other organisms he experimented with the comma bacillus, the Bacillus typhosus, and the Bacillus coli communis. In one method Esmarch roll tubes were used. The medium inoculated with the organism forms a solid lining to the tube. A current of carbonic acid gas was then passed constantly through the tube during the experiment. At the end of a stated time the gas was discontinued and the tube was incubated in air. Observations were recorded as to the growth of the organism in the jelly. In another method Esmarch roll tubes were used, but the medium was inoculated and kept at first in a liquid condition. Through the liquid jelly carbonic acid gas was passed for a considerable time; it was then discontinued, the jelly allowed to solidify on the walls of the tube, and the growth watched.

Fraenkel's experiments enabled him to classify the observed organisms into three groups:—(*a*) those that are killed, (*b*) those that are arrested in growth but not killed, (*c*) those which develop as well and almost as quickly in carbonic acid gas as in air. Amongst those whose development is absolutely arrested Fraenkel places the bacillus of Asiatic cholera. Of it he says: "The bacilli of cholera perish quickly and almost totally in carbonic acid gas." He regards as exceptional those instances in which individual colonies of the organism develop after the above exposure to the gas. However, he was sometimes able to obtain a feeble growth of the cholera bacillus by incubation in air subsequent to the exposure to carbonic acid gas.

On the other hand, the Bacillus typhosus and the Bacillus coli communis occupy places in the group of those which develop in carbonic acid gas almost as well as in air. Fraenkel found that the growth of strictly anaerobic species was restricted in an atmosphere of carbonic acid. Since Fraenkel also used a gelatine medium, his experiments are open to the same criticism as those of Frankland in respect of the temperature of incubation. Neither endeavoured to grow the Vibrio choleræ in carbonic acid gas at the optimum temperature for the organism. Accepting the fact that both these experimenters found that an atmosphere of carbonic acid gas, under the conditions of their experiments, killed, or, at all events, prevented the growth of, cholera organisms, one must next consider how far the result was due to exclusion of oxygen, and how far to a toxic effect of carbonic acid.

The conclusion in both cases is that carbonic acid gas has a direct deleterious action on cholera bacilli, and that the result was not simply due to exclusion of oxygen. This conclusion is based upon the fact that the Vibrio choleræ is a facultative anaerobe. This is now well known, though at first the organism was believed to be strictly aerobic. Its growth as

an anaerobe is certainly feeble compared to its aerobic growth, but mere exclusion of oxygen does not prevent all growth. This has been demonstrated by the growth of comma bacilli in an atmosphere of hydrogen.

In this method of anaerobic culture the organism is unaffected as regards its vitality, though its growth in colonies in gelatine plate is retarded, and the colonies are smaller than in air incubation. In the absence of oxygen the usual liquefaction of the jelly is much retarded.

Additional evidence in favour of the toxic influence of carbonic acid gas is that in Frankland's experiments the atmosphere used must have been air with a large percentage of carbonic acid gas, and not pure CO_2, so that in his case all oxygen was not excluded, and yet no growth occurred.

Fraenkel also takes the view that carbonic acid has a direct toxic effect, because certain organisms, affected by it in a similar manner to that shown by the cholera bacillus, and arrested entirely by it, were not also arrested by an atmosphere of hydrogen gas, as they would have been if the apparent action of CO_2 had merely been due to exclusion of oxygen.

Sabrazes and Bazin, experimenting with carbonic acid gas under pressure, found that it was not fatal to the B. typhosus or to B. coli communis even after several hours' exposure. Moreover, they state that the organisms were not notably altered in biological, morphological, or developmental powers. They do not seem to have tested the other organism in which we are interested, viz., the cholera bacillus.

D'Arsonval and Charrin differ from the above in regarding carbonic acid gas under pressure as a germicidal antiseptic, but their experiments did not include the organisms at present under consideration.

Hamburger finds that carbonic acid gas increases the bactericidal power of the blood, and believes that its action in this respect is due, not only to the fact that carbonic acid gas itself possesses bactericidal properties, but also that in its presence the corpuscles take up more water, and the serum is thus more concentrated. Further, in its presence the diffusible alkalis of the serum are increased. The serum of venous blood is said to possess higher bactericidal power than that of arterial blood.

Guyon relates experiments with carbonic acid gas upon cholera cultures. He exposed broth cultures of cholera bacilli to the action of the gas in a desiccator. Drops of the culture were placed on glass plates, and the dessicator filled with the gas. He found that when a young culture, such as a 24-hour growth of Vibrio choleræ, was used, the carbonic acid gas destroyed the organisms in twenty-four to forty-eight hours. When, however, the culture had been desiccated for one to two days previous to exposure to the gas, the organisms were not destroyed even in fifty days. Guyon concludes that a certain amount of desiccation seems to augment the resistant powers of the Vibrio choleræ to carbonic acid gas, and that carbonic acid gas has a distinct toxic influence on the organism. When cultures in a liquid medium, such as broth, are dealt with, the effects of carbonic acid gas must not be confused with results due to the evaporation of the medium and desiccation of the bacilli.

Opinions differ very markedly concerning the effects of desiccation on the cholera microbe, and one cannot help concluding that the different writers use the term desiccation without distinct definition of what they mean by it. So different are the results arrived at by different bacteriologists, that one finds Koch stating that the Vibrio choleræ will not resist desiccation longer than twenty-four hours, while Finkler and Prior, having desiccated cultures over sulphuric acid, were able to obtain pure subcultures after three and a half months. It is hardly necessary to quote

the work of Kitasato, Watson Cheyne, Hueppe, Guyon, and Berkholtz upon this point, as the possible error of desiccation affects very few of my experiments. It is sufficient to note that this possible fallacy was recognised.

ACCOUNT OF RESEARCH.

An experimental research was undertaken in the Laboratory of the Royal College of Physicians of Edinburgh, with the object of testing the effects which the different forms of carbon mentioned by Dr Parkin might have upon cultures of the Spirillum Asiaticæ Choleræ, and upon cultures of some other organisms.

The forms of carbon which I submitted to investigation were:—

I. Carbonic Acid Gas.
II. Charcoal (both Animal and Vegetable).

A few experiments are also included in which I used—

III. Naphtha.
IV. Creosote.
V. Yeast.

Naphtha and creosote were tested because they are in use as remedies for cholera, and because Parkin attributes their alleged efficacy to the amount of carbon they contain. Yeast was included on account of its production of carbonic acid gas when grown in suitable media.

The organisms with which I conducted the experiments were:—

(A) Spirillum Asiaticæ Choleræ of Koch.
(B) Bacillus Typhosus of Eberth.
(C) Bacillus Coli Communis of Escherich.
(D) Bacillus Diphtheriæ of Klebs-Löffler.

The media used, either in the investigation or in subsequent tests, were 1 per cent. peptone broth, 1·5 per cent. agar, 10 per cent. gelatine, 2 per cent. glucose jelly, milk, and blood-serum.

I. Carbonic Acid Gas Experiments.

The carbonic anhydride was obtained from the action of dilute hydrochloric acid upon marble chips in a Kipp's apparatus (figs. A 1 and B 1). The gas was passed through a saturated solution of sodium carbonate in a wash-bottle (figs. A 2 and B 2), and then through sterilised cotton-wool (figs. A 3 and B 3), into the chamber containing the cultivation media. This process was used to purify the gas given off in the Kipp apparatus. The chamber used was in one of two forms, either a Novy's jar or a glass cylinder which could be securely sealed.

Novy's jar (fig. A 4) consists of a lower half composed of a cylindrical glass jar with a flat ground-glass rim, upon which fits a similar rim belonging to the upper half. The upper half takes the form of a glass dome surmounted by a neck, into which fits a ground-glass stopper. The stopper and neck are laterally perforated, so that gases may be passed in or out and the openings apposed or otherwise by revolution of the stopper.

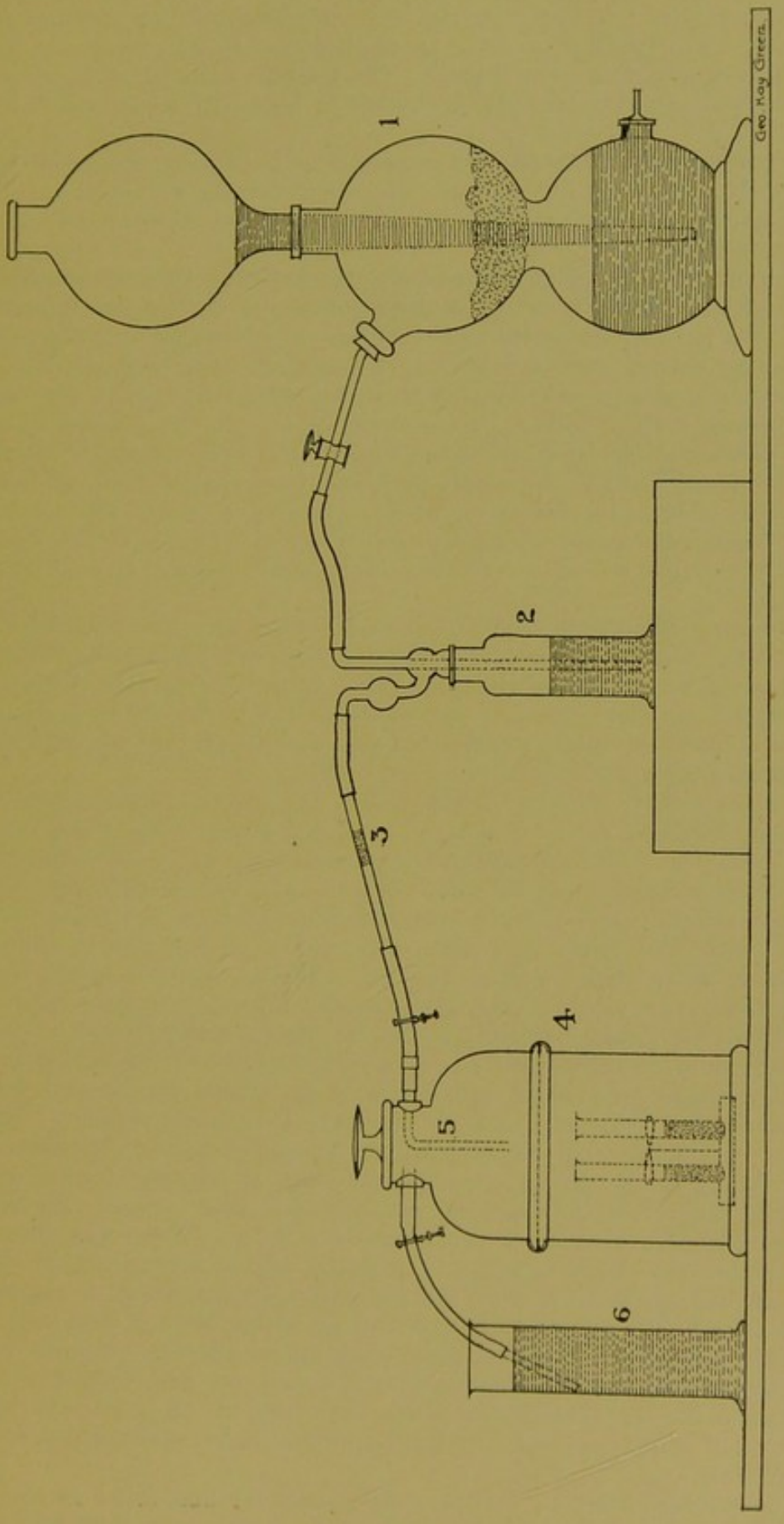

Figure A.

By means of a glass tube continuance of one of the stopper apertures its opening is conducted low in the jar (fig. A 5), while the other aperture is near the top. The two halves of the jar are held together by clamps or by a rubber band round the opposed rims. The bottom of the jar was padded with cotton wool, and the jar is large enough to hold both plate and tube cultures.

The alternative apparatus (fig. B 4) consisted of a wooden stand closely covered with a tight-fitting rubber plate, and having a central raised circular portion of wood on which the plates or tubes were placed in a movable wire cage.

Resting on the rubber plate and covering the central portion of the stand was a cylindrical glass jar having a thick lower rim with a flat inferior surface. This jar was perforated in two places—(1) laterally near the foot by an opening corked with a rubber cork: the cork was pierced with a metal pipe which was provided with a screw tap (fig. B 5); (2) at the top by an opening corked with a rubber cork: this cork was pierced by a piece of glass tubing bent at right angles, and was carefully luted. Rubber tubing attached to the metal pipe and to the glass tube allowed the apparatus to be connected as required. The glass rim and the rubber plate were sealed and made adherent to each other with the help of vaseline, but mainly by a firm collar of modeller's wax, which proved an excellent seal.

Figures are given on pp. 23 and 25 showing these two forms of chamber, each connected with the charging apparatus and the purification wash-bottle. During the process of charging either form of apparatus, the exit tube, by which air and excess of gas escaped, was immersed to a slight depth in a cylinder of water, which acted as a seal to prevent air rushing backwards into the apparatus (figs. A 5 and B 6).

It was customary, after preparing either chamber for experiment, to exhaust the contained air by means of a suction pump. The pump was provided with a mercury manometer attachment, so as to ascertain the extent of exhaustion achieved. When this was satisfactory, the tubes were clipped, and the chamber disconnected from the pump and connected with the Kipp apparatus. The object of this was to obtain as far as possible an atmosphere of carbonic acid gas, and not merely air with a moderate percentage of the gas. It was found by control experiment that this preliminary exhaustion had no effect on the growth of the organisms if air were subsequently admitted instead of carbonic acid gas.

The percentage amount of carbonic acid gas which was present was calculated at the beginning and at the end of each experiment. The method of volumetric analysis adopted was to collect some of the atmospheric contents of the jar in a eudiometer over a column of mercury. The sample was often obtained by displacement with water. Having noted the cubic centimetres of gas taken as a sample, there was passed into the eudiometer some caustic soda solution of the strength of 50 per cent. This was shaken up with the gas and the diminution in volume of the gas noted. The percentage absorbed by caustic soda is then calculated. This is practically the carbonic acid gas which was present. In the ordinary atmosphere carbonic acid gas forms ·04 per cent., but in these experiments the atmosphere of the jar contained 20 to 90 per cent. of the gas, and sometimes was practically pure carbonic acid without any other constituent.

The plates incubated in carbonic acid gas were uncovered so as to allow the gas to get fully at them, and similarly the tube cultures had

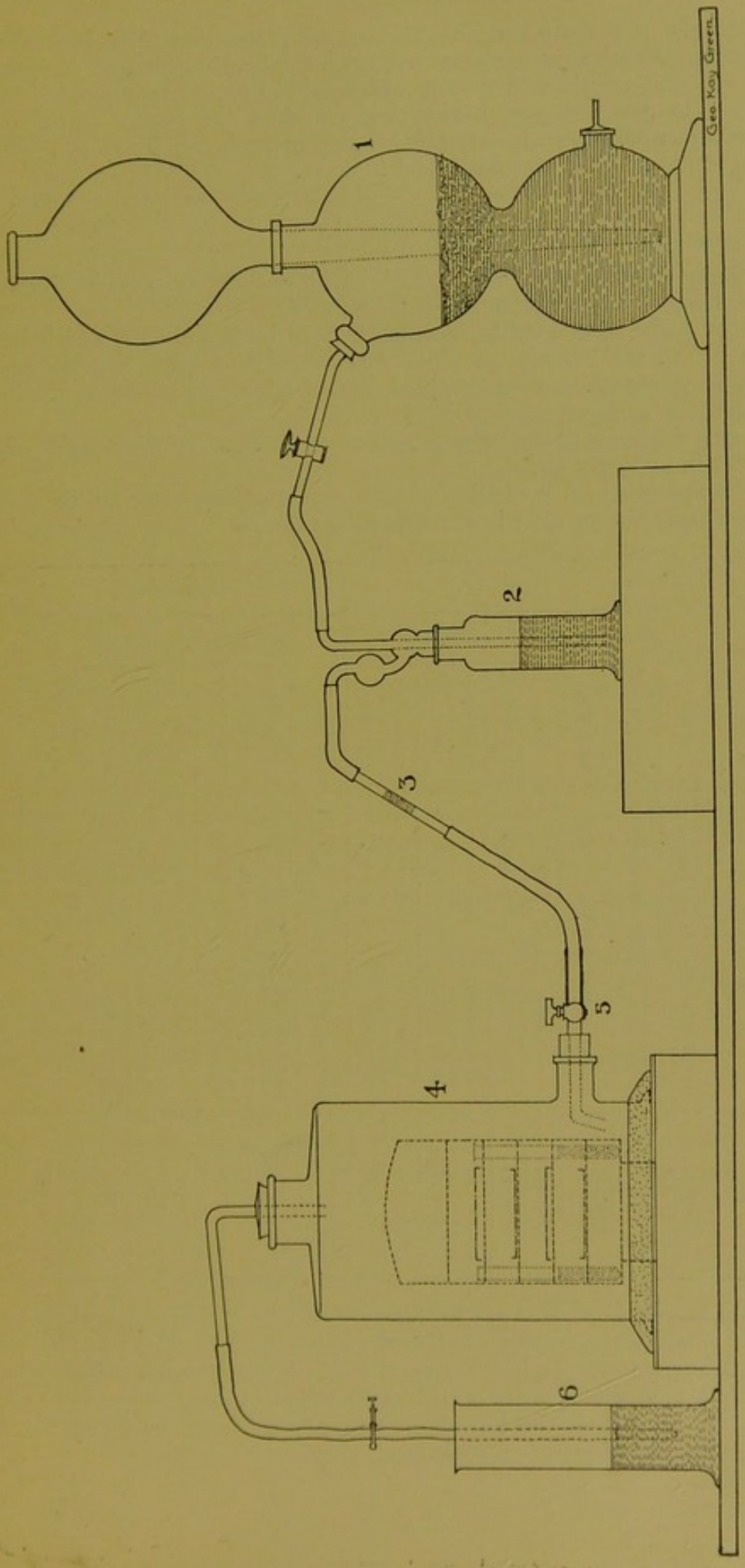

Figure B.

their cotton-wool plugs removed. The control plates and tubes incubated in a similar chamber in air were not uncovered and unplugged, because the air was not sterile and they would have become contaminated. The carbonic acid gas, however, having been washed and passed through a sterile cotton-wool plug while freshly disengaged, was not likely to render cultures impure, and it was not found to do so.

Experiment 1.

To ascertain the effect of carbonic acid gas on cultures of Vibrio choleræ, Bacillus typhosus, and B. coli communis.

The cultures were freshly inoculated from broth cultures which had been at 37° C. for 1 hour. A broth tube, a gelatine plate, and an agar plate of each organism were prepared, and set to grow in air containing 30 per cent. of carbonic acid gas.

Exactly similar cultures were prepared and set to grow alongside the above, but in ordinary air.

The temperature of incubation of both sets of cultures was 20° C.

The growth is compared in the following table:—

Time.	Air.									Air and CO_2.								
	V.C.			B.T.			B.C.C.			V.C.			B.T.			B.C.C.		
	Broth.	Gelatine Plate.	Agar Plate.	Broth.	Gelatine Plate.	Agar Plate.	Broth.	Gelatine Plate.	Agar Plate.	Broth.	Gelatine Plate.	Agar Plate.	Broth.	Gelatine Plate.	Agar Plate.	Broth.	Gelatine Plate.	Agar Plate.
In 3 days.	Cloudiness and distinct pellicle.	Colonies liquefying.	Few colonies.	Normal.	No colonies.	No colonies.	Normal.	Normal.	Normal.	Little cloudiness and no pellicle.	Small colonies. No liquefaction.	No colonies.	Normal.	No colonies.	No colonies.	Normal.	Normal.	Normal.
In 7 days.	Growth increased.	Plate completely liquefied.	Several colonies.	Normal.	Few colonies.	Few colonies.	Normal.	Normal.	Normal.	Traces of pellicle.	Colonies show traces of liquefaction.	Much less growth than in air.	Normal.	Few colonies.	Few colonies.	Normal.	Normal.	Normal.
In 14 days.	Normal.	Not kept after 7 days.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.	Some growth, but less than in air.	Increase of liquefaction.	A few colonies.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.

Result.—Carbonic acid gas in this amount and at this temperature has little or no effect on the B. typhosus or on the Bacillus coli communis. It has, however, a definite restraining influence on the Vibrio choleræ, retarding its growth in all media, and postponing both the liquefaction of gelatine and the formation of the usual pellicle on broth.

It was noticed that the broth cultures of V. choleræ grew relatively better in the presence of carbonic acid gas than the plate cultures of the same organism.

Experiment 2.

To ascertain the effect of carbonic acid gas on the growth in broth of Vibrio choleræ, B. typhosus, and B. coli communis.

Three broth tubes were inoculated, one with each organism, and set unplugged in air containing 40 per cent. of carbonic acid.

Control tubes exactly similar were grown in air.

The temperature of incubation was 20° C.

Observations were made as follows:—

In 48 *hours.*—Growth apparently taking place in all the tubes, but very slight in the tubes from the CO_2 jar, which showed no turbidity such as that seen in the control tubes. Subcultures in broth showed that the organisms were alive in the CO_2 tubes, though the gas had restrained growth.

In 3 *days.*—The cholera tube in CO_2 shows slight turbidity but no pellicle, and is less turbid than would correspond to one day's growth in air, and much less so than in the control tube which showed a profuse pellicle. The typhoid tube and the coli tube show slight turbidity, not quite so pronounced as in their control tubes.

In 7 *days.*—The cholera tube still shows no pellicle. It and the others all growing more feebly than the corresponding tubes in air.

The tubes were now all transferred to a temperature of 37° C. for 3 days. Growth was at once increased notwithstanding the CO_2 in the jar, and at the end of the 3 days a pellicle formed in the cholera tube, while the other organisms grew very rapidly.

Result.—The carbonic acid gas restrains cholera organisms much more than either of the others, but at this temperature and in this percentage does not kill all the organisms in the broth culture. Raising the temperature enhanced the growth to a very marked degree.

Experiment 3.

To compare the growth of the Vibrio choleræ anaerobically, aerobically, and in a moderate percentage of carbonic acid gas, so as to observe whether the effects of exclusion of oxygen were the same as those produced by carbonic acid gas.

Broth cultures of the organism were employed, and the temperature of incubation was 37° C. The anaerobic culture was placed in a Buchner's tube over pyrogallate of potash solution.

The carbonic acid chamber contained 40 per cent. of CO_2.

The tubes were compared as follows:—

In 24 *hours.*—The aerobic culture showed pronounced growth and some pellicle formation, the anaerobic culture showed little growth and no pellicle, while the carbonic acid culture showed less growth than either and had no pellicle.

In 3 days.—The best growth had taken place in the aerobic tube, and least in the anaerobic culture. In carbonic acid there was now some turbidity though little pellicle.

Result.—The carbonic acid gas was insufficient to restrain the organism altogether, though the first day's growth was retarded. The restraining influence of CO_2 seems less potent at 37° C. than at 20° or 24° C.

The effects of exclusion of oxygen were not the same as those of this amount of carbonic acid.

Experiment 4.

To compare the growth on agar of Vibrio choleræ in air and carbonic acid at varying temperatures.

Stroke agar cultures were used, one being set in air and the other in air containing 60 per cent. of carbonic acid.

Both tubes were incubated at room temperature (16° C.).

In 7 days.—The air tube showed normal streak growth, not so profuse as would have been the case at 37° C., but ample. The CO_2 tube showed no growth.

Both tubes were then removed to the incubator at 37° C., the one being incubated in air and the other in 60 per cent. of CO_2 as before. They were thus kept for 7 days more.

On the 14th day from the commencement the air tube showed good streak growth. The tube maintained in carbonic acid showed no definite streak growth, but there was some growth now noticed in the broth fluid expressed from the agar, and lying near the foot of the agar tube.

Result.—Carbonic acid gas in the proportion of 60 per cent. had practically restrained all growth of the comma bacillus, though it had not absolutely killed all the organisms. The higher temperature of incubation in the 2nd week assisted a few organisms to live. Probably at room temperature the tube would have remained sterile.

Experiment 5.

To compare the growth on agar and at blood-heat of Vibrio choleræ, B. typhosus, and B. coli communis in air and carbonic acid respectively.

Agar stroke cultures were used and the tubes incubated at 37° C. The percentage of carbonic acid gas was 60.

In 24 hours.—The tubes in air were all growing well, but of those in the CO_2 jar only the B. coli shows good growth. The others show only traces of growth.

In 3 days.—The cultures of V. choleræ and B. typhosus in air and CO_2 show marked differences in extent of growth.

In 9 days.—*The Vibrio choleræ* has grown as a narrow strip along the line of inoculation in the tube exposed to carbonic acid; its growth is meagre when compared with that in air.

The B. typhosus is not alike in the two tubes. That in air shows a streak of small blue-grey rounded dots, isolated from each other towards the margins and end of the streak.

The tube in CO_2 shows a narrower streak, similar to but less pronounced than that in the air tube; also it shows, at the margins of the agar, a more homogeneous smear. Perhaps this may have been due to the expressed fluid of the agar having been inoculated, and then the tube tilted so that this fluid ran along the edges of the agar.

The B. coli communis is normal and alike in both tubes.

Result.—Distinct restraint upon the growth of V. choleræ by carbonic acid. No effect by the gas on B. coli and no great retardation of growth of B. typhosus.

Experiment 6.

To compare the growth of V. choleræ and B. typhosus in air and carbonic acid gas respectively at 24° C.

Gelatine plates and agar cultures of each organism were used, and the percentage of carbonic acid gas present was 60.

On the 6th day.—The cultures were examined.

The agar stroke culture of B. typhosus in CO_2 gas was growing slightly, much the same amount of growth as in its control tube in air, and about as much as one would expect in air at this temperature. Not so, however, with the V. choleræ, which showed very scanty growth, much less than in its control tube in air. The jelly plates showed more distinctly the effects of the CO_2 gas. That which had been inoculated with cholera was sterile, no colonies were visible, and none appeared later although the plate was incubated in air for the next three days at 24° C. The control cholera plate incubated in air showed liquefaction of the gelatine in 48 hours, and was entirely fluid before the 6th day. Both plates inoculated with B. typhosus showed colonies, that in air most profusely, but that in CO_2 was well covered with colonies.

Result.—The toxic effect of 60 per cent. carbonic acid gas on V. choleræ is well shown in gelatine plate cultures. The same percentage of gas has little or no effect on B. typhosus.

Experiment 7.

To ascertain the effect of carbonic acid gas on the growth in gelatine plate of V. choleræ, B. typhosus, and B. coli communis.

Control plates were incubated in air for comparison.

Temperature of incubation, 24° C. Percentage of carbonic acid, 60.

After 1 week.—Vibrio choleræ.—The plate in air had entirely liquefied in 3 days, but that in CO_2 showed no liquefaction. The latter contained several colonies which were ascertained to be an impurity and to be of the colon type.

B. typhosus.—No colonies in the CO_2 plate, and but few in the air plate.

B. coli.—The plates were alike.

After 2 weeks.—V. choleræ.—There is no general liquefaction of the plate kept in CO_2. Individual colonies here and there show signs of commencing liquefaction, while others are non-liquefying and belong to the colon type.

B. typhosus.—The plate contains typical typhoid colonies in considerable number and of small size.

B. coli.—This organism seems unaffected by the CO_2 gas.

A few days later the Vibrio choleræ was recovered in pure culture from the plate incubated in CO_2. The plate rapidly liquefied when left in air. The recovered organism grew in gelatine stab culture somewhat differently from ordinary Vibrio choleræ. Instead of liquefying in the shape of a funnel along the stab, the organism liquefied the medium more especially at the surface. Perhaps the organism after its fortnight in CO_2 felt air-hunger and grew as near the surface as possible.

Result.—The carbonic acid gas had little effect on B. typhosus and B. coli communis. It, however, markedly retarded the growth and liquefaction of V. choleræ, though it did not entirely restrain it. The fact that the cholera organism was not completely arrested in this case may have been due to a variety of causes. There were not many cholera colonies in the plate, and these must have been derived from the more resistant individuals of the original culture. Now and then particular individuals resist, when the general bulk of the organisms are entirely inhibited and destroyed. Another influence may have been exerted by the simultaneous presence of B. coli, which, according to Metschnikoff and Wiener, facilitates the growth of V. choleræ in gelatine plates.

Experiment 8.

To ascertain the effect of carbonic acid gas on the growth in agar plate of V. choleræ, B. typhosus, and B. coli communis, and on the growth on blood-serum of B. diphtheriæ.

Control plates and tube were incubated in air.

Temperature of incubation, 37° C. Percentage of carbonic acid gas, 80 per cent. at first; 70 per cent. found present at the end of 5 days.

After 5 *days:*—

V. choleræ.—No colonies visible in the CO_2 plate, though the organism had grown well in the air plate.

B. typhosus.—Both plates contained a large number of small colonies, perhaps more plentiful in the air plate. The CO_2 plate, when examined with the low power of the microscope (× 105) showed colonies which were irregularly circular, not so uniform in shape as in the air plate similarly examined. Some of the colonies were oval, and many less coloured than in the air plate. They were tested by subculture in broth, gelatine stab, milk, and glucose gelatine, and found to be typical B. typhosus unchanged in morphological and cultural characteristics.

B. coli.—There was no notable difference in the plates in air and CO_2. The colonies in the CO_2 plate examined by the microscope (× 105) were often more oval and somewhat paler than in the air plate. The colonies were tested by subculture in broth, gelatine stab, milk, and glucose jelly, and found to be B. coli unchanged in morphology or reactions. The indol reaction was positive in broth after 48 hours at 37° C., thus showing that carbonic acid gas does not interfere with the production of indol by B. coli.

B. diphtheriæ.—This organism grows in streak on blood-serum, equally well in air, and in air with a large percentage of carbonic acid.

Result.—In a strength of 70–80 per cent. carbonic acid gas arrests growth of V. choleræ, but does not alter the characters and growth of B. typhosus, B. coli, or B. diphtheriæ in any important manner.

Experiment 9.

To ascertain the effect of an atmosphere wholly composed of carbonic acid gas upon the growth in agar plate of V. choleræ, B. typhosus, and B. coli communis, and upon the growth on blood-serum of B. diphtheriæ.

Control plates and tube were incubated in air.

Temperature of incubation, 37° C. Percentage of CO_2, 100.

After 4 *days:*—

V. choleræ.—Plate in CO_2 sterile, no colonies even on examination with microscope (× 105). Control plate in air showed numerous colonies.

B. typhosus.—The plate in CO_2 shows a few colonies, but fewer than in air.

B. coli.—Innumerable colonies in both plates. Those in CO_2 plate examined (× 105) show similar shapes to those mentioned in previous experiments.

B. diphtheriæ.—This organism has grown as well in carbonic acid as in air.

The cholera and typhoid plates from the carbonic acid jar were then incubated in air at 37° C. for 3 days, and at the end of that time the cholera plate was still sterile, but the typhoid plate showed numerous colonies of B. typhosus.

Result.—An atmosphere of pure carbonic acid gas acting on agar plate cultures completely arrests V. choleræ, retards somewhat the growth of B. typhosus, and has no effect on the growth of B. coli and B. diphtheriæ.

Experiment 10.

To compare the growth of Vibrio choleræ in air and carbonic acid gas respectively.

Cultures used: 4 agar plates, 2 agar tubes, and 2 broth tubes; two plates and one of each of tubes in air and CO_2 respectively.

Temperature of incubation: 38° C. for 24 hours, then 26° C. for 3 days.

Percentage of carbonic acid: 99–100 per cent.

In 4 *days:*—

Agar plates.—No colonies in either of the plates placed in the carbonic acid, but plenty of colonies in those aerobically incubated.

Agar tubes.—There is good streak growth in the tube exposed to air, but in that exposed to CO_2 there is only a very slight growth of a few isolated colonies.

Broth tubes.—There is no growth in broth in carbonic acid, but the tube in air shows both good growth and ample pellicle formation.

The agar plates, which had been in CO_2 and were apparently sterile, were now separated. One was incubated in air, and the other in pure carbonic acid gas. The temperature of incubation was 26° C. The agar and broth tubes were continued in their respective jars.

In 4 days (8 days from commencement):—

Agar plates.—Neither the plate which had now been in CO_2 for 8 days, nor the plate which had been in CO_2 for 4 days, and then in air for 4 days, showed any colonies of Vibrio choleræ.

Agar tubes and Broth tubes.—Much same as before.

The carbonic acid gas was renewed and incubation continued.

In 4 days (12 days from commencement):—

Agar plates.—No colonies in either plate.

Agar tubes.—Same as before.

Broth tubes.—The broth tube in air is normal, that in CO_2 shows no pellicle and no apparent growth, though perhaps not so clear as formerly.

Incubation in carbonic acid gas was now discontinued, and all the plates and tubes incubated in air at 37° C.

In 4 days (16 days from commencement):—

Agar plates.—The plate which had been in CO_2 4 days and in air 12 days was sterile. The plate which had been in CO_2 12 days and in air 4 days showed four colonies which were cocci. These had probably gained access to the plate during a process of photography on the 12th day.

Agar tubes.—The stroke culture which showed feeble growth during 12 days in carbonic acid developed well in air, and on subculture in broth and jelly proved to be Vibrio choleræ. In the former subculture it gave the indol reaction with sulphuric acid alone.

Broth tubes.—The tube which had been free from growth in carbonic acid for 12 days began to show signs of growth after 4 days in air, and after a fortnight in air at 37° C. was found to contain Vibrio choleræ, but even at that time it showed no pellicle or sediment which might have resulted from deposited pellicle.

Result.—The arrest of cholera organisms was very marked, especially so in the agar plates and broth tube. The growth in agar stroke culture was exceptional, since in pure carbonic acid one would expect no growth, but certainly the gas seems to retard growth in agar plate more completely than in agar stroke culture. Subsequent incubation proved that a few organisms might survive in broth and be able to grow in it in a modified fashion under more favourable conditions.

Experiment 11.

To ascertain whether small percentages of carbonic acid gas in air produced any noticeable effects on the growth of Vibrio choleræ and B. diphtheriæ.

Cultures used: Agar in plate and tube, broth, and blood-serum. The

B. diphtheriæ was grown on blood-serum and in broth, and the V. choleræ on agar and in broth.

Temperature of incubation: 37° C.
Percentage of carbonic acid: 7·3 per cent.
Period of incubation: 3 days.

At the end of 3 days.—The B. diphtheriæ had grown on blood-serum as well in air containing 7 per cent. of CO_2 as in the ordinary atmosphere with its ·04 per cent. Its growth in broth showed no special distinctions between the atmospheric influence of the carbonicised and ordinary air. The broth culture of V. choleræ in air showed a more pronounced pellicle than the similar broth culture in carbonic acid gas and air, but the latter possessed a pellicle and the organism had grown well. This was perhaps to be expected at this temperature of incubation, as other experiments had shown that at this temperature a much larger percentage was required to produce marked restraint of growth. The agar stroke cultures showed little difference between the two conditions, but in the agar plates there were fewer colonies in the one incubated in carbonic acid than in the one incubated in air. This may have been accidental, and in any case the difference was not very striking. The experiment was not continued, as there seemed no object in prolonging the incubation.

Result.—At 37° C. small percentages of carbonic acid gas produce little evident effects on the growth of Vibrio choleræ or of B. diphtheriæ.

Experiment 12.

This experiment is hardly worth giving in detail, as its results were entirely negative. It consisted in growing cultures of V. choleræ, B. typhosus, B. coli communis, and B. diphtheriæ in air containing about ·4 per cent. of carbonic acid gas, and comparing their growth with similar cultures in ordinary air. The media used were agar and blood-serum, the former for the first three organisms and the latter for B. dipththeriæ. The temperature of incubation was 37° C. and the period of incubation one week. There was no perceptible effect of the variation in atmosphere.

Experiment 13.

To ascertain whether carbonic acid gas has a lethal effect at ordinary temperature on mature broth cultures of Vibrio choleræ, B. typhosus, and B. coli communis.

The method used was to take well-grown but not old broth cultures of the respective organisms, and place a few drops in the wells of sterilised hollow slides. These slides were then exposed to the effects of the gas in a concentration of 60 per cent. Control slides were kept in a similar chamber full of air. Subcultures were made from each slide after the experiment. The slides were not incubated at a high temperature, but allowed to remain at room temperature (15·5° C.). This was done in order to minimise evaporation of the broth drops, and so to avoid the possible error of the effects of desiccation. Distilled water was also present in the jars to provide for evaporation; it was placed either in a beaker in the jar, or was used to moisten filter paper spread over the floor of the jar.

After 1 *week.*—Subcultures in broth were made from each slide in the CO_2 jar, and after 48 hours' incubation at 37° C. all the organisms were growing well. The slides kept in air gave living subcultures of all the organisms after 10 days.

Result.—One week's exposure to air containing 60 per cent. of carbonic acid gas did not suffice to kill the organisms in broth cultures which had already grown at 37° C.

Experiment 14.

This was a further experiment of the effect of carbonic acid gas on grown broth cultures of V. choleræ, B. typhosus, and B. coli communis. To obviate the difficulty of the small quantities of broth cultures held by the recessed slides, sterile Petri dishes were used in this case, and about 6 c.c. of each culture exposed to the gas. The broth cultures used were 24 hours old, and had been at 37° C. for that time. The dishes were exposed on wire shelves, and a similar dish of distilled water was inserted to provide for evaporation.

The temperature was 15·5° C., and the percentage of CO_2 present was 70. Similar dishes were kept in a similar chamber in air, but covered to avoid contamination.

In 1 *week.*—Subcultures in broth from all the plates all contained the respective organisms alive and well.

In 2 *weeks.*—A similar result was obtained. The CO_2 jar was then charged more fully with the gas, which reached 95 per cent.

In 2 *weeks and* 5 *days* (*i.e.*, 5 days after the increase of the percentage of CO_2).—Subcultures in broth yielded B. typhosus and B. coli communis without difficulty, but the V. choleræ had been almost killed by the five days in 95 per cent. of CO_2. In its case the broth subculture showed no growth and no pellicle in 24 hours at 37° C., but after 48 hours a feeble pellicle formed. A gelatine stab subculture, made at the same time as the above broth subculture, showed no growth or liquefaction at 24° C.

Further incubation of the broth subculture resulted in growth from which, on plating out, a vigorous specimen of Vibrio choleræ was recovered.

Result.—Carbonic acid gas, in a concentration of 95 per cent., has a deteriorating influence on flourishing broth culture of V. choleræ at room temperature, provided that the exposure be sufficiently long. The organism is with difficulty recoverable after 5 days in 95 per cent. CO_2, though it was recovered after a fortnight in 70 per cent.

Cultures of B. typhosus and B. coli are not affected by the gas in the same way, since they yielded pure subcultures without difficulty.

II. Charcoal Experiments.

Although Dr Parkin confines to carbo ligni his recommendation of charcoal as a cure for cholera, it was thought advisable in these experiments to include both animal and wood charcoal. The method adopted was to spread the charcoal in sterile Petri dishes and sterilise it *in situ*, then to pour over it broth cultures of the organisms under consideration, and to ascertain by subculture their period of existence in charcoal. Animal charcoal was first used by this method. Fresh purified animal

charcoal was taken, and sterilisation was accomplished by dry heat for one to two hours at 155° C. (or sometimes even higher temperatures) on each of three successive days. Broth in tube was then inoculated from the charcoal to see that the charcoal was sterile, and if no growth occurred the experiment proceeded. Over the sterile charcoal were poured broth cultures of the organisms, the dishes of charcoal were then incubated at 37° C., and subcultures made at varying intervals to ascertain whether the charcoal had the effect of destroying the organisms. In order to obviate the fallacy of possible destruction due to desiccation by the constant maintenance of temperature at 37° C., the charcoal was kept moist by occasional watering with sterile water or with sterile broth. Great difficulty existed in sterilising the charcoal before use; it appears to form a suitable medium for spore-bearing organisms whose spores are capable of great resistance to heat.

The same method was used with fresh powdered wood charcoal. Three experiments were conducted with each kind of charcoal. The organisms dealt with were the same in each case, viz., Vibrio choleræ, Bacillus typhosus, and Bacillus coli communis. The details of each experiment were as follows:—

Experiment 1.

Remedy tested: Animal charcoal.

Cultures used: Broth cultures of V. choleræ, B. typhosus, and B. coli communis of three days' incubation at 37° C.

Moistening used: None.

Temperature: 37° C.

Subcultures made from the charcoal: Broth and agar.

Result:—

In 1 *week.*—Each variety of organism recovered from its respective plate.

In 2 *weeks.*—Each variety of organism recovered from its respective plate.

In 4 *weeks.*—Each variety of organism recovered from its respective plate.

At later periods the subcultures obtained were impure, and on plating them out on agar the attempt to recover the specific organisms of cholera and typhoid failed, but the B. coli was recovered from its plate.

Experiment 2.

Remedy tested: Animal charcoal.

Cultures used: Broth cultures of the same three organisms. The cultures were 24 hours old, and had been at 37° C. for that time.

Moistening used: Sterile water.

Temperature: 37° C.

Subcultures made from the charcoal: Broth.

Result:—

In 1 *week.*—Each variety of organism recovered from its respective plate.

In 2 *weeks.*—Each variety of organism recovered from its respective plate.

In 3 *weeks.*—Each variety of organism recovered from its respective

plate, but the B. typhosus with difficulty, owing to its plate giving an impure culture.

In 5 *weeks.*—None of the three organisms were recovered, all the subcultures were impure, and the common impurity was a spore-bearing organism.

Experiment 3.

Remedy tested: Animal charcoal.
Cultures used: Broth cultures of the same organisms. These cultures were 24 hours old, and had been at 37° C. for that period.
Moistening used: Sterile water and sterile broth.
Temperature: 37° C.
Subcultures made from the charcoal: Broth.

Result:—

In 1 *week.*—Each variety of organism recovered from its respective plate.

In 2 *weeks.*—Each variety of organism recovered from its respective plate.

In 3 *weeks.*—The B. typhosus and B. coli were recovered easily, and the V. choleræ with difficulty.

In 4 *weeks.*—The V. choleræ was irrecoverable, but the other organisms were recovered The subcultures at this stage were impure.

Experiment 4.

Remedy tested: Wood charcoal.
Cultures used: Broth cultures of the same organisms. The cultures were 4 days old, and had been at 37° C. for that period.
Moistening used: Sterile water.
Temperature: 37° C.
Subcultures made from the charcoal: Broth.

Result:—

In 1 *week.*—Each variety of organism recovered from its respective plate.

In 2 *weeks.*—Each variety of organism recovered from its respective plate.

In 3 *weeks.*—The B. coli was recovered, but neither V. choleræ nor B. typhosus, though there was no impurity.

In 4 *weeks.*—The subcultures from the cholera and typhoid plates were sterile, but B. coli was recovered.

Experiment 5.

Remedy tested: Wood charcoal.
Cultures used: Broth cultures of the same organisms. The cultures were 24 hours old, and had been at 37° C. for that period.
Moistening used: Sterile water and sterile broth.
Temperature: 37° C.
Subcultures made from charcoal: Broth.

Result:—

In 1 *week.*—Each variety of organism recovered from its respective plate.

In 2 weeks.—Each variety of organism recovered from its respective plate.

In 3 weeks.—Vibrio choleræ was irrecoverable, but B. typhosus and B. coli were recovered.

Experiment 6.

Remedy tested: Wood charcoal.

Cultures used: Broth cultures of the same organisms. The cultures were 24 hours old, and had been at 37° C. for that period.

Moistening used: Sterile water.

Temperature: 37° C.

Subcultures made from charcoal: Broth.

Result:—

In 1 week.—Each variety of organism recovered from its respective plate.

In 2 weeks.—Each variety of organism recovered from its respective plate.

In 3 weeks.—Neither V. choleræ nor B. typhosus recovered, but the B. coli was recovered.

General Result of Charcoal Experiments.

Neither animal charcoal nor wood charcoal, when sterilised, appear to exert any very deleterious influence on V. choleræ, B. typhosus, or B. coli. The latter organism seems quite unaffected even after several weeks, but the V. choleræ and B. typhosus can be recovered easily after a fortnight. Wood charcoal is a less favourable medium than animal charcoal for the Vibrio choleræ, and also appears to be slightly less favourable for the B. typhosus. If the charcoal of either kind had any germicidal influence on these organisms, one would certainly expect to have seen this effect within a fortnight. The Petri dish, having its bottom freely covered by a layer of charcoal, contains a considerable quantity of the material, while the amount of broth culture added (10 c.c.) was relatively small. It is therefore remarkable that the organisms were easily recoverable for so long a period as a fortnight. The conclusion is inevitable that charcoal is not bactericidal to these organisms.

III. Naphtha Experiments.

Remedy tested: Pure medicinal naphtha.

Medium used: Plate preparations of 10 per cent. gelatine peptone, 10 c.c. in each. Inoculation in all cases very free, much more so than in ordinary plate preparations.

Organisms tested: V. choleræ, B. typhosus, and B. coli present in one-hour broth cultures.

Temperature of Incubation: 20° – 24° C.

Period of Incubation: One week for V. choleræ, a fortnight for B. typhosus, and one week for B. coli.

The results of experiments are tabulated, and control plates of the same medium inoculated similarly but unmedicated were made:—

Experiment Number.	Naphtha Medicated Plates.				Control.
	I.	II.	III.	IV.	
Amount of Naphtha added to each Plate.	·5 c.c.	·3 c.c.	·2 c.c.	·05 c.c.	
Vibrio choleræ,	Sterile.	A few colonies. Liquefaction.	Numerous colonies. Liquefaction.	Many colonies. Liquefaction beginning in 48 hrs.	Normal. Liquefaction in 48 hours.
B. typhosus, .	Sterile.	Sterile.	Sterile.	Sterile.	Normal.
B. Coli, . .	Only 2 colonies.	Many colonies. Irregular in shape.	Numerous colonies. ×105 show central mass and clear outer ring.	Normal.	Normal.

Result.—Naphtha is very fatal to B. typhosus, much less so to V. choleræ and B. coli, though it retards their growth, especially in the larger amounts used.

IV. Creosote Experiments.

Though this is not one of the remedies primarily recommended by Parkin, it is mentioned by him as one whose good qualities in cholera treatment possibly depend on its carbon constituent. A few experiments were therefore made with it to ascertain its effect on the growth in plate cultures of the Vibrio choleræ, the B. typhosus, and B. coli. It was found that creosote in the larger quantities did not mix well with the solid culture media such as agar and gelatine. It produced a cloudy appearance, so that the plates had to be searched with the microscope for colonies. The cloudiness clears up in the course of two or three weeks, and the plates become transparent, but still show here and there a few clear globules of creosote. The following table shows the result of the experiments:—

Experiment Number.	Creosote Medicated Plates.						Control Unmedicated Plates.					
	I.	II.	III.	IV.	V.	VI.	I.	II.	III.	IV.	V.	VI.
Temperature of Incubation.	37° C.	37° C.	20° C.	22° C.	22° C.	22° C.	37° C.	37° C.	20° C.	22° C.	22° C.	22° C.
Medium.	Agar.	Agar.	Jelly.	Jelly.	Jelly.	Jelly.	Agar.	Agar.	Jelly.	Jelly.	Jelly.	Jelly.
Amount of Creosote added.	·3 c.c.	·05 c.c.	·05 c.c.	·01 c.c.	·01 c.c.	·005 c.c.	..	..	..	..	..	..
Vibrio choleræ, .	Sterile.	Sterile.	Sterile.	Sterile.	Sterile.	Sterile.	Normal.	Normal	Normal	Normal.	Normal	Normal
Bacillus typhosus,	,,	,,	,,	..	,,	,,	,,	,,	,,	..	,,	,,
B. coli communis,	,,	,,	,,	..	,,	A few colonies	,,	,,	,,	..	,,	,,

Result.—Creosote, even in small proportions, is very inimical to the growth of all three organisms. B. coli alone is able to grow even in the plates containing least creosote. The contrast between the effects of naphtha and of creosote upon V. choleræ is notable.

V. Yeast Experiment.

Since yeast growing in media containing sugar causes evolution of carbonic acid gas, it was interesting to see if it would in such media affect the growth of the organisms under consideration. The yeast used was termed real German yeast, such as is used largely in the culinary art in the preparation of cakes. It was quite fresh, and when inoculated into a tube of glucose gelatine, produced large quantities of gas, which broke up the medium considerably. The yeast grew well in the medium, conferring upon it a cloudy haze, and did not cause any early liquefaction. In plate culture, using the same medium, the yeast proliferated freely, with the same appearance, except that there was not the same evidence of gas production.

Experiment.

Glucose gelatine plate cultures were inoculated with V. choleræ, B. typhosus, and B. coli respectively, and each also simultaneously inoculated with yeast. Control plates of the same organisms were made without yeast. All the plates were incubated at 20° C., the cholera plates for 5 days, and the others for 8 days. The organisms all grew in the medium notwithstanding the presence of yeast.

With the low power of the microscope (× 105) the plates were examined, and the yeast cells could be seen here and there invading the colonies of the other organisms; perhaps this was less evident in the plate of B. coli than in the others. The B. coli colonies appeared to be lighter in colour by transmitted light than in the control plate, and also exhibited a less definite margin, their edges being very minutely crenated. The B. typhosus colonies maintained their natural outline, and did not appear so much decolorised in comparison with their control plate. The V. choleræ liquefied the gelatine in 4 to 5 days, as compared with 3 days in the control plate.

The experiment was not repeated, as plate cultures are not gas-tight, and no effect of the carbonic acid gas, presumably given off by the yeast, was observed.

It might be advisable to repeat the experiment with the Vibrio choleræ, using instead of plate culture a closed Esmarch roll tube.

SUMMARY OF RESULTS OF RESEARCH.

As each experiment has had its general result described already, it is only necessary here to emphasise the comprehensive results of the whole.

I. Carbonic Acid Gas.

This gas was found to destroy, or arrest the growth of, the comma bacillus in a very decided manner.

The conditions favouring the arrest of growth appear to be:—

(*a*) Atmosphere of pure carbonic acid gas or a high percentage of the gas in air.

(*b*) Low temperature of incubation, 15°–25° C.
(*c*) Plate cultures as contrasted with cultures in tube.
(*d*) Prolonged exposure to the gas.
(*e*) Scanty inoculation.

The conditions in which least effect of carbonic acid gas is seen are:—
(*a*) Small percentage of CO_2 in air.
(*b*) High temperature of incubation, 37° C.
(*c*) Tube cultures as contrasted with plate cultures.
(*d*) Temporary exposure to the gas.
(*e*) Very free inoculation.

Where growth was not completely stopped, the most noticeable effects of the gas were the retardation of liquefaction of gelatine and the imperfect formation of pellicle in broth. Small percentages of carbonic acid gas, up to, say, 5–10 per cent., appear to have little or no influence on the vitality of the Vibrio choleræ.

On the B. typhosus carbonic acid gas has no marked toxic influence; occasionally the growth of the organism was somewhat retarded, but not very decidedly, and not more than might be due to the diminished supply of oxygen associated with growth in an atmosphere of carbonic acid gas. Often the organism grew in CO_2 as well as in air.

Carbonic acid gas does not interfere with the growth of B. coli communis nor that of B. diphtheriæ.

In connection with the latter organism, it is interesting to note that Schierbeck has found that a weak acid medium through which carbonic acid gas is passed constitutes the best medium for the production of diphtheria toxin.

II. Charcoal.

There is nothing to show that charcoal has any destructive influence on the cholera bacillus, nor on the B. typhosus or B. coli. It should be pointed out that the wood charcoal to which good effects are attributed in cholera was unsterilised, that with which the experiments were conducted was sterilised, and in the process any contained carbonic acid gas would be expelled. Since it is to this that Parkin mainly sets down its possible therapeutic value, his conclusion is not controverted by these experiments, though, as the quantity of gas in charcoal must be small, the results of the coincident experiments with the gas itself may be taken as powerfully negative to his view.

III. Naphtha.

Naphtha does not appear to possess so great a lethal power on the V. choleræ as to promise great good from its use in the disease. It is much more fatal to the typhoid bacillus.

IV. Creosote.

This, even in very small amounts, is very fatal to both cholera and typhoid, and, moreover, it even restrains markedly the growth of B. coli.

V. Yeast.

The Vibrio choleræ shows signs of restraint in the presence of yeast, but not to any great extent. The growth of B. typhosus and B. coli is not prevented or much altered by growth with yeast.

CONCLUSION.

There are few things more difficult than to arrive at a just appreciation of the value of a remedy in the treatment of disease.

On the one hand, it is so easy to believe that *post hoc* is *propter hoc*, and either to assign solely to the remedy the improvement which has followed, or to attribute its failure to coincident malign circumstances. On the other hand, it is no less easy, when the case has done well, to find more than one influence which may claim to share with the remedy in the production of improvement, or when the case has done badly to feel that the remedy was useless and that something else might have acted better. These difficulties confront one very formidably in the endeavour to estimate the value of carbonic acid gas and the various forms of carbon. It will therefore be best to consider separately each of the remedies and diseases with which this investigation has been concerned.

Carbonic Acid Gas.

Cholera.—In the first place cholera deserves our attention. From the experiments of Frankland and Fraenkel, and from the results detailed above of my own research into the subject, it is apparent that, so far as cholera is concerned, Dr Parkin's treatment with carbonic acid gas may claim to have a basis in the antagonistic influence which this gas under certain definite conditions exerts upon the Vibrio choleræ. While recognising to the full the very definite toxic effects which carbonic acid gas can exert upon the Vibrio choleræ under the conditions of laboratory experiment, one realises that clinically in treating cholera cases the conditions, which are requisite in order to obtain in the laboratory these toxic effects, are not exactly reproduced.

In particular, the temperature of the human body is that at which in the laboratory a very high concentration of carbonic acid gas is necessary in order to exhibit any marked bactericidal effect. It is necessary to ask the question whether carbonic acid gas given as described by Parkin can attain this high concentration in the human intestine, and whether it does reach and destroy the cholera organisms in this situation. Further, one must inquire how, if the curative action of carbonic acid gas in cholera be granted, is this result achieved?

In cholera cases the organisms are mainly to be found in the intestinal contents and in the rice water dejecta, which are often almost pure cultures of the organism. The comma bacilli are not usually found in the blood or in the other organs of the body, nor is it very common for the stomach contents to contain them. As a rule, the vomited matters are bilious and devoid of comma bacilli, though, if the vomiting be very severe, the rice water material and its pervading organisms may occasionally reach the stomach. It is evident, therefore, that carbonic acid gas administered into the stomach will not there meet with the organisms, nor, if it be absorbed from the stomach, will it have much chance of doing so. For the carbonic acid gas to come in contact with the organisms it must reach the intestine. We may conceive that, when given in frequently repeated doses, some portion of the gas will pass out of the stomach into the intestine, and that the gas will not be wholly absorbed from the stomach. The peristaltic movements so continuous in cholera may assist in transferring some volumes of the gas to the lower portions of the alimentary tract. Even so

the air in the intestines is hardly likely to contain carbonic acid gas in anything like so large a percentage as experiment shows is necessary to prevent growth of the cholera organism at the body temperature. It is not easy to conceive that the carbonic acid gas given off from 180 grains of bicarbonate of soda (six doses of 30 grains each with sufficient acid usually producing marked effects according to Parkin) would impregnate the intestinal atmosphere to a percentage of 60 or 70, and less than this is not very powerful in restraining growth of the cholera organism at the temperature of the body.

Some of the gas is lost during imbibition of the mixture, more is probably absorbed by the stomach, and only a proportion of the gas administered may be expected to reach the habitat of the vibrios. Certainly if a sufficient quantity reach the organisms it will tend to check their development, and to this may be attributed part of the alleged efficacy of the gas in the disease.

It is quite possible to admit that if in our cases of cholera we could maintain in the intestinal cavity an atmosphere containing not less than 50 per cent. of carbonic acid gas, the cholera vibrios would be placed in an unfavourable environment, would have some difficulty in surviving, and would probably be overcome by the patient instead of overcoming him. Whether this is possible with safety, and how it is to be achieved in the stress of acute cholera, are subjects pertaining more to the clinical than to the experimental aspect of the question. The experiments of Rosenbach and Oliven, founded upon Fraenkel's statement of the antagonism between carbonic acid gas and the cholera bacillus, are stated to have shown that it is possible to inflate the intestine with carbonic acid gas either from the rectum by means of a rectal tube, or from the stomach by means of an œsophageal tube. At the same time, relaxation of the pyloric orifice and of the ilio-cæcal valve is produced by the administration of opium either internally or by injection.

In addition frequent repetition of the gas injections are required in order to produce in the small intestine an atmosphere highly charged with carbonic acid gas. The procedure does not seem to me to be one which lends itself very easily to adoption as a therapeutic measure in acute cholera.

The broad fact that pure carbonic acid gas inhibits the development of cholera organisms supports, in a way which Parkin could never have foreseen, his reiterated epithet of 'antidotal.' When the conditions of the application of this fact are investigated, the support given to Parkin's contention is seen not to be so conclusive as at first sight might seem to be warranted. As I have shown by experiment, and contrary to Guyon's conclusions, carbonic acid gas is not nearly so powerful in destroying grown cultures as in preventing development of the organism in newly-inoculated media. From this one might suspect that clinically carbonic acid gas would exert a more favourable influence in the very early stages of the disease than when symptoms were fully established.

A further point for consideration may take the form of this query—Is there any other way, in addition to inhibition of growth of cholera vibrios, whereby carbonic acid gas may cure choleraic illness? Without being prepared to assert that there is such a way, one may speculate on the possibility of carbonic acid gas, absorbed by the stomach and reaching the blood, meeting there a toxin produced by the cholera bacillus and neutralising its effects. That the cholera bacillus does produce a toxin is generally believed, both on account of certain symptoms and clinical features of cholera, and on account of the laboratory experiments of Pfeiffer and others with recent

aerobic cultures. Here lies work for future experiment, to obtain the toxin in virulent phase, treat it with carbonic acid gas, and compare the effects of the pure and of the carbonised toxin. So far only the administration of the gas by the mouth has been referred to in this discussion of Parkin's treatment. Of the other methods, rectal injection of the gas in suitable cases may possibly be helpful, but gas passed in per rectum is hardly likely to reach high up the intestine, owing to the frequent diarrhœa and downward peristaltic movements. In addition, the difficulty of administration would, in acute cases, be considerable. Inhalation of the gas offers no particular advantages, nor would it be likely to be of much use in cholera unless the gas were found to be antagonistic to a toxin produced by the organism.

On the whole, then, one may conclude that carbonic acid gas may be useful in cholera, though I am not sanguine enough to expect that it will cure all cases of the disease, or even ameliorate them. The difficulties of achieving a high concentration of the gas in the intestinal lumen detract very considerably from the possibility of benefit. If one may judge from laboratory experiment, minor quantities of carbonic acid gas, such as might be more easily achieved in practice, have small effect on the growth and vitality of the organism.

The bactericidal action of carbonic acid gas on the cholera organism suggests a possible explanation of the very remarkable experiments of Hankin on a similar action of the waters of the Jumna and of the Ganges. He shows that these waters contain a bactericide which is inimical to the comma bacillus. This bactericide is volatile, and is injuriously affected by heat or the addition of alkali. No mention is made of observations as to the gases present in the water, but the characters noted of the bactericide, and of its action on cholera organisms, suggest that perhaps it is to the presence of carbonic acid gas that the water owes its destructive power over the comma bacillus.

Though not quite germane to the therapeutic qualities of carbonic acid gas in cholera, there is another consideration which merits suggestion in connection with the influence of carbonic acid gas on the Vibrio choleræ. The conditions of life of the cholera bacillus in soil have mainly been studied in relation to the moisture of the soil and to the height and variations in level of the ground water. Also the condition of the soil as to organic impurity has been mentioned in relation to cholera epidemics. But nowhere have I found reference to the amount of carbonic acid gas in the ground air as influencing the vitality of the organism. The impurity of the soil and the amount of carbonic acid gas are supposed to be correlated, but, apart from that, there is no special note as to the latter. Now the amount of carbonic acid gas in the ground air is often considerable, and largely exceeds that in the atmospheric air. Moreover, the temperature of the soil is not that of the human body, and the influence of carbonic acid gas on the cholera organism is much greater at 15° C. or lower temperatures than at 37° C. Carbonic acid gas in the ground air may reach 3 to 5 per cent., and possibly this is insufficient even at low temperatures to be powerfully bactericidal, but in future experiments on the viability of the cholera bacillus in soil, I think it would be well to ascertain the percentage of carbonic acid gas in the ground air, or to supply to the soil under experiment air containing the percentage of carbonic acid gas which is natural in the soil at the place from which the sample is taken for experiment.

Fever.—With regard to the large number of other diseases in which

Parkin recommends similar treatment with carbonic acid gas, one is not in a position to affirm that there is any solid ground for the treatment. As to most of these diseases, I have no research results by which to judge the question. In the case of typhoid fever, however, I can confidently state from my experiments, supported as they are by those of Sabrazes and Bazin, that there is no reason whatever to believe that carbonic acid gas has any deleterious or bactericidal influence on the Bacillus typhosus. Nor has it any toxic effect on Bacillus coli communis, such as might influence the course of those anomalous cases of fever which are sometimes attributed to the assumption by Bacillus coli communis of pathogenetic properties.

Other Diseases.—Diphtheria also is not a disease in which carbonic acid gas is likely to be of any use. The diphtheria bacillus appears to thrive uncommonly well even in large amounts of carbonic acid. Among other diseases in which carbonic acid gas has been recommended whooping-cough takes a foremost place. For this disease Bergeon first recommended its use, and Norton has more recently described excellent results which in his hands the remedy has produced. It is fair to say that others, having tried the gas, report that they have observed no good effects.

Rose advocates the use of the gas in the form of rectal injections, not only in whooping-cough, but also in cases of dysentery. In both these diseases and in some others he believes the gas to be of great service. With regard to dysentery, he thus confirms the observations of Parkin, who specially commends carbonic acid gas in the treatment of this complaint, whether it be in the acute or chronic stages. With regard to this and other diseases, I have no experimental evidence to offer, and therefore restrict my reference to a brief mention of the above opinions. It would serve no useful purpose to give in more detail isolated instances of the therapeutic use of carbonic acid gas, as *e.g.*, in vaginitis, in the vomiting of pregnancy, or in impotence, since in none of these diseases is bacteriological evidence from my laboratory experiments available.

Other Forms of Carbon.

Charcoal.—There is no evidence revealed in the course of my experiments which supports in any way the theory that charcoal has any deleterious or bactericidal influence either on the Vibrio choleræ, the Bacillus typhosus, or the Bacillus coli communis. On the contrary, I believe it to be incapable of destroying these organisms, and devoid of curative properties in the diseases associated with them.

Naphtha.—This drug is not specially inimical to the Vibrio choleræ, but, on the other hand, it is powerfully destructive to the typhoid bacillus. There is no reason to suppose that its use in cholera would materially affect the comma bacilli. It is therefore not surprising to find Widal stating that Bouchard had tried without success to disinfect the intestine in cholera cases with naphtha, although he prescribed it in doses which he had already found effectual for that purpose in cases of typhoid fever.

Creosote.—There is more possibility of this drug being useful in cholera, for even small percentages of creosote suffice to inhibit growth of the Vibrio choleræ. Nevertheless, it is not possible to say that creosote owes this quality to the amount of carbon in its composition.

To the Royal College of Physicians of Edinburgh my sincere thanks are due for the provision of laboratory accommodation and equipment, for the use of apparatus, and for the assistance of the Staff of the Laboratory.

To Dr Noël Paton, the Superintendent of the Laboratory, I am greatly indebted for much courteous advice and help during the progress of the research.

The drawings of apparatus, reproduced on pp. 23 and 25, have been skilfully executed by Mr Geo. Kay Green, whom I desire to thank for the manner in which he has delineated these from my description.

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PRINTED BY NEILL AND CO., LTD., EDINBURGH.