Pleomorphism in bacterial protoplasm : a study in psittacosis / by Andrew Todd McClintock.

Pleomorphism in Bacterial Protoplasm

A Study in Psittacosis

Andrew Todd McClintock

Andrew J. McClintock

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Pleomorphism in Bacterial Protoplasm

A Study in Psittacosis

by

ANDREW TODD McCLINTOCK, M. D.

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ANDREW TODD McCLINTOCK

Andrew Todd McClintock was born in Wilkes-Barre, Pennsylvania, in 1885, (January 21), and died thirty-eight years later, (August 4, 1923). To those who really knew him, it is clear that he was one of the "glorious band, the chosen few on whom the Spirit came."

There were three things, three epochs, in his life; first his home with its environment of literature, science, art, music and philosophy, with its splendid library, its family councils and communions, its unusually close-knit family ties, its travels into far countries actually to see what had been studied at home; second, the profession which was his choice, vocation, and avocation, from boyhood, to which he gave himself utterly, heart, mind, and body; and third, his last four years and the gradual throttling of his tremendous activities by one of the intestinal diseases which he had made his chief study during this period.

His father dominated the first period; his ceaseless work and large practice the second; in the third stands with him his wife, untiring comrade, laboratory assistant, and nurse, selfless in loyalty, speaking his language, thinking his thoughts.

His father was Andrew Hamilton McClintock, (1852–1919, M. A., Princeton) a successful lawyer with a large practice, chiefly corporate, and a member of many directorates. He was a man of exquisite appreciation of the efficient, the just, and the beautiful, whose avocations were books, literature, art, and science. He was the constant companion, tutor, and inspiration of his two sons, passing to them the results of his deep reading and broad travels, his appreciations and aspirations.

As a result Dr. McClintock approached manhood not only with an unusual store of general information and an unusual maturity of judgment, but with an eagerness for professional accomplishment, a desire to undergo every travail, and an ambition for service, which made him a marked man in whatsoever his group.

And as a further result there was a mutual regard between

him and his father which amounted to adoration. Shortly after the father's death Dr. McClintock confided to one of his note books these verses entitled "To My Father":

I am awaking.
I have hardly lived.
These many years in larval state
Of parasitic childhood
My soul was tissued
To the pulse of winged you.

While your wings were drooping,
Your shell becoming less
Straining at your earthly ties
Enduring in distress,
You wound me in a chrysalis
Where, since you left, I've slept.

But at last I'm stirring.
Stirred by memories
Of your selflessness and truth
Of your fortitude and generousness,
On your wings I'll seek the highest air,
Where you were wont to soar.

Genealogically, Dr. McClintock was of Scotch-Irish forebears and of the best this country has. His grandfather, Hon. Andrew Todd McClintock, (1810–1892 A. B. Kenyon College, Princeton LL. D.), like the Doctor's father a lawyer of large practice, the leader of his County Bar, a director of corporations, and a man of learning and culture, was the grandson of James McClintock of the town of Raphoe, Donegal County, Ireland, and the son of Samuel McClintock, (1776–1812), both of whom emigrated to Northumberland County, Pennsylvania. Here Samuel McClintock married Hannah Todd, the daughter of Colonel Andrew Todd, a Revolutionary soldier. Their son Andrew Todd McClintock (for whom, of course, Dr. McClintock was named) moved to Wilkes-Barre. In 1841 he married Augusta Cist, a daughter of Jacob Cist, of Wilkes-Barre. Andrew Hamilton McClintock was the only son of this marriage, the other children being daughters. Andrew Hamilton McClintock married Eleanor Welles, of Athens, Pennsylvania, from which marriage there were two children, Dr. McClintock and

Gilbert Stewart McClintock, who has succeeded to his father's position in the law.

Dr. McClintock prepared for college at the Hillman Academy, a Wilkes-Barre school, and entered Princeton College. Here he specialized in zoology and biology, so that he had little time for extra-curriculum work, although he was a member of the Princeton Charter Club, the Triange Club, (playing in its orchestra), and was a member of the Fencing Team. He graduated in 1907 with the degree of A.B. and entered the Medical School of the University of Pennsylvania where he received his degree in 1911, ranking near the head of his class. In the Medical School he was a member of Alpha Mu Pi Omega, the H. C. Wood Medical Society and Sigma Xi, membership in the last resulting from research work in collaboration with Dr. Warfield T. Longcope of the University; and won the Pancoast medal in anatomy.

After receiving his doctorate he became interne at the Wilkes-Barre General Hospital, and upon completing this service went to Vienna, Berlin and London (St. George's Hospital) for clinical and pathological research. His work abroad was with such men as Professors Ghon, von Stejskal, von Albrecht, Kozak and von Schmitt, and bore particularly on pathology, diagnosis and blood analysis. He was elected a life member of the A. M. A. of Vienna.

In 1914, his European study being completed, he returned to Wilkes-Barre and began practising.

His splendid preparation, his thorough and scientific methods gave him an uncanny diagnostic ability which brought him such immediate success and such a large practice, that, within two years, notwithstanding small fees, many charity patients, careless bookkeeping, complete ignorance of the value of money, total indifference whether he received any or none, he found himself (we might as well be candid and direct!) with a professional income of twelve thousand dollars a year—to his own great amazement.

More and more of his time was now taken by consultations and for cases referred to him by brother practitioners, who sought him in perplexities, when diagnoses seemed uncertain, impossible or incorrect. His record of success was impressive even to the lay public.

He had found his work. As he wished, there was all that he

could do. He trained laboratory assistants. He had become pathologist to the Wilkes-Barre General Hospital; consulting pathologist to the Pittston City Hospital and attending physician State Tuberculosis Dispensary No. I. In addition to the hospital laboratory he operated one of his own. The influenza epidemic arrived and he did the bacteriological work in the local defense, in addition to his already heavy daily schedule. His work was his recreation; he had no other. He was now the man ready and able, dependent only on physical endurance for future accomplishment of the first magnitude. The days and nights were too short. His life was solely one of intellect. His mind drove with an open throttle. Again to quote a notebook confidence, "I learn," he wrote, "and develop; ideas have larger correlation, are more stimulating; maybe some day—! ! !

But then instead of that "some day" of attainment, came the last four years. His father, and then he, fell ill with the influenza. In the father's case this was followed by asthma, and colitis from which, after a long illness, he died. Dr. McClintock, of course, worked incessantly on the bacteriology of his father's case. During this, he himself had a severe infection of the hand and arm contracted in the course of duty, while making a post mortem examination. This was followed by appendicitis which developed into what he diagnosed as a colitis requiring surgical correction. But medical authority felt uncertain of this diagnosis; he took vacations and travelled to restore his health. From then he practised only intermittently.

Eventually his diagnosis of his own case proved to be correct. But it was then too late.

And so he died.

Wilkes-Barre, AUGUST, 1925. DORRANCE REYNOLDS.

FOREWORD

The manuscript from which this book has been made was found among the papers and laboratory records of the late Andrew Todd McClintock.* It was dedicated to his father. Owing to prolonged illness, it had remained untouched by the author for several years before his death. This occurred in August 1923. Doctor McClintock had hoped for the strength and time necessary to review these laboratory protocols and to confirm and compare many of the data, particularly those relating to protoplasmic mutation. Progressive illness, however, prevented this. Even less than a year before his death, and although very ill at the time, with that splendid courage so characteristic of him, he still hoped that he would recover sufficiently critically to revise the manuscript. This was denied him. The editor asks, therefore, that it be clearly understood by those of his friends or others who may read the book that the author realized and insisted that the manuscript would require much rearrangement to render it suitable for printing. He wished to subject the entire theorem to the improved and standard methods of the bacteriology of today. It is certain, therefore, that the book would not have been printed as it is, had Doctor McClintock lived.

However, it is equally certain that all progress is primarily dependent for its initiative upon the unaccepted and often derided outgivings of imaginative minds. Other and much commoner minds bring to fruition what they originate, often gleaning the popular credit belonging elsewhere. So there are different types of men. All who knew him are agreed that Andrew Todd McClintock belonged to the altogether rare, too occasional, creative type. He pointed the way. On reading the manuscript, therefore, the editor was not surprised that some of the ideas, unaccepted and unproved at the time they were written, are today accredited. Thus, for every reason, the manuscript should be placed on record

*The index to which the author refers in the preface was not found.—Editor.

just as it stands. Undoubtedly it will hearten the abstract thinker and stimulate further research. It is therefore with a reverent spirit of agnosticism and yet with an unyielding belief in the ultimate value of the abstract portion of his friend's contribution, and in frank acknowledgment of Andrew Todd McClintock's energizing effect upon his own thoughts, that the editor has with little change spread the unfinished laboratory minutes and the deductions upon these pages.

A specific reference to the manuscript will help to elucidate the editor's position. Andrew Todd McClintock wrote at a time (1917) when the now universally accepted theory of focal infection was regarded very dubiously by the leaders of the medical profession, and justly so, because it had not yet gone through the acid test of clinical and laboratory proof. Andrew Todd McClintock, however, with that clarity of vision and impatience of delay which characterized his work, not only accepted this theory but promptly extended its application into his own chosen field of gastroenteric pathology. Thus he was one of the very first to recognize the all important rôle played by the intestine in relation to general bodily disease. "Foci," he says, "in the present-day understanding of what the term represents, would seem to have been experimentally established in the chronic cases of enteritis. The already described mucus-filled sacs, formed by single loops of bowel, certainly acted as foci upon the basis of which, months after a rabbit had been inoculated, acute processes of disease appeared to develop." This view is just gaining recognition, as the segmental and selective character of bowel infection is being proven.

If correct regarding his advanced views of the importance of enteric pathology, it is a reasonable assumption that some part of the author's hypotheses concerning protoplasmic mutation may be equally correct. The somewhat cryptic nature of certain statements throughout the text, and which have not been altered, may in time, therefore, become more clear. Moreover, in weighing the degree of accuracy of the author's hypothesis regarding pleomorphism, one would do well to remember that not only were his deductions regarding enteric focal infection correct, but also

those which he formed regarding his own pathological condition.

Nor can one in reading these pages refrain from commenting upon the innate modesty of the author's presentation of his theorem; another quality which always speaks for accurate balance and good judgment. Throughout the pages one notes the continued use of the conditional, and not infrequently there occur clauses which show how eager he was to make it clear, even in his unedited laboratory notes, that what he was advocating was not yet proven. "Very soon," he says, "*in the imagination* these facts were linked together." And again in the second paragraph of Chapter nine, "What took place between the events of inoculation and death had been thus far left in the dark." Though striving to prove his hypothesis of protoplasmic mutation, under human case number five he says, "In bouillon cultures of the blood the assumption by bacterial units of a rod shape was unusually early, though the *metamorphosis was never really complete.*" Also, in discussing human case fourteen, he says, "The metamorphosis, while observed, never became complete. It is necessary to take a very broad view of the metamorphosis. Time, material and energy continually vary their relationships, providing such a great range of possibilities as are seen." Nor was he controversial. In the fourth paragraph of the preface he says, "It is by no means the intention of the writer to contest the work of the men mentioned or to complicate matters further by setting up still another cause for psittacosis, but *merely to report the local findings.*"

His flexibility and imaginative resourcefulness are well illustrated in his discussion of the findings in his comparative studies of canaries, pigeons and rabbits. "These otherwise uninterpretable findings are perhaps," he says, "to be explained because of the long continued residence of the canary *in intimate contact with human beings.*" We are not so much concerned with the accuracy of the explanation as with its versatility.

But if, as postulated, ambiguities in the text exist, there are also striking examples of clear reasoning, illustrative of the author's capacity for sustained and intricate thought. In paragraph one of Chapter two, note the philosophical trend in his in-

teresting and accurate definition of "environment," which he describes as "the temporary partial control of destiny." His high degree of technical skill may be well judged from the text bearing upon case thirteen. Here is a perfect description of a really complete autopsy. It seems only proper to assume that the bacteriological studies were conducted with the same skill and care. Certain it is that he reached very pertinent conclusions regarding the danger to the host of incursions of bacteria from the bowel into tissues, "far from the gut tube." (Part two, Chapter four). Pertinent also in the light of recent literature were his comments upon pigmentation of the bowel, and particularly those relating to pyloric obstruction and hypertrophy. (Page 176).

A definite light upon the author's ultimate expectations regarding the applied side of his abstract studies is to be found in the records of researches devoted to prevention of lesions in inoculated animals. This important portion of his life work was unfortunately arrested by his gradually increasing illness.

Finally, as cited, this monograph is printed not alone as a contribution to modern medicine, for it may contain germs of many truths as yet unproven, but also to record an otherwise hidden chapter in the short life history of a medical idealist, who, with splendid and unfailing courage and a full knowledge of his own physical condition, struggled on toward the Truth. Perhaps as a catalyzer of the spirits of future investigators this history of effort on the part of one of their most gifted but most unfortunate members will find its greatest worth.

JOHN WILLIAM DRAPER, *Editor.*

PREFACE

This investigation was first undertaken in order to determine if possible whether or not the epidemic of peculiar cases of human disease which occurred about the middle of March, 1917, in Wilkes-Barre, Pa., was really the result of contact with the group of sick parrots on exhibition and sale in that town near the beginning of the month. This opinion was fairly prevalent at the time. The diagnosis of Psittacosis was suggested by Dr. J. P. Higgins of Wilkes-Barre, this idea was further developed by Dr. Stanley Freeman, and the diagnosis was accepted generally by the practitioners of the town.

It very soon became evident to the writer that the human disease was the result of that of the parrots. At the same time it was clear that while resembling the classical form of Psittacosis, it was by no means one and the same with it, although the two might possibly be taken as representing different degrees or phases of the same entity. These ideas were presented in a preliminary report read by the writer before the Luzerne County Medical Society in the latter part of May, 1917.

With the further unfolding of the picture herein presented, the importance of finding merely a link between the parrots and human beings diminished, as knowledge concerning the bacterial protoplasm providing this link developed. The peculiarities of this extremely primitive form of life, the character of the lesions producible by it, the variations in the disease entities of the epidemic to which it gave rise, the close linking up through it of saprophytic and parasitic phases, all presented aspects of such fundamental significance that the value of investigating it for its own sake was realized.*

The term Psittacosis, as used in the title of this monograph, must be understood in its broader meaning as signifying any bacterial disease transmitted from parrots to men. In this sense its appropriateness is incontestable, even though the clear and

*And strengthened by the recent development in England of super-ultra-microscopic methods.—EDITOR.

exact descriptions by Dieulafoy of the disease in France (presented in detail in the introduction) fit the facts of our epidemic but loosely, and the bacillus of Nocard (described upon the authority of McFarland) bears only a remote resemblance to the bacterial protoplasm of our observations. In thus speaking, it is by no means the intention of the writer to contest the work of the men mentioned, or to complicate matters further by setting up still another cause for Psittacosis, but merely to report the local findings. The etiological factor of the local epidemic, it will be maintained, is not a specific bacillus with a fixed heredity. It is not even necessarily in existence at the present moment, except as a possibility. The generalized bacterial protoplasm of the parrot's intestine is not itself the cause, it merely provides susceptible material. The real cause is the enormous amount of energy which is capable of being rapidly evolved by the living chemistry of this bacterial protoplasm, when activated by outside factors; and which, when aroused by tissue material, will destroy, digest and replace living animal tissues. Reproduction associated with this energetic material merely provides generalized bacterial protoplasm capable of less activity, or inactive, unless fixation has occurred, in which case for a longer or shorter time a certain quantity of such energy will be found to be associated with the protoplasm reproduced.

The arrangement of data in the book, especially as regards simplicity and clarity in presenting the numerous autopsies of experimentally induced disease, has been difficult. In the introduction, the reader, after being armed with the classical facts, is presented with the story of the initial steps traversed by the author in the search for the link between the illness of the parrots and that of the men. Part I presents the studies of the epidemic material; Part II, studies in parallel, on the material from the intestines of healthy parrots. Part III correlates these two separate investigations in a summary, in which, with the aid of some additional general experiments, a continuous story of the events which took place in the production of the epidemic is hypothetically developed. Part IV is an appendix in which the results of the investigations presented in Parts I–III are linked

up to those of other investigators. In the Summary and Conclusions the entire structure of the work has been exposed to view.

The table of contents presents a chronological analysis of the work; the index, the same alphabetically arranged. The text has been supplied with frequent summaries, distributed especially among the protocols. These protocols, or autopsies, have been grouped together, before or after the summaries, as occasion demands. The chronology of events, as demonstrated in the autopsies, is indicated by their position upon the page; the passage of time is indicated by a position from left to right. The more important facts are brought out in italics.

A glossary has been appended to explain the terms employed to which special meanings have been attached, as well as to define terms in common use in connection with the subject for the benefit of the lay reader.

The literature consulted has been for the most part noted in the front of the book. No report, however, of an investigation involving the study of variability and pleomorphism in bacterial life, would be complete without giving credit to Rosenow (now of Rochester, Minn.) for opening up the way in his remarkable researches, mainly upon cocci. After the whirl of forms brought to light by these studies had been observed by the writer, a report by Dr. Noguchi of a similar pleomorphism in a microorganism concerned with the decay of human teeth came to his notice. In July, 1917, while in the midst of the investigation of the development of the units in the *Typical Culture,* the remarkable work of F. Lohnis and N. R. Smith, of Washington, D. C. was also read and proved of great assistance in the difficult task of analysing the intricate processes in bacterial form development, especially in connection with the beautiful picture of the crystallization of amorphous masses into units.

In conclusion, the author wishes to record his indebtedness to his assistant and associate throughout this work, Miss Isabel Graham Cairns*, who prepared an immense amount of material,

*Now Mrs. Andrew Todd McClintock (Ed. Note).

registered independently a great many facts concerning the cultures and animals inoculated, and rendered invaluable aid in the preparation and care of the manuscript; to Miss Margaret Williams, for the large share she assumed in the latter task; to Mr. Michael Turpak, for his untiring devotion and efficient coöperation as laboratory assistant and technician, as well as to various others of the writer's immediate friends and associates who were of great assistance throughout the entire work.

Thanks are also due to Mr. Burnside, manager of the store in which the sick parrots were unwittingly displayed, for his courtesy in providing every facility for a survey of the premises in his charge, and in furnishing material for study.

Finally, the writer wishes to express his appreciation of the coöperation of Dr. Lewis H. Taylor, president of the board and one of the directors of the Wilkes-Barre City Hospital, without whose permission to utilize the laboratory facilities this investigation could hardly have been carried to completion.

ANDREW TODD MCCLINTOCK,

BEAR CREEK, PA., *September,* 1918.

TO MY FATHER

ANDREW HAMILTON McCLINTOCK

WITHOUT WHOSE MORAL SUPPORT, MENTAL SUGGESTION, AND ACTUAL PHYSICAL AID, THIS BOOK WOULD NEVER HAVE BEEN WRITTEN.

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The Epidemic Involving Parrots and Human Beings.

PART I

INTRODUCTION

On March 2, 1917, during a period of intense cold a large number of parrots arrived in Wilkes-Barre, Pennsylvania. They were on a circuit, and they had been long en route from the previous stopping place. They were exhibited for sale, grouped together in the open, in the basement of a large department store. Many of these parrots were ill when they arrived, appearing to be "frozen," according to store employees. They drooped, their wings were ruffled, mucus ran from their noses, and many had diarrhoea. A large number soon died. Great crowds came to see the parrots, forty-eight were sold and taken to homes, a number died, some recovered, some never appeared ill. The parrots were in the store forty-eight hours, and were then sent on. They showed some signs of illness in the town where they were exhibited previous to their arrival here. After leaving this city there were no further evidences of the epidemic. The acme must have occurred en route, and in the store.

In about ten days, the sick list of the store employees began to grow. At about the same time, many peculiar cases of illness appeared in the practices of physicians in Wilkes-Barre and the surrounding towns. The patients usually gave a clear history of contact with the sick parrots, either at the store or at home.

These cases were variously diagnosed as influenza, pneumonia, and very often as typhoid fever. But in all, an element of uncertainty was strong. Many of the symptoms of one or all of these diseases were present, but the grouping and degree of intensity of the different symptoms differed from those of any disease so far observed in this community. In a little while, the diagnosis of Psittacosis was suggested by a clinician. Although certain cases fitted exactly the accepted symptom grouping of this disease as a whole, the clinical pictures in this epidemic varied more than in those epidemics previously described, and the mortality was distinctly lower; not above five per cent.

In this epidemic the incubation period appeared to be about ten days. The duration of the disease varied from one to four weeks. Three groups of cases were easily differentiated. In all three, a terrific headache was a common symptom; this was associated with a most profound prostration. The pulse rate was not often above one hundred. The temperature curves were often irregular; most frequently they simulated those of typhoid fever. The leukocytes, when counted, were nearly normal in number, and all Widal tests taken were negative. The majority of cases resembled either influenza, with rhinitis, conjunctivitis and cough; pneumonia, with a high sustained fever, but a nearly normal pulse rate; or typhoid fever, both with and without a splenic tumor, and without rose spots. Some showed a combination of well marked symptoms, involving the entire respiratory and gastrointestinal tracts. In all the cases there appeared to be some involvement, however slight, of the upper respiratory tract, the lungs, the gastrointestinal tract. A peculiar odor, somewhat resembling that observed in typhoid, pervaded the surroundings of these ill human beings. The lung signs were very baffling, exhibiting scattered areas of dullness, often wandering, with moist râles, and frequently without any abnormality in the breath sounds. The lung signs rarely balanced one another, rarely were typically pneumonic.

Though the courtesy of Mr. Burnside, the manager of the store, a brief survey was made of the building. The parrots had been located in two places, a show window, and in the basement in the centre of a large area. A careful analysis was made of the clerks located near the parrots, including one who had charge of those birds which were ill. These individuals were compared in groups for the incidence of disease, with those at various distances, upon the same floor, and those upon different floors. Like the public, practically all the clerks made a point of seeing the parrots. There must have existed in that basement a zone of virus loaded air about the group of parrots. Some people touched the parrots, but in the vast majority of instances, the inspiration of the air of this zone was the common factor. As ever, under such circumstances, there must also have been a

personal factor. The young woman caring for the sick parrots did not become ill.

There were a number of human cases, in which the disease in its epidemic form must have been contracted from sick parrots, after the birds had been taken to the homes.

As far as could be determined, the disease was not, in its epidemic form, transmissible from human being to human being. This also was true of previous epidemics.

HISTORY OF SIMILAR EPIDEMICS

To quote from Dieulafoy's *Text-Book of Medicine,* pages 1853–1856:

> "The term psittacosis is applied to an infectious disease transmitted to man by parrots. The infection is due to a specific microbe—Nocard's bacillus."
>
> The disease "has become endemic" (*in France*) "since the Paris epidemic of 1892. The direct transmission from the parrot to man has often been recognized. A bird affected with psittacosis is listless and sleepy; the feathers stand on end and the wings droop. This condition lasts from a week to a fortnight, during which period diarrhoea and anorexia are present. The wasting is rapid, and the disease ends fatally in nearly every case. Contamination from the bird to man takes place in various ways. Thus, in order to make the sick bird eat, the evil habit of feeding from mouth to beak is employed—a dangerous method, which explains the frequent onset of the affliction, with transient oedema of the face and diphtheroid patches in the mouth or the pharynx, or else with angina. Other persons do not go so far as to gavage, but they fondle the sick creature, and warm it under their clothes—a practice that exposes them the more readily to contagion, as the feathers of the bird are soiled by the infected excreta.
>
> "Transmission may occur indirectly through objects soiled by the dejecta (cages and perches). The disease

very rarely spreads from man to man (Dujardin-Beaumetz, Peter, Nicolle).

" 'Nocard's microbe' was isolated in 1892 from the bone-marrow of the wings of parrakeets which died during the voyage from America to France, * * *

"*Clinical Picture.* The general character of the disease is that of a typhoid infection, rapidly complicated by pulmonary troubles. * * *

"The incubation seems to last from seven to twelve days. In a case published by Dubief, this period was found to be exactly nine days.

"The onset is insidious. The patient complains of malaise, anorexia, lassitude, and pains in the limbs, kidneys and trunk. The morbid process commences with peribuccal oedema, prostration, and headache, which may be accompanied by epistaxis, nausea, and vomiting. Rigors are constant, and the temperature may exceed 104° F., with a slight morning remission.

"After four or five days the stationary stage declares itself. The symptoms of the onset become more marked; the patient is prostrated, and lies in a state of muttering or of violent delirium, with restlessness and incoördinate movements. The tongue is coated, the thirst is acute, and the loss of appetite is absolute and lasts until the fever falls. Vomiting of food and of bile is frequent, the stomach is slightly distended, and diarrhoea or constipation may be present. The liver is normal, but the spleen is generally enlarged.

"Pulmonary troubles are early and of the highest importance. At the onset the cough is paroxysmal, and distresses the patient greatly. The dyspnoea is severe if the pulmonary lesions are extensive. According to the case, we may find on auscultation general bronchitis, lobar or lobular pneumonia, or pleural effusion. The urine is scanty, dark-colored, and often albuminous. Nervous troubles (headache and delirium) increase during the course of the disease, especially when thoracic complications appear; and we may

then note hallucinations, carphologia, and subsultus tendinum.

"The disease remains stationary for eight to ten days, and then in favorable cases the fever diminishes, the other symptoms improve, the patient passes out of his stupor, and enters on convalescence, although he remains feeble and anaemic for several weeks. * * *

"PROGNOSIS.—The mortality amounts to 30 per cent, and the prognosis is made worse by pulmonary complications. The age of the patient must also be taken into account. The disease is relatively benign in children, but is very deadly in old people."

McFarland, in the eighth edition 1915 *Pathogenic Bacteria and Protozoa* mentions Psittacosis as "an epidemic, infectious disease with pneumonic symptoms and a high mortality." He states that "its origin has been traced to diseased parrots, and from them, Nocard isolated Bacillus psittacosis, supposed to be the cause of the disease in man." This was substantiated by Gilbert and Fournier. The same author, in grouping bacilli of the typhoid-colon type, placed Bacillus psittacosis between the meat poisoning and the typhoidal groups, under the designation of the pneumonic, or psittacosis group.

NOCARD'S BACILLUS

According to Nocard's work, the organism is a short, stout, motile, flagellated rod, rounded at the ends. It forms no spores. It is aerobic, optionally anaerobic, non-chromogenic, aerogenic, pathogenic. It is not stained by Gram's method. It ferments dextrose, not lactose, milk is not coagulated, nor is gelatin liquified. The colonies, on agar-agar, resemble those of Bacillus typhosus. Bouillon becomes clouded. With regard to its pathogenesis, it is peculiar. It is extremely virulent for parrots, for white and gray mice, and pigeons. Ten drops of bouillon culture injected intravenously kills a rabbit in from twelve to eighteen hours. It has been found in the blood of human patients, ill

with a peculiar pneumonia. It is only slightly agglutinable with antityphoid serum.

Other workers have attributed the disease to cocci. Gilbert and Fournier found a closely allied bacillus in the feces of normal parrots.

Dieulafoy, whose clinical description was of great aid, records as a rare occurrence, the finding of Nocard's bacillus in the blood of human patients.

METHODS EMPLOYED

This investigation was entered upon without any fixed ideas as to just what was to be looked for, other than disease producing material, transmitted from the sick parrots to the sick human beings.

Blood specimens were taken by the syringe and citrate method, and grown in neutral, 2% glucose bouillon, in the proportion of equal parts of bouillon, and of both serum and whole blood. The plain bouillon used for subculture was always simple, neutral, beef-extract broth. The saccharose, lactose, and glucose broths were merely composed of 2% of the carbohydrates in beef-extract bouillon. The Russell's Double Sugar medium was made according to the standard formula. The nutrient gelatin, and litmus milk, were obtained from H. G. Mulford & Co., and Parke, Davis & Co.

Twenty per cent blood agar plates were used. Whenever mention was made of the use of a definite form of organism, characterization was established upon a basis of "pure" blood-agar colonies. Agar, of course, merely a solidified plain meat extract bouillon, is considered always as providing the same environment as the latter. Blood, frequently from sterile human cultures, merely intensifies the animal extract factor.

For mouse inoculation, by the peritoneal route, 1 c.c. of a bouillon culture was at first used; later .5 c.c. was employed, although 1 c.c. of the cultures from some individuals was insufficient to kill. Two c.c. were almost universally injected into the ear vein of rabbits. In later studies the bacterial organisms will be shown to vary enormously in richness of growth, according

to stage and virulence, hence any general standardization of the material employed was difficult.

Care was taken with regard to the cages of the animals used for inoculation. They were sterilized preliminary to use. Accidental inoculation was guarded against by the isolation, or segregation, of active cases of disease, and tests were made for this by close contact with controls, but it appeared to be a negligible factor.

MATERIAL

For this investigation, three parrots were studied. These had been purchased at the store, and had become ill. There were twelve bloods from eleven patients, giving a history of contact with parrots, nine feces from nine patients, three sputa from one patient, and one from another. Five cases were studied, for which no history of contact was obtained.

INTRODUCTION TO THE INVESTIGATION

The impression had been received, however correctly or incorrectly, that there existed uncertainties, and conflicting testimony, as to the bacterial factor in Psittacosis.

The first idea that occurred, upon hearing of the epidemic, was, that because of the long incubation period, there was a strong chance that the micro-organismal cause, if truly bacterial, would be a rod of the Bacillus coli type.

The first observation was made prior to the main investigation, upon the sputum of Case No. 1, which was obtained rather early in the course of a disease resembling pneumonia. A smear showed nothing but oval cocci in chains, which were not agglutinable by Cole's anti-pneumococcus sera I and II. One c. c. of an emulsion of the sputum was injected into a mouse. This caused death with pneumonia after thirty-six hours. Smears from the mouse showed various Gram negative rods and oval cocci. The finding of such rods under such circumstances was unusual.

The second observation was that the first three or four blood cultures all showed, within twelve to twenty-four hours, very curious rod forms. These were very delicate, often curved,

often nodulated, varying in length, grouped in clumps, and intensely Gram positive. Such a rod has been observed and described as one of the forms assumed by variable streptococci. Smears were made of these cultures, often twice daily, and it was observed that within twelve hours the tubes would show forms resembling staphylococci, often mingled with faded primary rods which showed tiny points considered, as probably, the centres of the former's origin. There were observed variations in the cocci, elongations, a pairing of oval forms, and some were arranged in chains. There were many forms shaped like pears, and clubs. Then there would appear in a smear, forms grouped, or fused, or drawn out, creating regular or irregular rods, some Gram positive and some Gram negative, varying in thickness, length and regularity. Sometimes the transition would be observed; sometimes, very suddenly, various types of rods would appear without transition forms. The change from the primary rod stage to the various coccal forms was not unknown to the author, but the variation from the coccal forms to the terminal rod forms was entirely new.

The third observation was made upon a parrot. The parrot was etherized while ill and a blood culture was taken. Death then occurred. There was no disease under the wing, such as had been described in previous epidemics. The brain was normal. The parrot showed congestion of the lungs, abscess formation of the surface of the liver and peritoneum. Cultures were taken from the bone marrow, from the heart blood, while living, from the lungs, feces, liver, and peritoneum. Smears from the various areas showed the primary rods, diplococcal forms, and large and small Gram positive rods. The blood cultures, injected into a mouse, caused death with pneumonia, but there was regained a very large rod, causing a coarse, saprophytic, furry growth upon agar, and showing unstained, central areas in smears. This was, at first, considered a contamination. It was, actually, as will be demonstrated, one of the forms the bacterial protoplasm assumed. Exactly the same blood culture was injected into a second mouse. Death was the result, with the same pathology, but a fine Gram negative rod was obtained.

The fourth observation was upon three specimens of human feces. Primary rods, in large collections, were found about the outer edges of stained smears. Cultures showed a pure growth of primary rods in twenty-four hours. In forty-eight hours there were no primary rods, but all forms were large rods, Gram positive, and very like those seen in the smears of specimens from the parrot. There were then injected into mice, the cultures from two feces, and the liquids from three different blood cultures. All died. Hemorrhagic spots were observed upon the lungs. The organisms regained were variable; diplococci, rods, Gram positive and negative, small and large, with or without central unstained spaces.

The fifth observation was upon a second parrot, which died a natural death in forty-eight hours. The pathology was slight, there was some congestion of the lungs, and an excess of peritoneal fluid. The brain was normal. There was no disease beneath the wings. Mucus from the nose showed large collections of delicate Gram positive rods, cocci, and primary rods. Cultures were taken as from the first parrot. Smears and cultures showed diplococci and rather large Gram positive rods. These cultures were plated out upon blood agar. There it was noticed that all the colonies were "pure," and composed of Gram negative rods, and those from the larynx and the nose, haemolized. Then a very beautiful green pigment was found to be spread out over the culture. At the same time the bouillon showed the same coloration. The other cultures also haemolized, but showed no green coloring. About the same time, cultures from the mouse first described, injected with the first sputum of Case No. 1, caused this same green coloration of the bouillon. The organism causing this coloration, in both cases, was a delicate, motile, Gram negative bacillus. The original culture from the sputum of Case No. 1 never showed this, although it did show various rod forms. So we see the first instance of a characteristic form in cultures from both parrot and man, obtained, as it was thought, directly from the parrot, indirectly from the man.

AGGLUTINATION TESTS

Agglutination tests were now tried with these bacilli, using the sera of different patients, in 1 :20 and 1 :40 dilutions. Positive results were obtained. It was naturally hoped that a diagnostic test could be worked out. Normal sera were then employed and those used gave even more brilliant results.

The sera of fifteen convalescents, taken while at work in the store, were obtained through the courtesy of the manager, and the agglutinating power of these was compared to that of seven sera from individuals in the store, who had not been ill, and to the thirteen sera provided by the epidemically ill patients studied by the author, and to those of persons encountered in his office practice. The sera were employed in dilutions of 1–10 up to 1–70. In a dilution of 1–20, it was found that many human sera, absolutely irrespective of previous contact with the epidemic disease, agglutinated the organism obtained from the parrot. Some sera showed no agglutinating power at all, some even operated in a dilution of 1–70. As the metamorphoses became complete, the resulting rods were found to bear this same relationship to normal human sera. Thus, very soon, agglutinability by normal human sera was studied as a character in comparing the forms in the states resulting from the completed metamorphoses. Of course, a negative result was checked by the use of a standard form, such as the typical bacillus from the parrot.

Interesting observations were made upon the type of bacterial organism susceptible to agglutination. Typically, the most agglutinable, was the small, highly motile, Gram negative type of rod, but rarely, much longer forms were observed to agglutinate, as well as were, also, some non-motile units.

Following the injection into a rabbit, of a culture of one of these rods, it was observed that subcultures from different areas of the rabbit might contain rods, some agglutinable, some not agglutinable. It can only be asserted that agglutinability by most human sera, in a dilution of say, 1–10, was typical for rod forms, exemplified in the Gram negative bacillus obtained from the parrot.

Nicolle found an agglutination of Nocard's bacillus by the sera of two human cases of psittacosis, one reaching a fiftieth, another a tenth dilution.

CHARACTERIZATION OF TYPICAL BACILLUS FOUND IN CASES OF BOTH PARROT AND HUMAN DISEASE

The specimen obtained from the second parrot was studied exhaustively.

The bacillus was a very delicate, slim, needle-like, Gram negative rod, of extraordinary motility. It grew upon all artificial media. In plain bouillon, it caused a delicate pellicle, intense clouding, and a most disgusting odor. In glucose bouillon, it caused an acid reaction. On blood agar the colonies were rather coarser than that of the colon bacillus, moist, and somewhat slimy. The degree of transparency varied. Runners, or extensions, were at times present, otherwise the margins were definite and regular. The blood was haemolized. Usually, it produced an exquisite green pigment, which involved the colonies and surrounding media. Upon Russell's double-sugar medium, the bacillus frequently seemed to remove all color from the material; then, while developing rather heavy whitish colonies, it colored the medium green. When chromogenesis was not present, various pictures were painted at different times, resembling those executed by different members of the typhoid-colon group. It was agglutinable with almost all the normal human sera employed, in degrees of concentration between a 1–10 and 1–70. It was not agglutinated by the few sera of rabbits and mice tested. It was not agglutinated by an anti-typhoid serum (with a titre of 1–1800) in a dilution above 1–40, nor by anti-paratyphoid sera A and B (with a titre of 1–2000) in dilutions above 1–70.

This form, within moderate limits of variation, already indicated, and later on, to be further studied, will hereafter be designated as the *"Typical" Gram negative bacillus* or rod.

Thus far, there had developed, as leads, these facts: There were common initial forms in human bloods. These appeared

to change. Could they be considered as the real cause of the human disease? Then, a green-producing organism, another kind of rod, was found in a parrot, and almost the same day an old human culture had turned green. Very soon, in the imagination, these facts were linked together. Changes more and more extraordinary occurred in the blood borne organisms, and rods were developing there.

After the realization of the presence of this bacillus, four blood cultures from the original tubes, a culture of the sputum of Case No. 1, one feces culture, and a culture from the marrow of the above described parrot, were injected into mice. All the mice died, showing hemorrhagic spots throughout the lungs. Cultures revealed rods, some Gram positive, and some Gram negative. On blood agar, they developed the moist colonies of the above described bacillus in varying degree of resemblance, causing green tints, also of different shades. Later on, these cultures showed the complete, or nearly complete, characteristics of the *typical bacillus*. At the time, the uniformity of results aroused suspicion. The possibility was considered of an impartation to the media of a bacillus by the mice, through their injury at the time of injection. Up to this time, most of the mice had shown dark, hemorrhagic spots upon the lungs, or pneumonic consolidation, but in many mice the viscera were black and slimy and gave off a most foul odor, resembling, of course, the plain bouillon cultures. This intense putrefactive capacity was not always present, but resulted most frequently from the activities of the more terminal rod-like forms.

For the last observation, therefore, three rabbits were selected. One was injected with a bouillon subculture from agar colonies of staphylococcal-like forms developed from the blood of Case No. 2, one with the culture of the green producing bacillus from the second parrot, one with the bouillon culture of the feces of Case No. 3. This last culture contained a large, Gram positive bacillus, also primary rods. The bacillus was only slightly motile, and not agglutinable with normal sera. These three rabbits became sick within three hours, and died within eighteen hours. The rabbit injected with the bacillus from Case No. 2 showed

markedly congested lungs, and scattered through them were large areas of hemorrhage. Sub-mucous hemorrhages existed also in the stomach and hemorrhages were found all through the appendix, and in some parts of the large intestine. The autopsy of the rabbit injected with the culture of the parrot's bacillus was practically a duplicate of the above. From all these areas in both animals, by smear and culture, was obtained a Gram negative bacillus with all the characteristics of the *typical bacillus*. The rabbit injected with the feces of Case No. 3, showed a few lung hemorrhages, but most of the visceral hemorrhages were lacking, while the putrefactive change was very marked. From the rabbit inoculated from Case No. 3 there was obtained the variable, large bacillus with which it had been injected. After a number of days this bacillus developed the characteristics of the *typical bacillus*. Clinically Case No. 3 exhibited mainly septicemia. Case No. 2 showed especially marked intestinal symptoms.

SUMMARY

Now, therefore, in studying sick parrots and sick human beings, between which two classes the definite associations previously cited had existed, the following bacterial forms were found. There were the primary rod forms, cocci, round and oval, in pairs or chains, cocci blended into rods, long, short, thick and thin rods, Gram positive, negative, mixed, or intermediarily stained, very large Gram positive rods, and very fine Gram negative rods. These forms seemed all interchangeable. They were often represented in the same area, growing in definite clear-cut colonies on agar, but individuals frequently assumed a new form when transferred to bouillon.

In parrots, in smears from the organs, although it was associated with different forms, the large Gram positive rod predominated. Cultures, on the other hand, almost uniformly within a short time showed the motile, slim, Gram negative rod. In human blood, the primary rod form first appeared; then in more or less definite order, with varying speed and according to the media employed, a round, or oval, or very irregular coccus devel-

oped. Then the various rod forms appeared as described, and, finally, in most cases, a slim, motile, Gram negative rod. This change in form, then, appeared to obey some law. The organism involved did not seem to be merely variable. In conjunction with the fact of the agglutinability of the fine Gram negative rod by human sera, it was for a time thought that the primary rod shape was a cloak worn for protection.

The imagination could hardly be expected to lay hold immediately upon such evanescent bacterial forms, as fundamental units. Behind the veil of mere form, another unit was sought. The various forms or stages of organisms, wherever obtained, each showed standard or universally recognized colony characteristics. Most specimens of all types exhibited easily recognizable degrees of pathogenicity. These degrees differed very greatly. In them there existed, apparently, no common link. But from both birds and men, specimens of bacterial forms in cultures which were not alike, following their inoculation into the circulation of rabbits, had caused similar disease reactions. The classical localization for the disease known as Psittacosis, is pulmonary. Here, in the lungs of the rabbits, the great hemorrhagic lesions lay. Were these not, also, comparable to the poorly defined, half obscured spots in the lungs of the mice? Did they not represent the pneumonic pathology, which underlies the peculiar clinical pictures of human pneumonia?

Chapter I

THE DESCRIPTION OF THE DISEASE PICTURES WHICH AROSE DURING THE EPIDEMIC IN THE PARROTS AND HUMAN BEINGS STUDIED AND OF THE FORM AND FUNCTIONS OF THE ASSOCIATED BACTERIAL PROTOPLASM

In the detailed descriptions to follow, the cases of parrots and human beings are grouped separately. Brief histories and clinical descriptions will be presented, in the instances where they were obtainable. In the cases of the three parrots, and of one human being, the post mortem findings are included. The bacteria will be considered under headings, designating the sources from which the materials containing them were obtained. The bacterial forms will be described as found directly in the materials, and in artificial or animal media. Then first, as exactly as possible, the changes relating to their unit morphology will be related as they were observed, in the tubes of bouillon, and on agar plates. Secondly, by the detailing of the autopsies of inoculated animals, the capacities for disease production and the changes in function relative to these will be indicated. The protocols are arranged, as well as possible, to bring out the various steps which it may have been necessary to institute, before a common end result was attained. In some of the cases there are shown a number of different courses in form and functional changes, as often observed in the case of bacterial units from but a single specimen.

In the descriptions of the bacterial unit forms, the terms in ordinary usage are employed with the following qualifications:

The first form, the fine, Gram positive rod, will be referred to as the *"primary rod."* The round forms are called cocci, if single, and diplococci, if double. If in chains they are designated as streptococci. When coccal colonies resemble those of

the staphylococcus the organisms will be so named. The various rods are described as they appeared and in order not to cause a misunderstanding no specific species have been named. Change in form is referred to as a "metamorphosis." Temporary states of uniformity, assumed by the bacterial units, will be referred to as "stages." The final stage has been described as the *"typical rod, or bacillus."* A culture will also be called *"typical."* For clarity, italics will be used for these terms, and they are intended to embrace the fact that the organism compares to that already described as a standard. But the formation of green pigment, motility, and agglutinability by normal sera previously tested and found to agglutinate the more typical strains, were characters not always present. Even when a culture was perfectly adjusted, in finest units, colonizing homogeneously upon a plate, there were individuals rounded, or larger than others, or even diphtheroid, and more or less Gram positive. Cultures, less well fitted, environmentally, might exaggerate any of these exceptions. All individuals might be rather large, less slim, less uniform, either in size, thickness, shape, or in all three characters. So might vary the degree of positiveness to Gram's stain. Or the units were divided, half positive, and half negative according to this differential test. Deviations from the standard will be indicated. When the final or terminal functional stage is shown to be reached, the bacterial protoplasm must be conceived of as having reached a state of more suitable adjustment. This adjustment is represented in the reactions of the cultures in animal tissues, by the production, in the host, of rapid death. This occurred at most within twenty-four hours, and was associated with hemorrhages not restricted to the gastrointestinal tract. Both this adjustment and the reaction will be referred to as "typical."

While performing these earlier experiments, some idea of the underlying truth had occurred to the writer, but time could not wait for correlations. Therefore, cultures were kept and restudied as new relationships were realized. The lapses of time will be shown. Time, as a factor in the functional readjustments, of course, was relatively of prime import.

The descriptions of the autopsies are simplified to bring out

the stages in the courses of functional change, or readjustment. Especially in mice, putrefaction was frequently so rapid, that the tissues of the thorax alone showed a differentiation. As time passed, this phenomenon was raised out of the category of accident, as unrelated to disease, and placed in the all-embracing realm of true characters. In the descriptions of the autopsies representing the *typical reactions,* the fact of hemorrhages, acute, and associated with death, is of principal interest. Their location points may be roughly grouped either as in the lungs and the gastrointestinal tract, or diffusely, even including the skeletal muscles. The essential tissues of the kidneys, liver, and heart, irrespective of hemorrhagic lesions, will be referred to as the *"parenchyma."* The condition of the spleen will be observed merely as to the presence and degree of "tumefaction." The variety of degeneration of the essential cells varies, but it is, primarily, cytoplasmolytic in the rapid deaths. In the exudative lesions, and the sub-acute and chronic cases, a more delicate process exists. The delicate variations in degeneration will not be indicated in the protocols of each animal, as a needless complexity would result. A separate chapter will be devoted to the finer pathological findings. They can be thus, far more easily correlated, and understood. (Illness prevented the completion of this part of the work. Editor.)

The registration of the rapidity of putrefaction was difficult to achieve with anything like precision. There lies no doubt in the writer's mind, that the onset of the process was variable, or that it was associated with the final stages of the adjustments of the bacterial protoplasm during the accretion of power. It was practically always more rapid, the smaller the animal employed. In the larger animals, the relation between the degree of lesion formation and the putrefactive reaction-intensity, was more clearly observed. They are now considered to be complementary.

PARROT NO. 1

PAST HISTORY:

Was obtained March 31, 1917. It was bought at the store and the disease under discussion occurred in the family to which it was sent.

CLINICAL HISTORY:

The bird appeared droopy and had diarrhoea. There was little molting. Blood cultures were taken during life, but death immediately subvened.

AUTOPSY:

The brain and skin showed nothing. There was congestion of the lungs and intestine, with *hemorrhages.* There were greenish plaques composed of a cellular exudate, spread over the liver.

HISTOLOGICAL EXAMINATION:

The parenchyma showed cytoplasmic destruction with clear nuclei, and the kidneys, glomerular congestion. The lungs appeared congested.

BACTERIOLOGICAL FINDINGS:

SMEARS:

Droppings: Large, Gram positive rods, rare cocci.
Chyme: Large, Gram positive rods, rare cocci.

CULTURES:

(Glucose Bouillon)
Larynx: Same as smear. Plate: Grey, staphyloid cocci.
Chyme: Same as smear. Plate: White haemolizing Gram positive rod.
Heart blood: Primary rods. 72 hours: Coarse rods, central areas unstained.
Later, on blood agar:
Lung: Grey, moist, non-haemolizing, colon-like, Gram positive and negative rods.
Chyme: Same.
Feces: Same.
Liver: Sterile.
Marrow: Sterile.

ANIMALS:

MICE:

No. I. 1 c.c. heart blood, 72 hr. Glucose bouillon culture.

DEATH:

36 hours.

AUTOPSY:

Peritonitis. Congestion of lungs. Parenchyma degenerated.

SMEARS:

Peritoneum: Coarse large Gram positive rods.
Spleen: Coarse large Gram positive rods.
Heart: Coarse large Gram positive rods.

CULTURES:

Bouillon, on blood agar. All, at first dry and furry forms, coarse, unstained central areas. Later, haemolized, became green and moist, typical Gram negative rods.

No. II. 1 c.c. heart blood 96 hour glucose bouillon culture.

DEATH:
18 hours.

AUTOPSY:
Putrefaction very rapid. Hemorrhages of lungs.

SMEARS:
Fluids: Large Gram positive rods.

RABBITS:

No. III. 2 c.c. lung culture, 24 hours bouillon, of a 24 day old culture, forms typical.

CLINICAL HISTORY:
24 hours, prostrated with intense rhinitis, and evidences of pneumonia. Recovered. 60 days interval.

DEATH: 61 days.

AUTOPSY:
Acute *rhinitis, pleuritis,* and *pericarditis.* Small hemorrhages of lungs, with hemorrhagic and fibrinous *pneumonia,* and compensatory emphysema.

SMEARS:
Lungs: Cocci, mixed rods, diphtheroids.

CULTURES:
Lungs: Rods, Gram positive and negative, varying in length.

No. IV. 4 c.c. 24 hour subculture in plain bouillon, of a 20 day old culture, forms typical.

DEATH: 24 hours.

AUTOPSY:
Hemorrhages of lungs, adrenals, and duodenum. Parenchyma degenerated.

CULTURES:
Hemorrhagic areas of,
Lung: Typical rods, some cocci.
Adrenal: Typical rods, some cocci.
Duodenum: Typical rods, some cocci.
Heart blood: Typical rods, some cocci.

No. V. 2 c.c. feces culture, 24 hour subculture of a 105 day old culture.

DEATH: 96 hours.

AUTOPSY: *Peritonitis:* Parenchyma degenerated.

SMEARS: Peritoneal fluid: Gram positive, rather large rods.

CULTURES: Peritoneal fluid: Gram negative rods.

No. VI. 2 c.c. feces culture, 96 hour subculture in plain bouillon of a 105 day old bouillon.

Heart: Primary rods.

DEATH: 12 hours.

AUTOPSY:

Hemorrhages of wall of thorax, stomach, duodenum, colon, mesenteric nodes. Pleuritis with bloody exudate, congested lungs.

SMEARS:

Pleura: Gram negative rods.
Lungs: Gram negative rods.
Intestine: Gram positive rods.
Node: Gram positive rods.

No. VII. Two c.c. marrow, 24 hour subculture, in plain bouillon, of a 105 day old bouillon, coccal forms.

DEATH: 6 days.

AUTOPSY: *Acute gastroenteritis* with hemorrhages.

SMEARS:

Kidney: Gram negative rods.
Intestinal blood: Gram positive rods.
Chyme: Gram positive rods.

CULTURES:

Bile: Cocci and Gram negative rods.
Urine: Cocci and Gram negative rods.

SUMMARY:

The bacteriology of this parrot is especially interesting because of the relative slowness of the metamorphosis. Various stages stand out. Especially important is the transition in the heart blood, from the primary rod to the large rod with unstained central spaces.

In mice, in one case, on blood agar, a dry, furry, coarse growth was seen to change to a haemolizing, moist, smooth green color.

The first rabbit experiment provided, primarily, only clinical results, with a picture of pneumonia and recovery. This was followed by a relapse two months later, with death, and typical pneumonia. Re-inoculation of a subculture into a second rabbit resulted in a *typical reaction,* by a fine, Gram negative bacillus.

Rabbits Nos. II, III, and IV, were injected with subcultures of old, original cultures. No. II showed the primary rod in the heart blood. No. III exhibited a remarkable persistency in function on the part of the Gram negative bacillus, in plain bouillon, i. e. a *typical reaction.* No. IV showed a metamorphosis taking place beginning with the coccal stage, in which up to this time, the units had remained unchanged as cocci for one hundred and five days, undoubtedly because they had remained in glucose bouillon. Metamorphosis certainly did take place in the presence of glucose but this substance tended to prolong the intermediate, or coccal, stages. It is questionable if the full course would have been understood, if ordinary plain bouillon had not been employed for subcultures.

PARROT NO. 2

CLINICAL HISTORY:

Was obtained April 1st, directly from onset of illness. Bird was quiet and droopy, ate little, but did not appear very ill. It died naturally in 48 hours.

AUTOPSY:

There was no disease of the skin; the brain was normal. There was much mucus in the nose. An excess of peritoneal fluid was present, as well as some congestion of the lungs. There was congestion of the intestinal tract.

HISTOLOGICAL EXAMINATION:

The parenchyma showed cytoplasmic destruction with clear nuclei, and the kidneys glomerular congestion.

BACTERIOLOGICAL FINDINGS:

SMEARS:

Mucus of nose: Masses of Gram positive rods, a bunch of fine, Gram negative rods. Many cells, amœbae, and cocci.

Feces: Large, Gram positive rods, pneumococci and primary rods.

Larynx: Same.

Lung: Same.

Peritoneum: Same.

CULTURES:

Heart blood: Cocci to plump rods, grey on plate.

Marrow: Grey staphylococci on plate.

Bronchus: Fine, Gram negative rods, green, haemolizing on blood agar.

Larynx: Fine, Gram negative rods, green, haemolizing on blood agar.

Feces: Plump, Gram negative rods. No haemolysis.

ANIMALS:

MICE:

No. VIII. 1 c.c. heart blood culture.

DEATH: 48 hours.

AUTOPSY: *Pneumonia.*

SMEARS: Gram positive and negative rods and cocci. Later, Gram negative rods.

ORIGIN OF TYPICAL CULTURE NO. 1522

No. IX. One c.c. marrow, 96 hour culture.

DEATH: 48 hours.

AUTOPSY: *Lungs congested.* Organs pale. Parenchyma degenerated. Splenic tumor.

SMEARS: Cocci to plump rods.

PLATE: In six days: Grey, furry, dry colonies. In seventeen days: Gram negative rods with moist, greenish, *typical colonies.*

RABBITS:

No. X. Two c.c. bronchus subculture, in plain bouillon, from plate colony, after eleven days.

DEATH: Eighteen hours.

AUTOPSY: Putrefaction rapid. Rhinitis. Hemorrhages of the lungs, stomach, appendix, and colon.

SMEARS: Typical Gram negative rods.

CULTURES: Typical Gram negative rods.

NOTE: .05 c.c. of this culture killed mice in from eight to eighteen hours.

ORGANS: Slimy. Lungs very dark and congested.

SMEARS AND CULTURES: Typical bacilli.

Smears from this parrot showed the standard stages in the usual course of the metamorphosis.

Cultures from the heart blood indicated a course somewhat resembling that in human beings, but it was more rapid.

The marrow forms, as usual, were primarily coccal, the change to rods took place upon a plate, the change from a coarse Gram positive growing in dry, furry, green colonies, to smooth, green, typhoid-colon type of colony, composed of fine, slim, Gram negative rods. The respiratory tract provided *typical cultures,* with *typical powers* from the beginning.

PARROT NO. 3

PAST HISTORY:

This parrot was presented by the store and secured from a house where the disease had followed its entrance.

CLINICAL HISTORY:

It did not appear very ill, but was irritable, quiet, dirty, and had some diarrhœa. After twenty-four hours it died, although the night was warm.

AUTOPSY:

The skin was normal. The brain was normal. There was much mucus in the nose. The lungs were congested. The liver was dark with clots, and pus was upon the surface. The intestine was inflamed. The peritoneal cavity contained an exudate.

HISTOLOGICAL EXAMINATION:

The parenchyma showed cytoplasmic destruction with clear nuclei, and the kidneys, glomerular congestion. The lungs were only congested.

BACTERIOLOGICAL FINDINGS:

SMEARS:

Mucus: Primary rods, Gram positive and negative rods, cocci and mononuclear cells.

Feces: Mixed forms, cocci and various rods.

Peritoneal exudate: Mononuclear cells, irregular cocci, fairly slim, Gram positive and negative rods, also primary rods.

CULTURE:

Nose:	All	On plate,	12 days, plate and
Bronchus:		colon-like,	bouillon, (nose),
Heart:	rod	slimy,	green and entirely
Liver:		haemolizing,	characteristic of ty-
Feces:	forms	not green.	phoid bacillus.

ANIMALS:

RABBITS:

No. XI. Two c.c. bronchus, 48 hour culture, in plain bouillon, from eleven day old plate streak.

CLINICAL HISTORY:

Profoundly ill, rhinitis, wheezing, progressively weaker.

DEATH: 6 hours.

RABBIT: No. XII:

AUTOPSY: Brain congested. *Rhinitis* and *laryngitis.* Lungs showed hemorrhages and brown colored, *pneumonic* lower lobes, with fibrinous exudate. *Appendicitis,* and *nephritis.* Parenchyma degenerated. Splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Mucus: Primary rods, cocci, and Gram negative rods.

CULTURES:

Blood: Immediately, cocci, and Gram positive and negative rods. Later, *typical, slim, Gram negative bacilli.*

This parrot showed the most definite, gross pathology. Smears showed the usual stages, or mixed bacterial forms. The cultures were all staged parallel, a phenomenon probably associated with the more marked pathology present, a condition imitated in many acute reactions in rabbits. The respiratory specimens turned the media green. A culture, in form a typical rod and

of very foul odor, nevertheless had a very high pathogenic power in rabbit tissues. This resembled case No. 12, only, probably, here elements of lower grade caused a much shorter course. Here the functions were comparable, but the forms of these two specimens of the human and parrot bacterial units were quite divergent.

About this time, in a nearby town, occurred an epidemic of diarrhoea. A stool from only one human being, the last one of the four mentioned, was obtained. This was cultured in plain bouillon and caused a green growth. Following injection into a rabbit, death occurred in twelve hours, with hemorrhages. This culture was carefully compared to No. 1522 and No. 24, and found to be identical in character, especially as to its variability and latency. The possibility of such a connection between the epidemic and post epidemic diseases, whether direct or indirect, of course, suggested itself. It was realized, however, that the organism might be either a primary or secondary cause, or that even the evidence of a disease producing power, following inoculation, might be merely the result of an inflammatory reaction in its surroundings in the host, arising from another cause. In just this way the latent powers of normal fecal flora in parrots, were found to be aroused by plain bouillon.

So much for the findings of August and September.

SUMMARY OF THE THREE PARROT CASES

These, presumably, did not represent the severe type of the avian epidemic disease. They were studied after the crisis. Disease of the gastrointestinal tract constituted the predominant gross pathology. Histologically, as has been indicated, there were no practical points of difference between the individuals. In general, the bacterial forms found in smears were more mixed than was usually the case in human material. The metamorphoses and functional adjustments were much more rapid. The quick transitions which took place in two instances in plate colonies were not observed in the case of specimens from human beings. In the beginning of the study, the metamorphosis

on the part of the bacteria obtained from parrots was overlooked. The fine Gram negative rod was at that time considered in the light of a fixed infective bacterial agent.

CASE NO. I

PAST HISTORY:

This patient had attended cases of "parrot" disease and had played with a parrot which had been bought at the store.

CLINICAL HISTORY:

He began to feel ill about a week after the main epidemic. He slowly developed malaise and a severe headache. He was admitted to the hospital 3/21/1917. There he developed indefinite pneumonia, with moist râles, areas of dullness and some bronchial breathing, without a definite correlation of signs. For thirteen days he carried a sustained fever of from 101° to 103°. The pulse rate averaged about 80. The respiration rate rose as high as 34. The blood pressure gradually sank to 90 mm. Hg. Lysis occurred. All signs were normal on the seventeenth day.

Specimens for study were taken frequently during the acute course of the disease.

FECES: SMEAR: Nothing peculiar.

ANIMALS:

RAT: 2 c.c. of a 48 hour culture. No results.

First blood taken 3/31/17. Acme. In glucose bouillon.

METAMORPHOSIS:

24 hours. All tubes showed primary rods.

48 hours.	Tube No. 1.	No. 2.	No. 3.
	Cocci.	Primary rods.	Course regular rods, unstained central areas.
72 hours.		Cocci.	
96 hours.	Staphylococci.		No. 4. Primary rods.
108 hours.		Cocci fused to coarse irregular rods.	

23 days.

Bouillon culture (18 days) of staphylococcal plate colonies became green, and contained, besides some cocci, great numbers of regular slim, Gram negative rods.

The data upon the blood culture tubes exhibited the great variations demonstrated in different tubes in the initial forms, and in the time required for metamorphosis. The latter was very slow but complete.

ANIMALS:

MICE:

No. 1502. One c.c. of a 72 hour culture showing large, Gram positive rods.

DEATH: In 5 days.

AUTOPSY: Viscera distended and slimy. Hemorrhages of the lungs. Parenchyma degenerated.

SMEARS: Primary rods.

No. 1511. One c.c. of a 48 hour reculture of a 5 day old culture showing primary rods.

DEATH: Some days. (Records uncertain).

AUTOPSY: Organs pale. *Peritonitis.* Parenchyma degenerated.

SMEARS: Cocci to Gram negative rods. Primary rods.

CULTURES:

Heart: Same.

As will be shown, specimens from this case of human disease provided wide sweeps in the metamorphic picture, and are thus of great interest. The first mouse exhibited in smears an interesting retrogression to primary rod forms, although the degree of putrefaction was abnormal. The second, on the contrary, linked the primary rods to much later forms, displaying the whole course in smears. They demonstrated the lack of a definite relationship between form and function.

RABBITS:

No. 1543. Small amount of a 15 day old culture showing primary rods. (Amount unknown because of leakage.)

DEATH: Three months.

AUTOPSY: *Gastroenteritis.* Congestion of the lungs. Nephritis. Sclerosis of the aorta and valves of the heart, with decomposition. Parenchyma degenerated.

SMEARS:

Spleen: Gram negative rods.

Heart: Irregular cocci, to Gram negative rods.

CULTURES:

Spleen: Same.

Heart: Same.

No. 1785. Two c.c. of a 24 hour subculture of a 10 day old culture of heart of No. 1543.

DEATH: 24 hours.

AUTOPSY: Many hemorrhages of appendix, colon, and stomach, and a few of the lungs. Splenic tumor.

SMEARS: Large Gram positive rods.

CULTURES: Quite *Typical Gram negative rods.*

In these two rabbits is shown the whole course. The first one, a chronic carrier, auto-infected later, showed bacteria in terminal rod stages, in its mortal reaction. These forms, in culture, reacted typically in the second rabbit.

No. 1578. Two c.c. subculture of 27 day plate culture of staphylococci which had changed, in form, to Gram negative bacilli, developing green pigment.

DEATH: Six days.

AUTOPSY: *Pneumonia, pleurisy* with fibrin. *Gastritis,* and *cholecystitis.* Parenchyma degenerated. Huge spleen.

SMEARS: Various organs. Great mixture of forms.

CULTURES: *Lung:* After 72 hours, a *Typical Gram negative rod.*

No. 1613. Two c.c. re-bouillon of a 6 day lung culture of No. 1578.

DEATH: In 12 hours.

AUTOPSY: Hemorrhages of lungs and endocardium.

SMEARS: Gram negative bacilli.

CULTURES: *Typical Gram negative rod.*

Begun with cultures in the terminal stage, the functional result is of great interest. In the first case, we have a green producing, quite *typical* rod causing a finely cut disease picture, as did an organism from parrot No. 1. But smears of the lung showed the forms to be mixed. Cultures in animals gave the reaction typical of a complete adjustment.

Second blood taken 4/3/17. In glucose bouillon.

METAMORPHOSIS:

	Tube No. 1.	Tube No. 2
24 hours:	Primary rods.	
48 hours:	Primary rods and cocci.	Cocci, irregular and elongated.

MICE:

No. 1517. One c.c. of a 72 hour white staphylococcic culture.

DEATH: 48 hours.

AUTOPSY: Organs slimy. Congestion of lungs with hemorrhages.

CULTURES:

Peritoneum: Primary rods and large Gram positive rods.

RABBITS:

No. 1561. Two c.c. of a subculture of a staphylococcus from a plate, fourteen days.

DEATH: 19 days.

AUTOPSY: *Peritonitis. Nephritis.*

SMEARS: Cocci to rods.

CULTURES:

Heart: (48 hours) *Fairly Typical Gram negative rods.*

Again there is seen in the mouse a retrogression in the metamorphosis. In the rabbit alone, the main stages are all included.

FIRST SPUTUM:

SMEAR AND CULTURE: All chained cocci. The culture later showed various rod forms.

No. 1501. One c.c. of an emulsion of the sputum.

DEATH: 36 hours.

AUTOPSY: Parenchyma degenerated. Lungs red and *pneumonic.* Abscessed abdominal wall.

SMEARS: *Lungs:* Rare, narrow rods.

CULTURES: Cocci to rods.

SUBCULTURES: On plates, later, *Typical Gram negative rods.*

No. 1563. Two c.c. 17 day old subculture of No. 1501.

DEATH: 18 hours.

AUTOPSY: Beginning pneumonia with hemorrhages of endocardium, intestine and kidneys. Parenchymatous degeneration.

SMEARS: Cocci to rods and primary rods.

CULTURES: Slow development of coccal forms to slim Gram negative, fairly *typical rods.* After 8 days: Colonies: green, slimy, haemolysis. Paratyphoid type.

RABBITS:

No. 1501B. Two c.c. 24 hour glucose bouillon, showing all chained cocci.

DEATH: 36 days,

AUTOPSY: *Pleuropericarditis. Enteritis and peritonitis.* Parenchyma degenerated.

SMEAR:

Bronchi: Diphtheroids.

Heart: Cocci to rods.

CULTURES: *Typical Gram negative rods.*

The mouse No. 1501 injected with the emulsion was the first experiment of the investigation. The mixed cultures in time showed the green pigment-producing bacillus.

The rabbit No. 1501B received a bouillon culture of the same sputum, and represented the wide step in metamorphosis from chained cocci to the *Typical Rod form,* associated with a subacute disease.

SECOND SPUTUM:

Glary, whitish.
SMEAR: Diplococci and irregular rods.
CULTURE: Gram negative rods.

ANIMALS:

MICE:

No. 1523. 1 c.c. re-bouillon of *typical rod* from a plate culture.
DEATH: 72 hours.
AUTOPSY: Organs slimy. Congestion of lungs. Peritonitis. Parenchyma degenerated.
CULTURES: *Very typical Gram negative rods.*

THIRD SPUTUM:

Bloody.
SMEAR: Negative.
BLOOD PLATE: Cocci to Gram negative rods. No further study.

CASE NO. 2.

PAST HISTORY:

Patient was a salesman, 25 years of age.

CLINICAL HISTORY:

About 3/10/17 patient suffered general malaise and headache. Three days later there was epistaxis. Five days from commencement of symptoms patient was unable to leave his bed. When first seen he did not cough and had no pain, only great lassitude. No chill had taken place. Patient was constipated.

Pulse 100.
Temperature 104 degrees for 6 days.
Temperature 102 degrees for 7 days more.
Temperature lysis 5 days later.
Temperature normal in 4 weeks.

During the third week there was dullness and moist râles in the lungs and some abnormalities of breathing at the base of the right lung. Otherwise the course of the disease closely resembled typhoid fever.

LABORATORY FINDINGS:

"Pathological Laboratory, Department of Health, State of Pennsylvania" reported a negative Widal at the end of the second week. Reported members of the typhoid group absent from a specimen of feces taken about the termination of lysis. At the same time in the hands of the author the serum failed to agglutinate Bacillus typhosus in the dilution of even 1:10. The leukocyte count at the end of the first week was 9,600.

Blood taken 3/30/17. In glucose bouillon.

METAMORPHOSIS:

24 hours: Sediment of centrifuged fluid from all tubes, rare short plump rods.

48 hours: Same. Some primary rods found.

72 hours: Tube No. 1, negative. No 2, negative.

96 hours: Primary rods. Subculture on plate, streptococcal colonies. Forms: Cocci.

120 hours: Largely short thick rods.

ANIMALS:

MICE:

No. 1527. 4/19/1917. .5 c.c. subculture of a re-bouillon culture from a staphylococcus colony on a plate.

DEATH:

18 hours.

AUTOPSY:

Organs not very dark. Reddish spots of hemorrhages on the lungs. Splenic tumor.

SMEARS:

Peritoneum: Cocci to Gram negative rods.

CULTURES:

Typical Gram negative rods.

RABBIT:

No. 1527. 4/19/1917. 2 c.c. reculture of subculture No. 1527.

DEATH:

18 hours, dying. Etherized.

AUTOPSY:

Dark area in left lung. Hemorrhages of appendix and large bowel. Parenchyma degenerated. Splenic tumor.

SMEARS:

Lung: Thick Gram positive-negative rods.

Hemorrhages: Various rods.

Spleen: Negative.

Blood: Gram negative rods.

CULTURES: *Heart blood: Typical Gram negative bacillus.*

RABBIT:

No. 1533. 4/11/1917. 3 c.c. same culture as in No. 1527.

DEATH:

18 hours.

AUTOPSY:

Laryngitis. Huge hemorrhages of lungs, also appendix and stomach. Parenchyma degenerated. Splenic tumor.

SMEARS:

Lungs: Gram negative cocci.

CULTURES:

All areas, *Typical Gram negative rods.*

RABBIT:

No. 1701. 6/12/1917. 2 c.c. re-bouillon of subculture of No. 1533.

DEATH:

24 hours.

AUTOPSY:

Rhinitis: Congestion of lungs.

Hemorrhages of duodenum and small bowel. Congestion and rare hemorrhages in the appendix. Splenic tumor.

SMEARS:

Practically all *Typical Gram negative rods.*

CULTURES:

Practically all *Typical Gram negative rods.*

FECES: Nothing typical, not injected.

In the blood cultures, the metamorphosis was slow. In a mouse injected with a culture of a pure staphylococcus, there resulted a rapid reaction and a considerable change in form. The cultures were typical. The reaction in the rabbit, caused with bouillon cultures of the patient's blood. was absolutely typical. Two months later, a subculture showed no changes in animal reaction power or bacterial form.

The mouse No. 1552 was injected with a culture plus human serum. Although living 48 hours, the reaction was attended with great putridity and the bacterial forms were *typical.*

CASE NO. 3

PAST HISTORY: Patient was one of the owners of the store.

CLINICAL HISTORY:

Following the usual prodromal symptoms, there was a long course which resembled a septicemia. Several transfusions of blood were necessary. A corneal abscess developed. The man's life was held by a thread for days.

Blood taken 4/8/17. In glucose bouillon.

METAMORPHOSIS:

24 hours: Slim rods, no primary rods or cocci.

48 hours: Large, Gram negative rods.
72 hours: Various types of rods.
9 days: Fine and coarse Gram negative rods, motile and agglutinable.

ANIMALS:

RABBITS:

No. 1564. 4 c.c. bouillon subculture from a plate colony of a large Gram positive bacillus.

DEATH: 60 days.

AUTOPSY:

Gastroenteritis: Cardiac degeneration and sclerosis. Parenchyma degenerated.

No. 1541. 2 c.c. of an original blood serum tube. Colonies "hairy" on a plate.

DEATH: About one month.

FECES: Liquid:

SMEARS: Various Gram positive rods.

CULTURE: Same, plus primary rods.

MICE:

No. 1525. 4/9/17. .5 c.c. plain bouillon culture.

DEATH: 18 hours.

AUTOPSY:

Organs moderately dark. Viscera distended. Lungs spotted with hemorrhages. Parenchyma degenerated.

SMEARS:

Peritoneum: Gram negative rods and staphylococci.

No. 1534. 3 c.c. plain bouillon culture.

DEATH: 18 hours.

AUTOPSY:

Putrefaction rapid. Few hemorrhages of appendix. Parenchyma degenerated.

SMEARS:

Various rods.

CULTURES:

Developed *Typical Gram negative bacilli* in green cultures.

RABBIT:

No. 1702. 6/13/17. 2 c.c. re-bouillon of 2 month old subculture of No. 1534.

DEATH:

12 hours.

AUTOPSY:

Rhinitis. Few hemorrhages of the lungs with areas of congestion. Hemorrhages intense in the duodenum, jejunum and appendix. Splenic tumor. Feces negative.

SMEARS:

Rich Gram negative rods.

CULTURES:

Typical Gram negative rods.

This case is instructive because of the possible connection between the intensity of the disease and the development, in bouillon cultures of the blood, of slim rods in twenty-four hours, as seen in the case of parrot No. 3. Although a smear of the feces showed mostly coarse rods, the culture also showed primary rods. The mouse reaction was rapid, but was not attended with much foulness; the rabbit reaction was typical and was attended with considerable foulness. The metamorphosis was complete. Two months later, the only variation in the reaction induced by this specimen was an increased speed, and less associated foulness.

CASE NO. 4

PAST HISTORY:

Patient was a saleswoman in the store.

CLINICAL HISTORY:

Eight days after the arrival of the parrots, March 10, 1917, a grippe-like disease began with headache, only moderately severe, with great prostration, high fever, delirium, but very little cough. Bowels were normal. Widal negative.

Specimens were taken late in convalescence.
Blood taken 4/3/17. In glucose bouillon.

METAMORPHOSIS:

12 hours:	Tube No. 1.	Diplococci.
24 hours:	Tube No. 1.	Cocci.
48 hours:	Tube No. 1.	Primary rods.
	Tube No. 2.	Primary rods and cocci.
72 hours:	Tube No. 1.	Irregular cocci.
	Tube No. 2.	Subcultures on plate, grey colonies, cocci and many rods, Gram positive and negative.
10 days:	Tube No. 1.	Irregular cocci.
14 days:	Tube No. 1.	Same.

ANIMALS:

MICE:

No. 1521. 1 c.c. serum tube containing staphylococci and primary rods.

DEATH:

Some days. (Record uncertain).

AUTOPSY:

Lungs three-fourths blackened with *hemorrhages*. Intestine slimy. Parenchyma degenerated. Splenic tumor.

SMEARS:

Primary rods.

CULTURES:

Gram negative rods.
6 days: Green haemolysis.
12 days: Gram positive-negative rods.
17 days: Trace green pigment.
20 days: Coccal rods. Motility, trace.
21 days: Gram negative rods, some cocci. Motile.

RABBIT:

No. 1567. 2 c.c. 24 hour re-bouillon culture from a plate, subculture No. 1521.

CLINICAL HISTORY:

Very ill. High fever. Dyspnoea.

DEATH:

72 hours.

AUTOPSY:

Brain suggested *meningitis*. *Rhinitis*. *Trachitis*. Lungs showed *pneumonia* with areas of suppurative *pericarditis*. Suppurative *nephritis*. Catarrhal *gastritis*. *Duodenitis* with *hemorrhages*. Parenchyma degenerated. Slight splenic tumor.

SMEARS:

Spinal fluid: Cocci.
Trachea: Mixed, and huge bunches of Gram negative rods.
Lung: Negative.
Pericardial fluid: Negative.
Heart blood: Negative.
Spleen: Negative.
Hemorrhages of duodenum: Gram negative bacillus.

CULTURES:

6 days: Gram negative rod. Slimy. Haemolysis. Green. Motile. Not agglutinable by human sera.

RABBIT:

No. 1560. Two c.c. re-bouillon of staphylococci from a plate culture of blood culture tubes.

DEATH:
Killed accidently in 43 days.

AUTOPSY:
Profoundly anemic. Evidence of sclerosis and degeneration of essential tissues.

SMEARS:
Indefinite.

CULTURES:
Indefinite.

NOTE: The blood showed nearly a complete metamorphosis in artificial media. The mouse in its reaction apparently reached the first stage of metamorphosis. The cultures therefrom, showed interesting periods of alternate retrogression and progression. The rabbit inoculations from these cultures exhibited a very complete imitation of the human disease type. Smears from it also showed the various stages, while the cultures completed the *typical picture.*

CASE NO. 5

PAST HISTORY:
Patient was in the store every day the parrots were there.

CLINICAL HISTORY:
March 18, 1917, patient began to feel very tired and weak. These symptoms grew worse until March 21st when patient came to the doctor's office. At that time she complained of the above symptoms, also severe headache, constipation and loss of appetite.

Temperature 102°. Pulse 104. Blood pressure 120. Examination of chest negative. Abdomen negative. Urine normal.

On March 26, above symptoms were intensified. March 28th, bronchial breathing and râles at the base of the left lung. Tongue heavily coated and dry. Troublesome dry cough. On March 29th marked consolidation of lower lobe of the left lung was observed. March 31st, lung symptoms had almost entirely disappeared. Temperature dropped to 99.8. From that time on the temperature was normal.

Widal negative at the beginning of lysis.

Blood taken 4/4/17. In glucose bouillon.

METAMORPHOSIS:

	Tube No. 1	Tube No. 2	Tube No. 3
24 hours:	Primary rods		
48 hours:	Cocci, some fused to rods.	Short plump rods.	
72 hours:	Same.	Primary rods. Diptheroids. Cocci.	Cocci, single rods.

96 hours: Mixed cocci and irregular rods. Same as No. 1. Cocci, single and fused in chains.

13 days: Subculture on plate, colonies grey, flat, moist, of rod type. Forms: cocci and rods which were rather irregular but motile and agglutinable.

MOUSE:

No. 1519. One c.c. original serum tube 96 hours old.

DEATH:

Not more than 3 days.

AUTOPSY:

Organs slimy. Hemorrhagic spots on the lungs. Peritonitis. Parenchyma degenerated.

CULTURES:

Developed Gram negative rods. Plate, medium colored light green.

RABBIT:

No. 1565. Two c.c. reculture from a plate culture of Gram negative rods. (Blood culture.)

CLINICALLY:

Culture of nose: All forms.
Smear of nose: Typical rod.

DEATH:

47 days, very ill. Etherized.

AUTOPSY:

Severe anemia. *Enteritis. Cholelithiasis.* Congestion of lungs. Splenic tumor.

SMEARS:

Gall bladder: Typical rods.
Lung: Irregular cocci.
Spleen: Irregular cocci.
Marrow: Negative.

CULTURES:

Gall bladder: Typical rods.

RABBIT:

No. 1703. 6/12/1917. Two c.c. 8 day subculture of gall bladder of No. 1565.

DEATH:

14 days.

AUTOPSY:

Enteritis. Cholecystitis. Parenchyma degenerated.

CULTURES:

All sterile.

No. 1749. 6/26/1917. Four c.c. suspended sediment of 24 hour subculture of 21 day culture of gall bladder of No. 1565.

DEATH:
12 hours.

AUTOPSY:
Rhinitis. Hemorrhages of the lungs, adrenals, stomach, ileum, appendix, and cecum. Parenchyma degenerated. Splenic tumor.

SMEARS:
Rather irregular positive-negative rods.

CULTURES:
Generally typical.

No. 1754. 6/27/1917. Three c.c. supernatant fluid, same gall bladder culture of No. 1565.

DEATH:
Few weeks. (Time record lost).

AUTOPSY:
Enteritis. Parenchyma degenerated.

SMEARS:
Heart: Negative.
Spleen: Negative.
Bowel wall: Negative.
Urine: Variable Gram negative rods with cocci.
Bile: Same.

CULTURES:
Fairly typical.

SPUTUM: Thick brown.

SMEAR: Mixed and primary rods which developed fairly *typical Gram negative rods,* forming a green color, and haemolyzing.

FECES: Constipated and yellow.

SMEAR: Excess of cocci, some Gram positive rods.

CULTURE: 24 hours, primary rods. In 48 hours developed instead thick rods and cocci.

In bouillon cultures of the blood, the assumption by bacterial units of a rod shape was unusually early, though the metamorphosis was never really complete. In the mouse the reaction was rapid. The first rabbit showed a very slow reaction, although inoculated with a Gram negative rod culture. Gall bladder cultures here first showed the *typical rods.*

The rabbit inoculated with these, exhibited only a somewhat more rapid reaction. When a double dose was used, there was a *typical reaction,* as well as a typical unit form demonstrated in the culture. A culture from these feces was important in showing an initial growth of primary rods, which exhibited a well marked metamorphosis.

CASE NO. 6

CLINICAL HISTORY:

This patient was seen in the early stages of a disease course which resembled grippe.
Blood taken 3/30/17. In glucose bouillon.

METAMORPHOSIS:

	Tube No. 1	Tube No. 2	Tube No. 3
24 hours:	Negative.	Primary rods.	
48 hours:	Negative.	Staphylococci.	Primary rods.
96 hours:	Rare primary	Regular staphylococci.	Variable staphylococci, some rod forms
9 days:	rods.	Cocci becoming irregular.	

ANIMALS:

MOUSE:

No. 1506. 4/2/1917. One c.c. original blood culture tube, 72 hours old, showing large rods.

DEATH:

5 days.

AUTOPSY:

Organs very foul.

SMEARS:

Peritoneum. Gram positive rods.

CULTURES:

Irregular staphylococci and large Gram positive and negative rods.

FECES:

SMEARS:

Excess of cocci, and primary rods.

CULTURE:

Primary rods and cocci in 48 hours.

MOUSE:

No. 1505. About 1 c.c. of a 48 hour old plain bouillon culture.

DEATH:

48 to 96 hours.

AUTOPSY:

Organs slimy, Peritonitis. *Pleuritis,* and *congestion* of the lungs.

SMEARS:

Peritoneum: Cocci and rods.
Pleura: Cocci and rods.

CULTURE:

At first the same. Later: Green haemolysis. Agglutinable, Gram negative, rather large rods.

In bouillon the blood specimens showed a very slow, incomplete change. In the mouse as well, the change was slow and incomplete. On the other hand the fecal culture showed nearly a complete metamorphosis, the final Gram negative rods being merely rather larger than usual.

CASE No. 7

CLINICAL HISTORY:

Patient was a woman between sixty and seventy years of age. The course of the disease had a remarkable resemblance to typhoid fever.

Blood was taken early in convalescence. (Third week.)

3/30/1917. In glucose bouillon.

METAMORPHOSIS:

24 hours: Sediment of centrifuged fluid from all tubes, primary rods and rare cocci.

72 hours: Tube No. 1. Diplococci. Subculture on plate, coarse moist colonies, short thick Gram negative rods.

96 hours:	Tube No. 1	No. 2	No. 3	No. 4
	Diplococci.	Cocci.	Negative.	Very small cocci.

NOTE: In the bouillon cultures of the blood there was little change, yet after 72 hours a subculture on an agar plate, in 24 hours provided nearly *typical Gram negative rods.*

ANIMALS:

MOUSE:

No. 1504. One c.c. of a 72 hour original blood culture tube showing a thick rod.

DEATH:

48–96 hours.

AUTOPSY:

Organs slimy. *Peritonitis.* Lungs negative. Parenchyma degenerated. Splenic tumor.

SMEARS:

Peritoneum: Cocci to fine rods.
Heart: Cocci to fine rods.
Spleen: Cocci to fine rods.
Lung: Cocci.

CULTURE:

Irregular Gram negative rods on a plate, not haemolyzing, moderately green in color.

RABBIT:

No. 1542. Two c.c. of original blood tube culture (sterile ?).

DEATH:

3 months.

AUTOPSY:

Red blotches in lungs. Right, yellowish. *Gastroenteritis.* Parenchyma degenerated. Splenic tumor.

SMEARS:

Spleen: Negative.
Heart blood: Negative.
Bowel: Coarse Gram positive rod.

CULTURE:

Heart blood: Cocci to rods of various lengths and Gram positive and negative.
Urine: Same.
Bile: All rods of same variations.

RABBIT (Pregnant):

No. 1805. 7/30/1917. Two c.c. of a 10 day old reculture of the heart blood of No. 1542.

DEATH:

12 hours.

AUTOPSY:

Hemorrhages of the appendix, small intestine, and nose. Peritonitis.

SMEARS:

Heart blood: Gram negative rod.
Peritoneum: Same.
Hemorrhages of appendix: Same.
Bile: Negative.
Nose: Negative.
Spleen: Negative.

CULTURES:

Heart blood: Rather irregular Gram negative forms.
Bile: Same.
Peritoneum: Gram positive forms.
Nose: Same.

The mouse reaction was typical. The metamorphosis was fairly complete. The rabbit reaction was profoundly slow, but the rabbit, although pregnant, inoculated with an old subculture of a very old culture, presented a typical clinical picture, yet the bacterial forms were not typical. A pregnant animal can be, as is well known, most easily killed. This result, with incompletely adjusted units providing a relative result, seems of value in itself.

CASE NO. 8

CLINICAL HISTORY:

March 15, 1917 patient began to feel weak, with pains all over body. On March 18, she went to a doctor's office, complaining of headache, weakness, and general muscular pains. Temperature was 102°. Pulse 118. Blood pressure 125. Physical examination negative. Urine normal.

Patient was ordered to bed. Temperature and pulse remained the same until March 30th, when they dropped to normal. While in bed she did not complain of pains or headache and had no cough, but had diarrhoea.

Patient recovered slowly. Blood was taken early in convalescence;

4/3/17. In glucose bouillon.

METAMORPHOSIS:

24 hours:	Negative.		
48 hours:	Tube No. 1	Tube No. 2	Tube No. 3
	Rare short rods.	Negative.	Primary rods.
72 hours:		Negative.	Cocci and rare fine rods.
10 days:			Same.
14 days:			Subculture plate, cocci, many fused to rods.

NOTE: As in case No. 9 here were extended time intervals, and only a partial metamorphosis was observed.

CASE NO. 9

PAST HISTORY:

Patient was an engineer in the store. He became ill about five days after the arrival of the parrots. March 8, 1917.

CLINICAL HISTORY:

Ill with a disease resembling, first pneumonia, then typhoid, lasting for about twenty-seven days. Widal negative.

Beginning of convalescence.

Blood taken 4/3/17. In glucose bouillon.

METAMORPHOSIS:

24 hours: Negative.
48 hours: Light suspension. Primary rods, rare cocci.
72 hours: Heavy suspension. Very *sheer* Gram positive rods.
6 days on a plate: Green staphyloid colonies.
23 days: Mostly long Gram negative rods, motile, not agglutinable. Also rare cocci.

ANIMALS:

RAT:

No. 1581. .5 c.c. blood culture tube.
DEATH: 7 days.

AUTOPSY:

Lungs showed large dark areas of *pneumonia*. Slimy intestine. Abscessed kidneys. Parenchyma degenerated. Immense splenic tumor.

SMEARS:

Lung: Cocci.
Heart: Cocci.
Spleen: Cocci and primary rods.

CULTURES:

Lung: Gram negative rods and some cocci, which in four days became very typical and green in colonies.
Heart: Same.

The metamorphosis is clearly shown.

CASE NO. 10

PAST HISTORY:

In this patient's house there were two parrots. After the first died, a second one was bought but it died also. (One week later the son and wife became ill.)

CLINICAL HISTORY:

Two weeks after arrival of first parrot patient became ill, suffering from insomnia, delirium, mild headache and malaise. While the lungs were clear, there was a slight cough. Temperature was 102° to 103°.

Blood was taken during the first week of the disease, 4/3/17.
In glucose bouillon.

METAMORPHOSIS:

24 hours:	Primary rods.
48 hours:	Vine like suspension, all tubes. Primary rods. Tube No. 3, diplococcal forms. No. 2, same. Fused, heavy rods.
72 hours:	No. 3 and No. 4. Coccal and diplococcal forms. Haemolysis.
6 days:	No. 3, forms fused to heavy rods.
10 days:	Same state.
18 days:	Same.
23 days:	Rods never Gram negative, motile, or agglutinable.

ANIMALS:

Mice were injected without any result within a reasonable length of time.

NOTE: The findings were incomplete.

CASE NO. 11

PAST HISTORY:

Patient bought a parrot at the store. This bird developed a discharge from the nose. It suffered one week from anorexia, and sneezing, then improved. It was well for a week, sick again for a week, and finally died.

CLINICAL HISTORY:

Five days after this the patient became ill. She had a sustained fever, headache and constipation. The disease ran a typhoidal course. The lungs were somewhat indefinitely involved.

A daughter had a very slight attack, mainly headaches, which lasted a few days and then passed away.

Specimens taken in the later acute stages.
Blood taken 4/4/17. In glucose bouillon.

METAMORPHOSIS:

24 hours: Diplococci, some already fused to coarse rods.
96 hours: Tube No. 1. Primary rods, immense oval cocci.
9 days: No. 3 Primary rods, subculture on plate, Gram negative cocci which changed to fine rods, both Gram positive and Gram negative, plus much smaller Gram positive cocci.

Here after 9 days a subculture was required to demonstrate a metamorphosis almost complete.

ANIMALS:

MOUSE:

No. 1518. One c.c. of the above serum, tube.

DEATH:

48 hours.

AUTOPSY:

Intestine slimy and dark. *Congestion of lungs* with dark areas. Parenchyma degenerated. Splenic tumor.

SMEARS:

Peritoneum: Negative.

CULTURES:

FECES:

Yellow, semi-fluid.

SMEAR: Indefinite.

CULTURE: Mixed cocci to rods, some primary some rare Gram negative rods.

This culture developed a typical Gram negative rod; motile, agglutinable and forming a green pigment.

NOTE:

This case is interesting mainly for the reaction in the bouillon tube inoculated from the feces. This at first showed all the various bacterial forms. Then it was observed that it became green in color. And this,

the very tube previously showing mixed forms, now was observed to contain only Gram negative, motile rods.

CASE NO. 12

CLINICAL HISTORY:

The sputum was received for the designation of the type of pneumococcus. Suspicion was aroused by the bright, cherry red color. The patient when seen was profoundly dull and gave forth a peculiar, disagreeable odor. He coughed and sustained a high fever. His pulse was failing under the stress of an irregular pneumonia. His lungs showed some indefinite areas of dullness, but especially diffuse large moist râles. Bronchial breathing was not in evidence.

Within 72 hours death had occurred.

Blood taken 4/29/17.

METAMORPHOSIS:

24 hours: Primary rods.

48 hours: Cocci in uneven chains.

72 hours: Oval cocci. Chains of astoundingly irregular cocci, long slim rather irregular Gram negative rods. Subculture on plate, moist green colonies, of cocci and long Gram negative rods, not motile but agglutinable.

Gram negative rods, unusually long, developed in original tube.

SPUTUM: Very red. No red cells.

SMEARS:

CULTURES: Blood agar: Slimy and greenish.

Bouillon: Large, agglutinable, but non-motile rods.

ANIMALS:

RABBITS:

No. 1610. Three c.c. re-bouillon of plate culture of the sputum.

CLINICAL HISTORY:

After 4 days became ill with fever. Was very quiet. Breathing was labored.

DEATH:

8 days. (Killed for comparison).

AUTOPSY:

Lungs were congested and showed large and small hemorrhages. Intestinal catarrh. Mucus and cells in exudate.

SMEARS:

Generally negative except chyme.

CULTURES:

Heart: Pneumococci, also rods, which were not motile, producing no green coloration.

RABBIT:

No. 1611. Three c.c. re-bouillon of plate culture of sputum.

CLINICAL HISTORY:

Never appeared very ill.

DEATH:

8 days.

AUTOPSY:

Pericarditis: Parenchyma degenerated. Splenic tumor. *Bronchitis.*

Lungs: Right: Solid grey, croupous *pneumonia,* covered with hemorrhagic areas, fibrin and *bloody* fluid.

Left: Showed various stages of *congestion,* small areas of grey and red *hepatization.*

SMEARS:

Lungs: Four different areas, pneumococci to diptheroids.
Pleura: Same.
Bronchi: Same.
Heart: Irregular, Gram positive rods.
Spleen: Rare cocci.

CULTURES:

9 days. Irregular, rather long Gram negative rods, hardly motile, but very agglutinable.

RABBIT:

No. 1799. Two c.c. subculture in plain bouillon of a 74 day old lung culture of 1611.

DEATH:

12 hours.

AUTOPSY:

Hemorrhages of the thymus, lungs, appendix, rectum and kidneys. Gas and mucus in the intestine. Parenchyma degenerated. Splenic tumor.

SMEARS:

Heart Blood: Gram negative rods.
Liver: Same.
Hemorrhage of appendix: Same.
Thymus: Negative.
Lung: Negative.
Rectum: Negative.
Kidney: Negative.

CULTURES:

Bile: Gram negative rods varying only in length.

RABBIT:

No. 1806. Two c.c. re-bouillon culture of 13 day heart culture of No. 1799.

DEATH:
8 hours.

AUTOPSY:
Hemorrhages of the heart, liver and large intestine.

SMEARS:
Hemorrhages of
Heart: Typical rods.
Intestine: Typical rods.
Spleen: Typical rods.
Liver: Typical rods.

CULTURES:
Heart blood: Typical rods, but many Gram positive.
Bile: Same.

In this patient's blood there was a regular but rapid metamorphosis although the cocci were chained. As usual, rods were in the sputum, and were here, immediately obtained in the cultures. They appeared as rather long Gram negative, slightly motile rods, very agglutinable with human sera. Also, coccal forms present were seen in transition stages, preliminary to rod formation, as in the blood. These forms had a most definite disease-producing capacity and in growth, assuming the characteristic pneumonia-producing cloaks, appeared as oval diplococci, some being fused or diptheroidal.

After two months, these organisms in bouillon, had all the characteristics of the *typical bacillus.*

CASE NO. 13

The author was fortunate in being summoned to perform a post mortem upon the body of this man. He had gone to work in the mines that morning. He had complained of a headache. Later he was found dead.

AUTOPSY:

Performed at the home of the deceased, Sugar Notch, at 5 P. M. April 16th, 1917.

PATHOLOGICAL DIAGNOSIS:

Status thymo-lymphaticus. General subserous hemorrhagic exudations. General peculiar, protoplasmic degeneration of the parenchymatous organs. Congestion, anthracosis and oedema of the lungs. Hemorrhagic nephritis, acute splenic tumor.

BACTERIOLOGICAL DIAGNOSIS:

Cultures from various portions including the brain, showed Gram negative bacilli of intense virulence.

EXTERNAL ASPECTS OF THE BODY:

Body was that of a powerful young man. Cartilages of nose appeared rather loose. There were several small bruises at the

base of nose and forehead. Beyond these there were no other marks of violence. Body had been embalmed. There was the usual undertaker's wound in the right axilla and the trocar wound in the centre of abdomen which did not penetrate to the thorax.

INTERNAL ASPECTS OF THE BODY:

HEAD:

Scalp: Intact.
Skull: Intact.
Meninges: Dura mater was normal.
Vessels: At the base were tremendously engorged.
Surface: Hyperemic, especially marked at the base.
Subarachnoid: Spaces contained blood clots.
Spinal Canal: Solid, with clots.
Brain Tissues: Cerebrum, cerebellum, medulla and stem, all were intact.

THORAX:

Thyroid Gland: Much enlarged, five times normal size. A simple goitre without pressure on the trachea.

Trachea: Showed intense congestion of mucous membrane and blood tinged froth.

Lungs: The right lung was adherent in its entirety. At its apex were some old tuberculous foci. Left lung here and there showed some evidence of rather recent pleurisy. Both lungs were markedly oedematous and congested. There were no signs of pneumonia. Bloody fluid was in the pleura.

Microscopic: Showed only congestion and anthracosis.

Heart: Pericardium contained bloody fluid. Heart was contracted and clotless. Walls were pale pink. Valves were intact. In some places there were subpericardial hemorrhages.

Coronaries: Normal.

Aorta: Small and showed some signs of fatty degeneration.

ABDOMEN:

Peritoneum: Contained a considerable quantity of bloody fluid.

Liver: Enlarged. Surface was smooth, color purplish. Structure much obscured. Gall-bladder was adherent to the colon. Otherwise normal.

Microscopic: There was moderate congestion. Cells showed a marked loss in homogenicity. The nuclei were not much obscured.

Spleen: Five times normal size, very dark. Structure was slightly fluid. Follicles evenly enlarged. Surface showed slight evidences of peritonitis.

Microscopic: Intensely hemorrhagic.

Kidneys: Showed considerable blood in peritoneal tissue. They were soft and very dark.

Glomeruli: Enormous, evidently intensely congested.

Microscopic: There was very marked diffused congestion especially of the glomeruli. These showed some increase in nuclei. Tubules showed very marked destruction of the cell bodies, but the nuclei remained very apparent.

Stomach: Contained a complete meal, practically undigested. Mucous membrane was everywhere congested, and there were many areas of small hemorrhages.

Intestine: Generally slightly congested. The follicles and patches were enormously enlarged, but not diseased.

Appendix: Free and apparently normal. Feces appeared yellow and rather soft. The retro-peritoneal tissue appeared generally to contain blood.

CAUSE OF DEATH: Acute bacillary septicemia of at least some days duration with the sudden death under physical activity so often seen in the individual of the thymo-lymphatic type.

SPECIMENS FROM HUMAN POST MORTEM:

SMEARS:

Brain: Gram negative rods.
Lungs: Cocci to rods.
Spleen: *Primary rods.*

CULTURES:

Gram negative rods.

ANIMALS:

RABBIT:

No. 1579. 4/27/1917. Two c.c. 10 day bouillon culture of lung.

DEATH:

24 hours.

AUTOPSY:

Large hemorrhages of the lungs, duodenum, appendix and small intestine, especially of the lymph patches. Also there were tiny hemorrhages in the muscles. The brain showed no hemorrhages. Splenic tumor.

SMEARS:

Typical Gram negative bacilli, generally, especially in the hemorrhagic areas.

CULTURES:

Typical Gram negative rods, whence grew a paratyphoid type of organism on Russells double sugar medium.

RABBIT:

No. 1568. Four c.c. mixed bouillon cultures of human post mortem spleen and lung.

DEATH:

5 hours.

AUTOPSY:

There was general intestinal and visceral congestion including the brain. Splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Spleen: Showed mostly primary rods, a few cocci, and Gram negative rods.

Heart: Same.

Brain: Same.

CULTURES:

Moderately agglutinable, motile, non-haemolizing, and non-green-producing rods, which grew as Bacillus paratyphosus.

The first rabbit showed the rather rare picture of general hemorrhages. The second rabbit, dying in five hours, showed, in the smears, the variations frequently seen in quickly terminating animal reactions. The smears showed the primary forms seen in the human autopsy. The man, likewise, died after only a few hours (12) illness. Of all human cases, his most closely resembled the animal reaction, classed generally, as *"typical."*

NOTE:

Investigation resulted in the discovery that in the house where this man lived there had occurred a case of the epidemic disease.

CASE NO. 14

CLINICAL HISTORY:

An appendix showed a perforation which was thought to be typhoidal in character.

APPENDIX:

CULTURE:

Gram positive-negative rod, which was motile and haemolyzing, agglutinable and producing in culture a green pigment.

ANIMALS:

MOUSE:

No. 1570. 4/20/1917. Two c.c. plain bouillon culture.

DEATH:

18 hours.

AUTOPSY:

Hemorrhages of the lungs and stomach. Intestine was slimy. Parenchyma degenerated. Splenic tumor.

SMEARS:

Peritoneum: Few large, oval, diplococci.

CULTURES:

Peritoneum: Cocci + Gram negative rods which were motile and agglutinable.

Lung: Same.

RABBIT:

No. 1612. 5/8/1917. Two c.c. re-bouillon culture of 18 day old culture of No. 1570.

DEATH:

12 hours.

AUTOPSY:

Some hemorrhages of stomach. Immense hemorrhages of the peritoneum. Bronchitis. Enteritis. Feces soft.

SMEARS:

Lungs: Gram positive rods.
Bronchus: Gram negative rods.
Heart: Gram negative rods.
Mucus of appendix: Negative rods.
Small bowel: Negative rods.
Stomach: Negative rods.
Spleen: Negative rods.
Hemorrhages: Negative rods.

CULTURES:

Heart: Gram negative rods.
Peritoneum: Gram negative rods.
Appendix: Gram negative rods.
Stomach: Gram positive rods.

After 48 hours a rod in cultures, colonies, slimy, colon-like, poorly chromogenic. After 6 days: Still not motile, not agglutinable, and poorly chromogenic.

NOTE:

In this case it is the twelve hour rabbit reaction which is the diagnostic key. It is interesting how this reaction, typical in itself, may be associated with such a considerable variation in the different smears and cultures provided by the animal. The metamorphosis, while observed, never became complete.

It is necessary to take a very broad view of the metamorphosis. Time, material and energy continually vary their relationships, providing such a great range of possibilities as are seen.

CASE NO. 15

CLINICAL HISTORY: (Date undetermined).

This patient was profoundly prostrated and had a fever. His symptoms were typical of no special disease. His tongue was coated with a whitish deposit, and the odor from his body was characteristic of cases seen during the epidemic. After an indefinite course he slowly recovered.

SPUTUM: Canary yellow.

SMEAR: Cocci and Gram negative rods, mainly rods.

CULTURE: Same.

ANIMALS:

MOUSE:

No. 1683. .5 c.c. culture in plain bouillon.

DEATH: 24 hours.

AUTOPSY: Very foul organs.

CULTURE: *Heart blood:* Typical Gram negative rods, but cultures were not green.

NOTE:

Smears and cultures of this sputum showed mainly the final stages of the metamorphosis. The mouse reaction was typical, and completed the metamorphosis.

CASE NO. 16

PAST HISTORY: Patient was a mail carrier.

CLINICAL HISTORY:

Had symptoms resembling typhoid fever with terrific headache. His lungs appeared practically normal. In this case, although there was some sputum, the lungs never showed signs of consolidation.

Specimens were taken in the beginning of the acute stage.

Blood taken 5/11/17. In glucose bouillon.

24 hours: Tube No. 1, cocci and streptococci.

48 hours: Tube No. 1, cocci and diplococci fused to short rods.

6 days: Tube No. 1, cocci, long and short Gram negative rods.
Tube No. 3, cocci.

Metamorphosis nearly complete in the original tubes.

ANIMALS:

MICE:

No. 1656. .5 c.c. 14 day old culture, tube No. 3.

DEATH: Few days (records uncertain).

AUTOPSY:

Organs dark. Hemorrhages of the lungs. Parenchyma degenerated. Splenic tumor.

SMEARS:

Peritoneum: Cocci.

Heart: Negative.

Lung: Negative.

Spleen: Negative.

CULTURES:

All cocci, for 6 days. Ten days: White on plate forms, cocci and some rods, not green, not motile.

No. 1655. One c.c. of culture tube No. 2.

DEATH: Few days (record uncertain).

AUTOPSY:

Same as above.

SMEARS:

Peritoneum: Variable Gram positive-negative rods.
Spleen: Same.
Lung: Same.

CULTURES:

Gram negative, non-motile rods. Very agglutinable.

NOTE:

This case demonstrated in the blood and in the first mouse reaction a metamorphosis, beginning at the coccal stage, which progressed very slowly. Only in the second mouse did it approach completeness. And yet the mice were similar except for the metamorphosis.

SUMMARY OF HUMAN CASES

The all too few human cases may perhaps claim attention, principally because of their comprehensive clinical variation and the multiplicity of the sources from which bacterial protoplasm was obtained.

There were represented clinical entities, already referred to as observed in the cases of human disease, and roughly grouped under the three headings; the influenzal, the pneumonic, the typhoidal. They were coarse enough in definition and sufficiently overlapping at their boundaries to indicate strongly that the disease was a general one with powers of local emphasis. The influenzal entities were represented by Cases Nos. 4 and 6; the pneumonic by Cases No. 1 and 12; the typhoidal by Cases No. 2, 7, 10 and 11. The rest proved links in varying degree; Case No. 3, septicemia, involving even the cornea of the eye, being most generalized. The pathology of the single human autopsy bore a striking resemblance to that in rabbits, resulting from the injection of cultures of the *Typical Gram negative rod.*

The comparisons of the initial findings from the sputa, feces, and bloods are interesting. The few sputa obtained, showed, in smear, the various stages of bacterial form, either greatly mixed, or with Gram negative rods predominating. Cultures in bouillon demonstrated in one case, (No. 1) streptococci, but slowly devel-

oping to rod forms. Another, (No. 12) exhibited rapidly developing rod forms, the third, (No. 15) from the beginning, contained rod forms. Cultures from the first caused a variety of slowly developing conditions, but reached a stage, in one animal, by which pneumonia was caused. The second, in the initial instance, induced a fine picture of pneumonia. The third, immediately killed a mouse after the manner of the *Typical Gram negative rod,* with rapid putrefaction.

The feces were hardly sufficiently investigated to reveal any definite facts, excepting that all the stages in form-evolution were frequently observable. One culture caused no effects in an animal, the others acted after the manner of the terminal stages. Later, facts will be added to show that, in general, the bacterial protoplasm under consideration, although aroused to a disease-producing activity when living in the more saprophytic environment of the gut tube, usually acted in this terminal stage manner.

The bloods showed much more uniformity in metamorphosis, although subcultures were frequently necessary to bring it out, and also, there were great variations in the time factor. The specimens varied in functional capacity, inducing pneumonic hemorrhages or pneumonia; gastrointestinal hemorrhages, or catarrh; very acute, and very chronic lesions.

Thus, the fairly regular metamorphoses in the bloods constituted the closest bacteriological links between the human cases. In other areas the same types of rods and cocci and transition forms were found, but in varying proportions.

Chapter II

A CORRELATION OF THE COURSES OF CHANGING BACTERIAL FORM AND FUNCTION OBSERVED IN RELATION TO THE BACTERIAL PROTOPLASM ASSOCIATED WITH THE EPIDEMIC DISEASE IN PARROTS AND MEN—NATURAL VERSUS EXPERIMENTAL DISEASE

Consideration of Form*

Since the complete course in metamorphosis suggested by the facts recorded cannot be minutely followed in any one case, it may be clarifying to demonstrate an ideal course in human blood cultures as shown in the series of plates, together with a shorter course, which actually took place in material from a parrot. As the plates demonstrate, the steps are by no means clear cut, nor can the methods of change in many cases be more than guessed at. The changes, apparently, take place either in small masses or en masse, slowly or suddenly, and it seems safe to say, absolute uniformity of morphology never exists. When the term "stage" is used, it has a relative meaning, referring to a tendency to a common form among the units of the colonies. This appears to depend upon a happy adjustment to the environment, the temporary partial control of destiny. Intermediate forms have no fixed shapes.

In plate cultures, frequently, the methods can be better understood. The various, relatively pure types, have definite colony characteristics. The first form seen in blood, the fine, often curved Gram positive rod, has very delicate, tiny streptococcal-like colonies. The staphylococcal forms exhibit coarser, whitish deposits. The large Gram positive rods grow in dry, hairy colonies. The finer Gram negative rods have colonies like the

* See plates at close of chapter.

colon bacillus. "Runners" may be associated with the different rod colonies, as well as a green pigmentation. Haemolysis may be accomplished by all forms.

But many colonies have a form all to themselves. A colony, pure to the naked eye, may show in smear, mixed forms. Every colony shows some exceptional forms. A frequent finding is a non-homogeneous, multitinted, rather granulary, hummocky streak, showing all the units as irregular masses, very poorly stained. Or a homogeneous colony may have scattered areas, raised and of different tints, refractability, or transparency, in which the units will be irregular; so to speak, pregnant points. Such a spot, near the edge of a staphylococcal colony, may in time, push out a mass of rod forms. Certainly, the well known forms are commonly assumed in stages. When under pressure, if conditions are at all ripe, uniformity results.

FUNCTION:

The change in function, paralleling the metamorphosis, but bearing no close and apparently no absolutely necessary relationship to unit forms, occurs in both artificial and natural media. In the test tube it may be observed as a slowly increasing cloudiness in pellicle development; the evolution of finely divided gases; of color changes, with the development of a significant green pigment.

DISEASE PICTURES INDUCED IN ANIMALS:

It has been concluded that the bacterial protoplasm found in connection with the epidemic disease in both parrots and men, although pathogenic for animals, did not, as obtained from a given host, necessarily induce, experimentally, a disease picture which resembled that seen in the host.

In mice, the peritoneal route for inoculation might well be objected to, as providing a factor leading to too generalized results. Certainly, this animal is too small to provide clear definition. But the pictures of pneumonia were indistinct, as were also the closely related hemorrhagic spots upon the lungs. Besides the autopsies, which exhibited these reactions, there were

others associated with peritonitis and pale, defined tissues, and, again others revealing a profound, general blackening and foulness. In mice, the terminal stage in functional adjustments, the *Typical reaction,* was represented by the last type. In this, frequently, the lung pathology was difficult of determination. The rapidity and intensity of the putrefactive changes causing in a short time this general blackening of the organs and a most terrific odor, as will be shown, has an important bearing upon the understanding of the functions of this form of bacterium. Examples of mice, injected with early rod and coccal stages, usually, did not show abnormal putrefaction. But the change in bacterial function at times occurred at such points, and putrefaction then resulted with inordinate rapidity.

In the case of the experimental rabbits, a much more differentiated picture could be obtained. Briefly to summarize the pneumonic cases:

No. 1. A typical, non-chromogenic rod, twenty-four days old, from the lung of a parrot, induced pneumonia, with recovery. This was followed by a relapse sixty days later, with fibrinous pneumonia, due to the same rods. Twenty days later, the same organism caused hemorrhages of the lungs, adrenals, and duodenum, followed by death in twenty-four hours.

No. 1578. A twenty-four hour culture of a twenty-seven day old staphylococcus culture, which had changed to a Typical rod, caused death in six days, due to croupous pneumonia, upon a basis of large hemorrhagic areas. The rod, regained from the lung, killed a rabbit in twelve hours with hemorrhages of lungs and endocardium.

No. 1567. A twenty-four hour subculture of a rod, from a seventeen day old culture which had metamorphosed in a mouse, caused a pneumonia of the same type; fibrinous, but constructed in large, round, hemorrhagic areas. Death took place in seventy-two hours. There were associated, complicated disease processes.

No. 1611. A forty-eight hour bouillon subculture of a plate culture from a sputum, showing a large but motile rod, caused the same type of fibrinous pneumonia, with death in eight days. However, another tube (1610) inoculated from the same plate, required the same time to cause death (eight days), but the autopsy showed only congestion and a few hemorrhages of the lungs, together with a catarrh of the gastro-intestinal tract. The resulting forms, pneumococci, and diphtheroids, over two months later, at this time Gram negative rods, destroyed a rabbit in twelve hours with hemorrhages of the thymus, lungs, appendix, rectum, and kidneys. The rods causing these, killed another rabbit in eight hours with hemorrhages of the heart, liver, and large intestine.

In these four definite cases of pneumonia, we have the nearest approach to the classical pathology of Psittacosis, but, as will be seen in the human cases, the human pathology in this epidemic must have been as variable as that of the rabbits.

SELECTIONS FROM ALL COURSES OF CHANGE OF STAGES IMITATING NATURAL EPIDEMIC CASES

Two examples may be compared to cases of human epidemic disease largely restricted to the intestinal tract.

No. IV. (Parrot I) A culture from the marrow caused death in 6 days with hemorrhagic gastroenteritis.

No. 1703. A culture from subcultures from Case No. 5 caused death in 14 days, associated with enteritis and cholecystitis.

The indefinite rather mild human cases exhibiting signs in both the respiratory and intestinal regions are represented well in this twin experiment to No. 1611.

No. 1610. A culture from the sputum of Case No. 12, killed animals in 8 days, just as in the case of No. 1611, but the lungs showed only hemorrhagic areas of congestion. There was also a low grade intestinal inflammation.

The more generalized types, the so-called connecting links, include one case also described in the pneumonic group.

No. 1567. Not only was there a *pneumonia* with *pericarditis* and respiratory inflammation, *rhinitis* and *laryngitis,* but also a suggestion of *meningitis, gastroduodenitis* and *nephritis.*

No. XI. A culture from a parrot bronchus caused a moderate degree of ill-defined *pneumonia, rhinitis* and *laryngitis.* The *brain* was congested. There was also *appendicitis* and *nephritis.*

CHANGES:

Compare these results with the initial action of a culture from the plate colony of rods from the bronchus of a Parrot (No. 2), a culture of coccus from a human blood, (Case No. 2) (No. 1533), and the long rod in a culture from a human feces, (Case No. 3) (No. 1534), all artificially grown for about 10 days. They all caused death in rabbits in about 17 hours, the first two

with very large hemorrhages, all disease foci full of rods. The first also showed gastrointestinal hemorrhages and rapid putrefaction. The second showed gastrointestinal hemorrhages but a slow putrefaction. The third, only appendiceal hemorrhages, exhibited most rapid degree of putrefaction of all.

Yet another bacterial specimen from the above human blood culture, (Case No. 2) following its passage through a mouse, was injected into a rabbit (No. 1566) which after 24 hours was etherized. The autopsy showed hemorrhages practically restricted to the bowel and not in the lungs and the appendix. While a subculture from the second rabbit destroyed in the first instance, No. 1533 caused a slower death. No. 1701 caused death in 24 hours, with rhinitis, congested lungs, nearly pneumonic, together with hemorrhages of the duodenum, ileum, jejunum and the appendix. A subculture of rabbit No. 1534, the third mentioned above, caused intense hemorrhages of the duodenum, jejunum and appendix, a few, of the lungs, and no abnormal putrefaction.

End of Changes:

In these short, hemorrhagic types, death came more quickly. All cultures progressed in their reactions, in time, towards this type. Some cultures primarily induced this type. The hemorrhages must be classed with regard to distribution, as general, or as indiscriminately located. The assumption of this common-ground type of culture adjustment appeared to be associated with a tendency more and more to a gastrointestinal location for the hemorrhagic lesions; at least large lung lesions became rare. Apparently, the whole change was a retrogression up to this point, except in the rapidity of death. As will be demonstrated, further use, at least within a reasonable period, induced no change, except slight variations in the speed of death, the intensity of hemorrhage-production, and the degree of putrefaction activity. The two latter phenomena appeared really to be complementary, as stated elsewhere.

The commonest location, therefore, of hemorrhages was in the gastrointestinal tract. Here, usually, selection was made

of seats in the stomach mucosa, the duodenum, especially at the caput, beneath the peritoneal coat of the appendix, and in the wall of the rectum. More rarely, in this small series, the hemorrhages were located very generally, even diffusely, throughout the skeletal musculature and viscera. (Nos. 1579 and 1799.)

TIME:

In the complex, picturing change, there is the thing, its time of beginning and its time of ending; its surroundings or environment. These are all of vast import; they have all been considered. They could never have been correlated unless the added factor of time had been fully realized.

Variations were so extensive as to comprise within this factor extremes embracing periods of disease courses between twelve hours and three months. The chronic types involved gastro-intestinal catarrh, with secondary anemia, cholelithiasis, peritonitis, etc. Cultures, sooner or later, exhibited *Typical Gram negative rods.*

The variations in the rapidity of change in function, in relation to this time factor, are well illustrated by two cases. Two c.c. of a twenty-four hour subculture of a hundred and five day old fecal culture, from the first parrot, killed a rabbit, No. II, in ninety-six hours, with gastroenteritis and peritonitis. Seventy-two hours later, the same culture killed a rabbit, No. III, in twelve hours, with hemorrhages of the gut tube. On the other hand, the culture of the rapidly developed rods in the blood culture of Case No. 5 (No. 1565) killed only after three months, a subculture then killed (No. 1703) in fourteen days, and finally a double dose of a culture from this animal (No. 1749) killed in twelve hours, *Typically.*

CORRELATION OF FORM AND FUNCTION

Illuminating differences in the function of like forms were evident. The primary stage rods, whether associated or not with cocci, varied in their action in mice, causing either a definite peritonitis with slow putrefaction (No. 1511), or inducing such foul changes as to render diagnosis indefinite. Staphylococcal

forms were much more frequently employed, as the stage was more enduring. Following the injection of cultures of these, we have produced pictures of pneumonia, variously placed hemorrhages, short and long disease courses. More or less Typical rods caused pneumonia, various pictures of hemorrhages; courses of disease in duration lasting from 8 hours to several months. Some appeared to have no effect at all, even in mice. These cases were later discovered to be established *"Carriers."*

GENERAL SUMMARY (CHAPTERS I AND II)

There was great difficulty in arranging the foregoing facts, grouped by cases and specimens, and demonstrated in terms of form and function.

Far greater difficulties impeded their correlation. There appeared to have entered this human community new life in the form of complex, chemical molecules, characterized by an extreme, almost abstract, generalization.

Variability of form and purposeful changes in form, were early observations. The production of hemorrhages and quick death in animals, by some cultures from the first, by many in time, led to an initial conception of an increase in virulence. But the most diverse varieties of culture could in common evolve into this hemorrhage producing type. Not only did those causing mild disease, thus apparently grow stronger, but those most exquisitely pathogenic, later, perhaps only under this cloak, grew stronger, although certainly less precise. Furthermore, animal passages provided for this common type, did not increase its strength. Small amounts did not, to any real degree, result in the more defined disease entities.

Gradually this type of culture, this state assumed in common, was recognized as probably neither the result of a rise or fall in power, but rather a state of adjustment.

Thus when fed upon meat substances (bouillon), or living animal tissues, the bacterial protoplasm showed a profound tendency both to assume a definite form and in large numbers to kill in a primitive manner. That is to kill by causing hemor-

rhages, and cytoplasmic destruction, with lesions indiscriminately located, apparently a process but one step removed from putrefaction. It is not desired to convey the idea that this state assumed in common, the end result, was any more than an adjustment. As will be seen in the following pages, the same flexibility and potentialities shown in the various steps, remained latent in this relatively final stage. Judged by the usual standards, this form cannot be classified as a species.

Thus far the actual artificial induction of the epidemic form of disease was not considered to have been satisfactorily accomplished. Of course the hemorrhagic lung lesions in animals were suggestive, when viewed together with the indefinite signs of lesions in the human lungs, attended with strong evidences of general moistness, and bloody sputa.

Although the form problem was perhaps partially soluble, the perfect maze of the experimentally induced disease pictures, caused by a type of bacterial protoplasm apparently common to all, was highly perplexing.

The bacterial matter was judged as common, because of the correspondence in the adjustments to an environment of this living material from all the different sources. But form and function had apparently no close relationships, not only in the bacteria as found in the hosts naturally diseased, but also as observed when cultures were experimentally manipulated.

Was it to be expected that such bacterial protoplasm, obtained from a case exhibiting a certain disease picture, should induce experimentally an exactly similar picture? It was impossible to characterize by the use of terms of shape, just what was expected to attain the results recorded. It must be admitted that the human disease was generalized, and that there were really many disease pictures, a state of affairs unlike that in the classical epidemics.

As has been already suggested, in the very maze of the various experimental disease pictures taken as a whole, may we not look for a relationship to the disease entity in the naturally arising epidemic?

CONCLUSIONS. CHAPTERS I AND II

I. Parrots and men in contact were ill of a general bacterial disease, characterized by poorly defined syndromes.

II. The parrots were ill first.

III. Bacterial protoplasm was present in large amounts in all areas studied in both parrots and men.

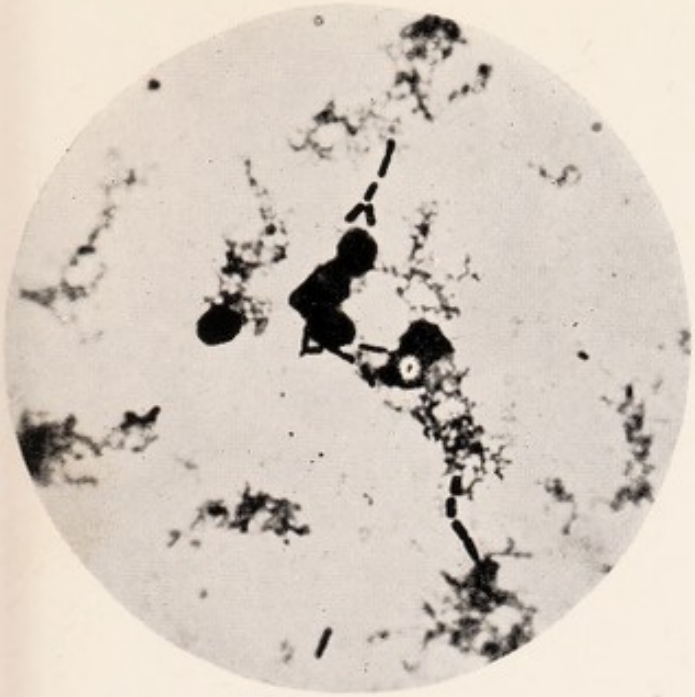
IV. In both form and function, and in their relationship, this bacterial protoplasm showed complete variability.

V. The function of specimens directly or freshly obtained, in its reactions in rabbits, bore no necessary relationship to that represented by the reaction present in the host from which it had originated, i. e. the parrot or human being.

VI. Practically all bacterial protoplasm studied did react in animal tissues.

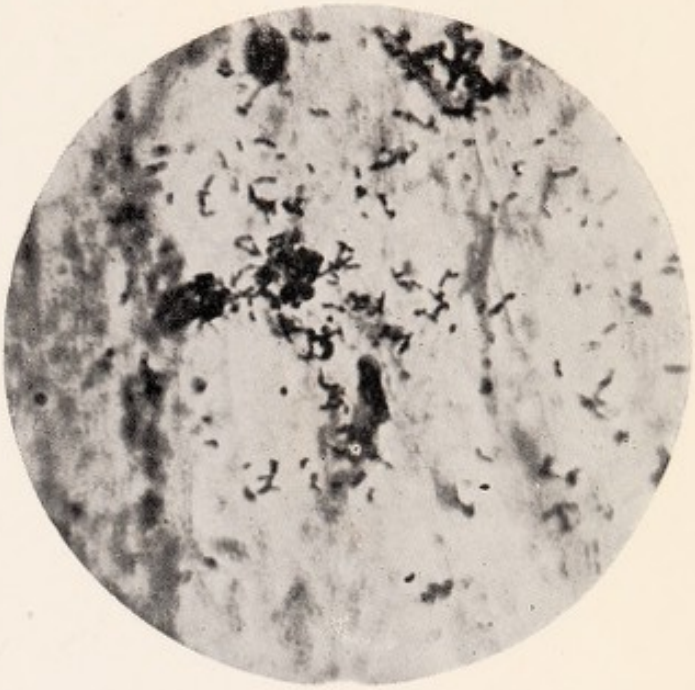
VII. Either with extraordinary rapidity, or with great slowness, protoplasm from all sources exhibited changes in forms and functions, all tending in the same direction with the assumption of a common state, called the *Typical culture*.

VIII. In the courses involved there were stages, either in the beginning or en route, in which the function of the bacterial protoplasm was such that following its injection into animal tissues, reactions resulted in disease pictures. These imitated those in human cases, in both the generalization, and the points of local emphasis.



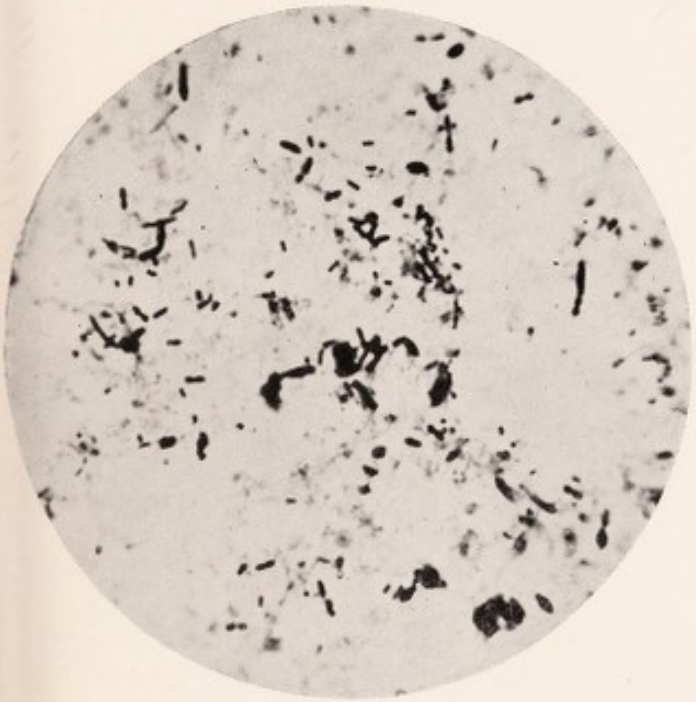
1

Coarse, Gram positive rod forms as seen in the blood culture, obtained during life, from "epidemic parrot" No. 1.



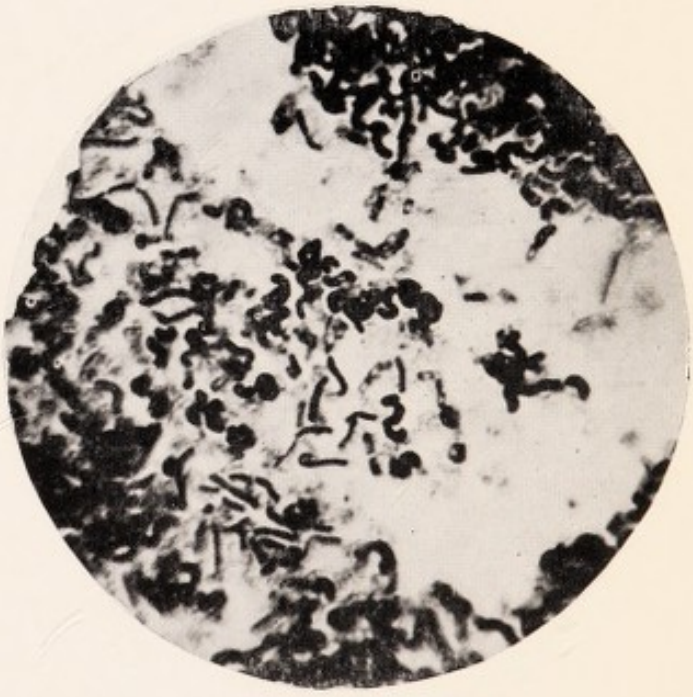
2

Forms in nasal mucus in an "epidemic parrot"; Gram positive, primary rods occupy the centre of the picture. They are recognized best by their curving shape. The type may be much better seen in plate No. 7.



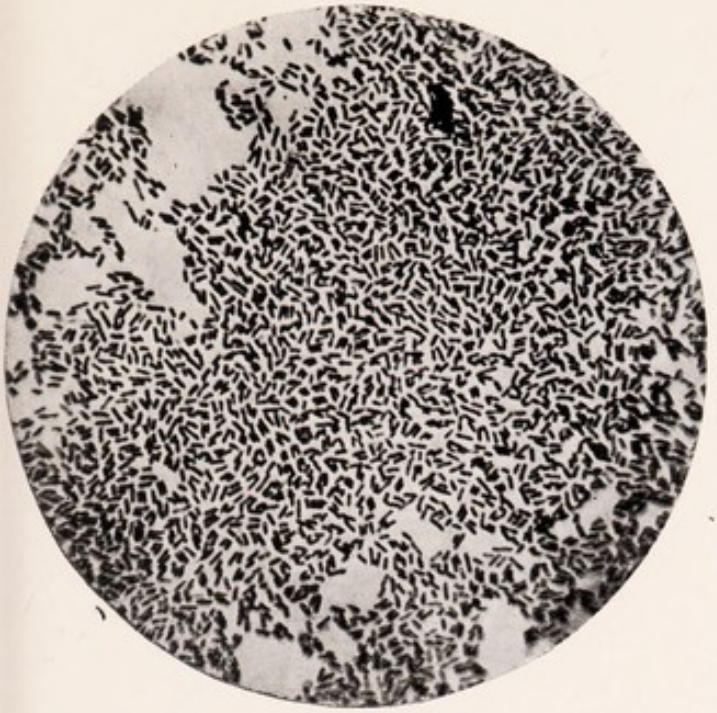
3

Bacterial forms in the droppings of an "epidemic parrot." Gram positiveness prevails. Irregularity is marked. The large rods, frequently referred to, are prominent, associated with various coccal forms.



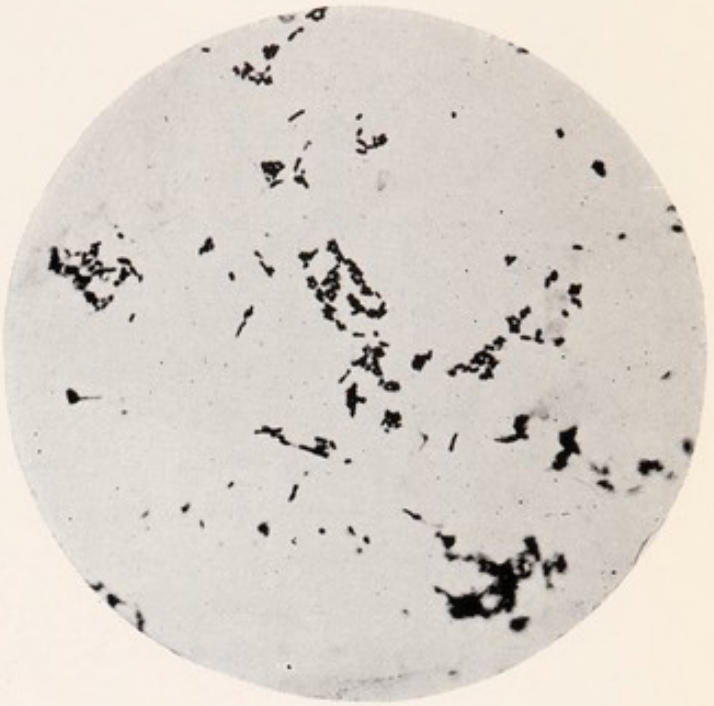
4

Remarkable exhibition of transition forms, as well as terminal, Gram negative rod forms, one of which lies to the right of the centre. These appeared in the course of a metamorphosis, involving bacterial protoplasm from an "epidemic parrot." This was growing on blood agar. The final result may be seen in the next plate.



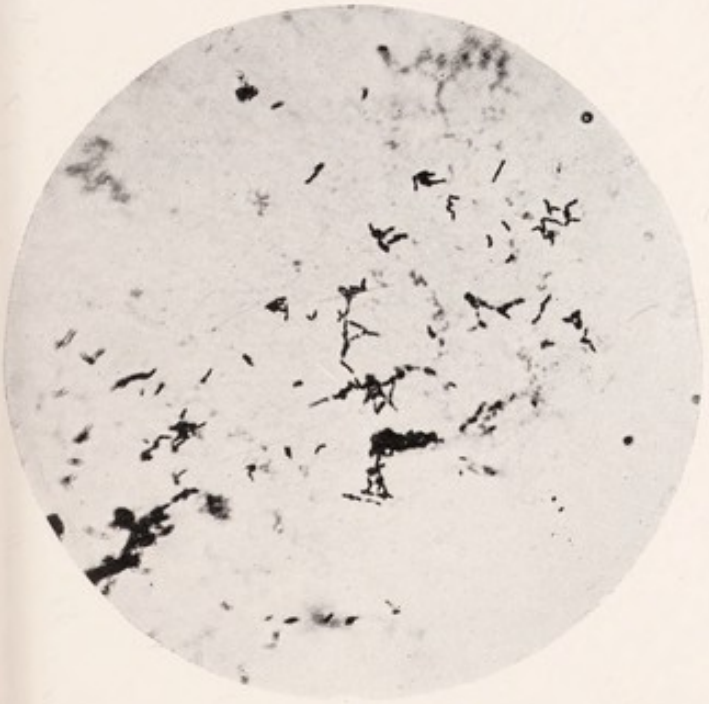
5

This shows the end result of the metamorphosis, exhibited in transition by the previous plate. These are very fine, slim, Gram negative rods, i.e., unit size is much reduced, but there is a rather unusual number of individuals, aberrant in shape.



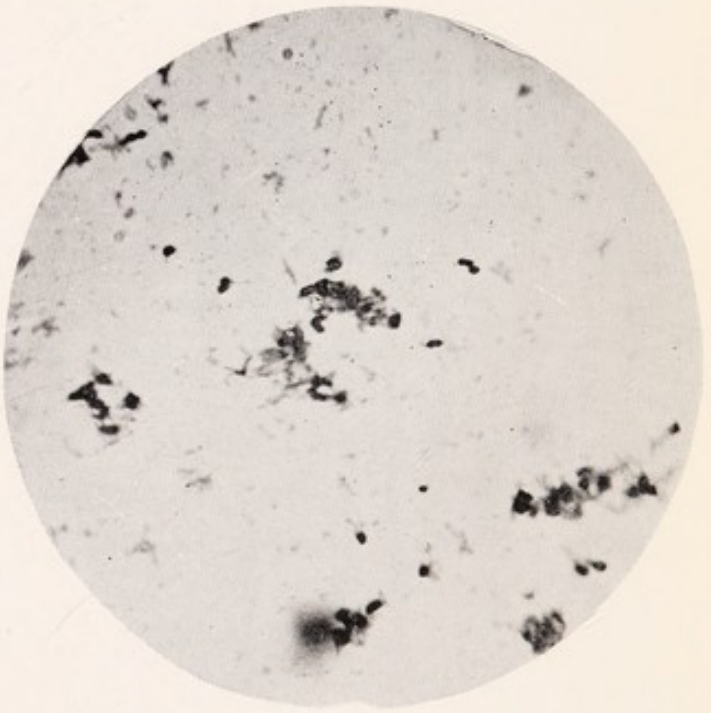
6

Primary rod forms: Initial stage of the metamorphosis of the blood culture from Case No. 7, in the human series.



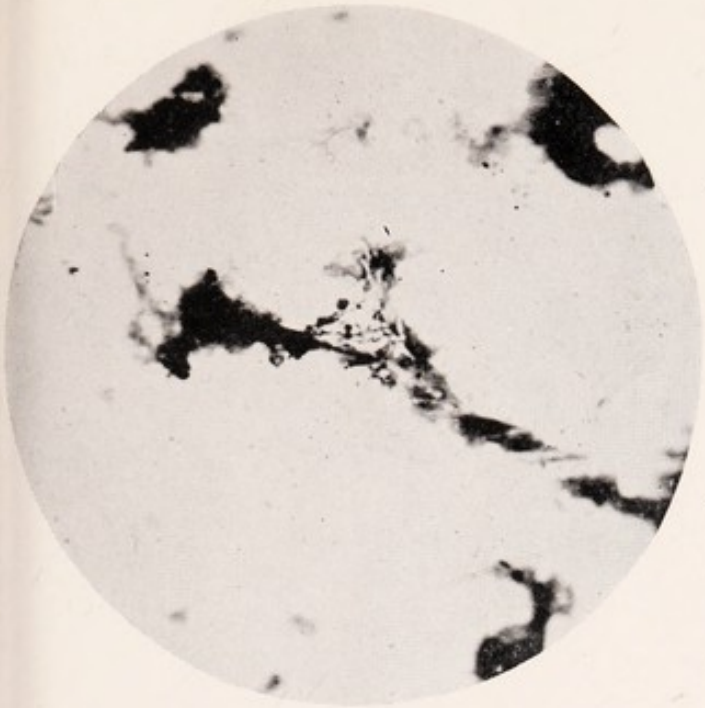
7

Primary rods, showing, in some areas, the beginning of coccal development. These were the first stages in the metamorphosis which took place in the blood culture from the first patient in the human series, Case No. 1.



8

Transition stage in metamorphosis, exhibited in its beginning, in plate No. 7. Faded primary rods are well shown, as are the rapidly developing coccal forms.

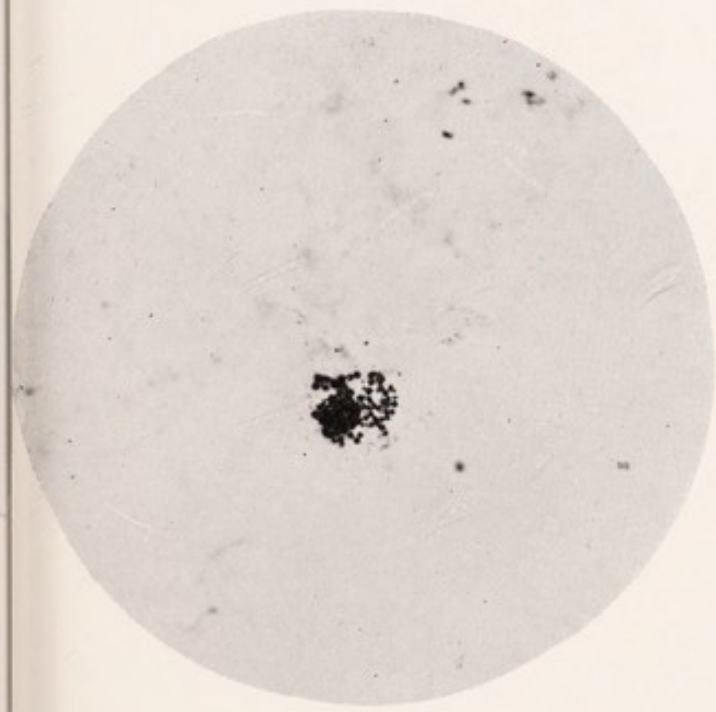


9

A transition stage, similar to that shown in plate No. 8, and taking place in the same culture. This shows in the bodies of the faded primary rods, tiny dots, at which point it seems likely the coccal forms, one of which is here shown, have their beginnings.

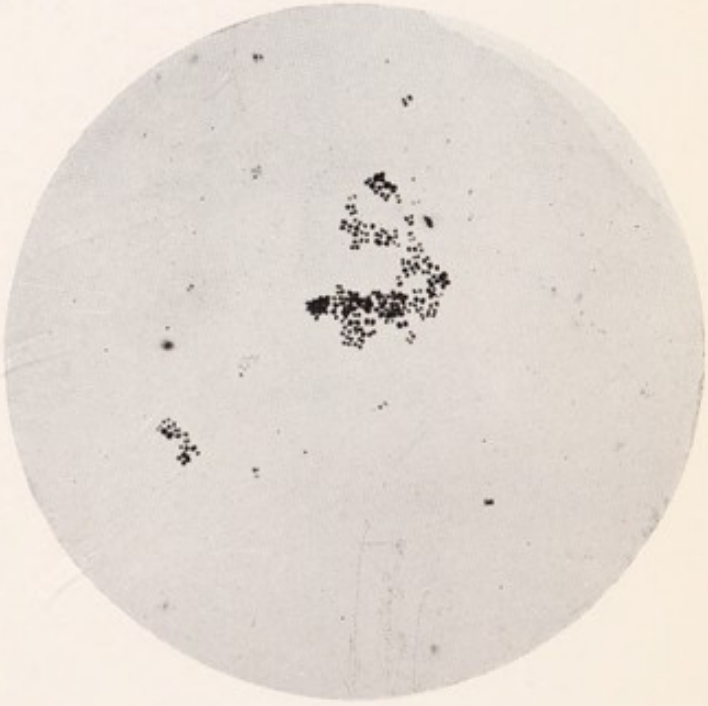
10

This is a stage between those represented in plates 9 and 11, a transition stage similar to that shown in plate No. 9. Here the ends of primary rods are becoming globular in an assumption of coccal shapes. The result is the Gram positive, pear shaped form, referred to in the text. The smear was from the blood culture of Case No. 5.



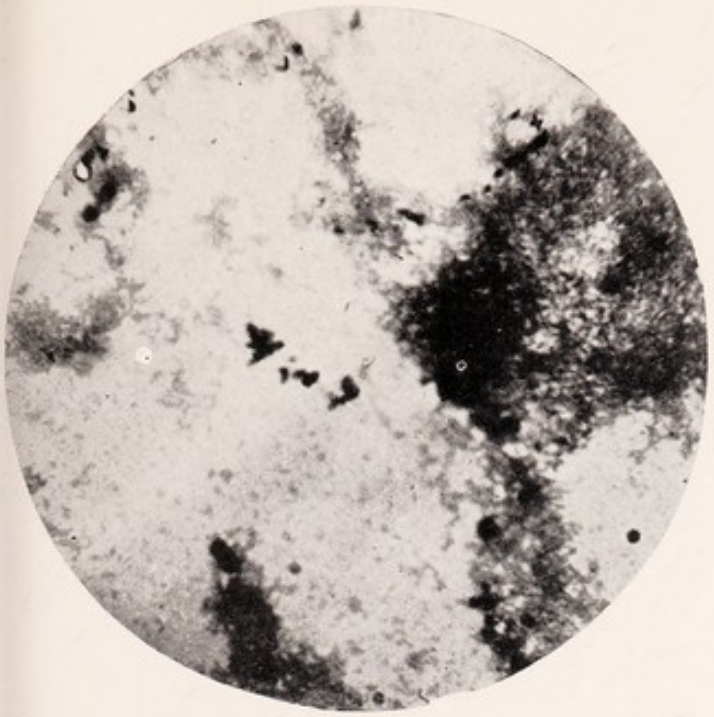
11

Gram positive cocci, seen in the second stage of metamorphosis which took place in blood cultures from the first patient in the human series, Case No. 1.



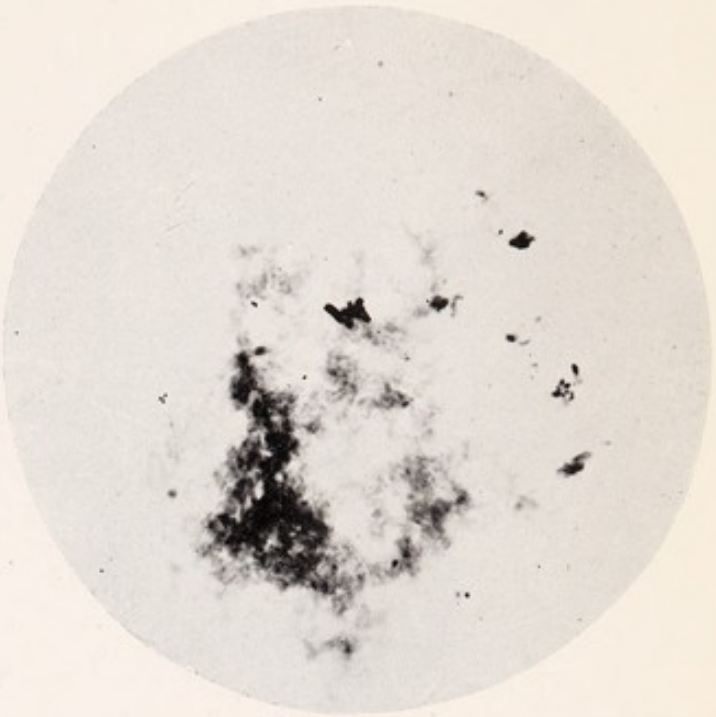
12

These are irregular, Gram positive, coccal transition forms, such as were frequently seen in the metamorphoses taking place in the blood cultures obtained from human cases during the epidemic.



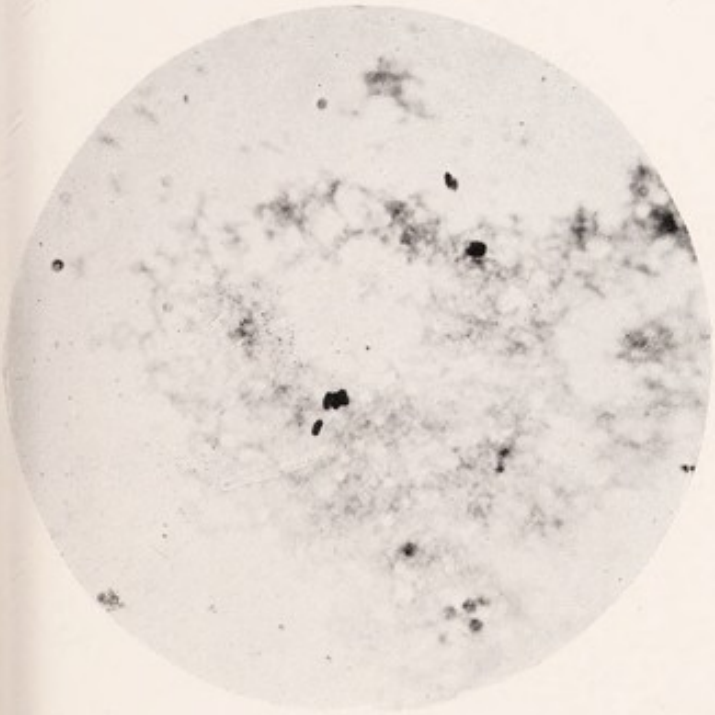
13

The transition stage in the same metamorphosis, in which coccal forms seem to be fused in very irregular but large and somewhat rod-like shapes. These were all Gram positive.



14

This is a transition state comparable to plate No. 13. It was observed in blood culture from the sixth patient in the human series.



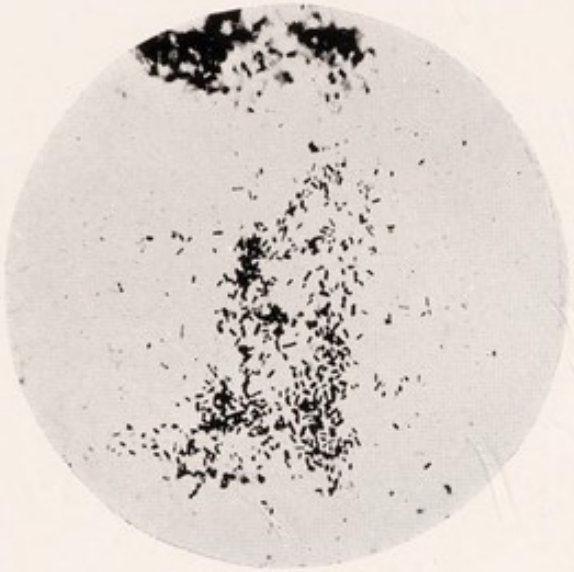
15

Stage in metamorphosis, observed in the blood culture of second patient in the human series. Forms are all short, plump, Gram positive rods.

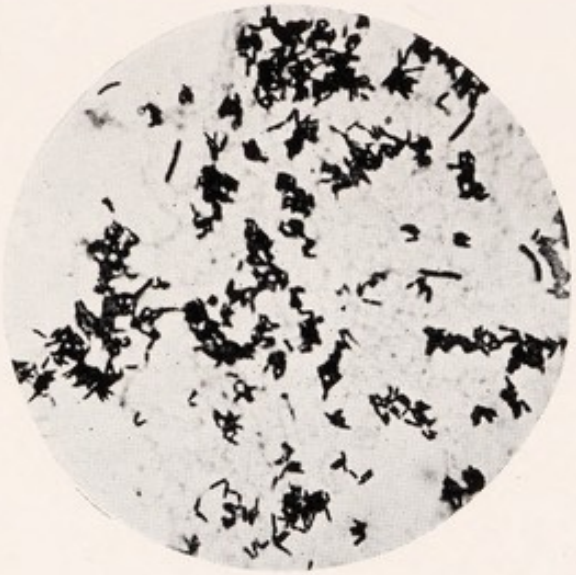
17

Here are to be seen, in the main, Gram negative rods. Although rather large and irregular in density, this was the end of the metamorphosis as it was observed in the blood culture alone, in Case No. 5.

Note: See following page for Plate 16.

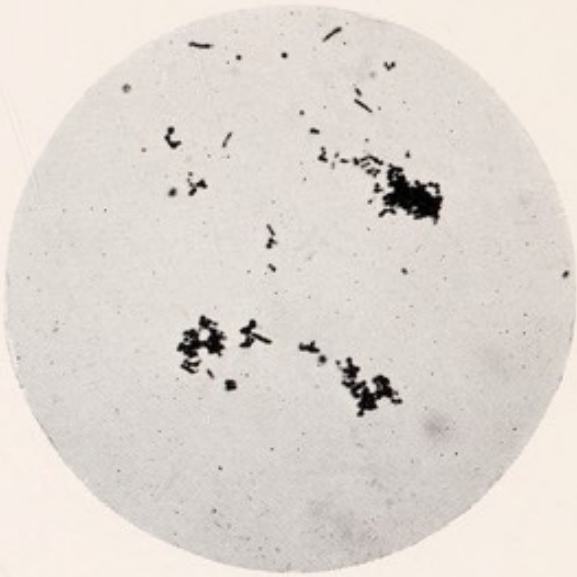


From blood cultures, human series, No. 13. In this plate all the forms are small Gram positive cocci and rods, existing in considerable numbers, but there are many individuals shown in which the assumption of a fine Gram negative terminal rod form appears very perfect.



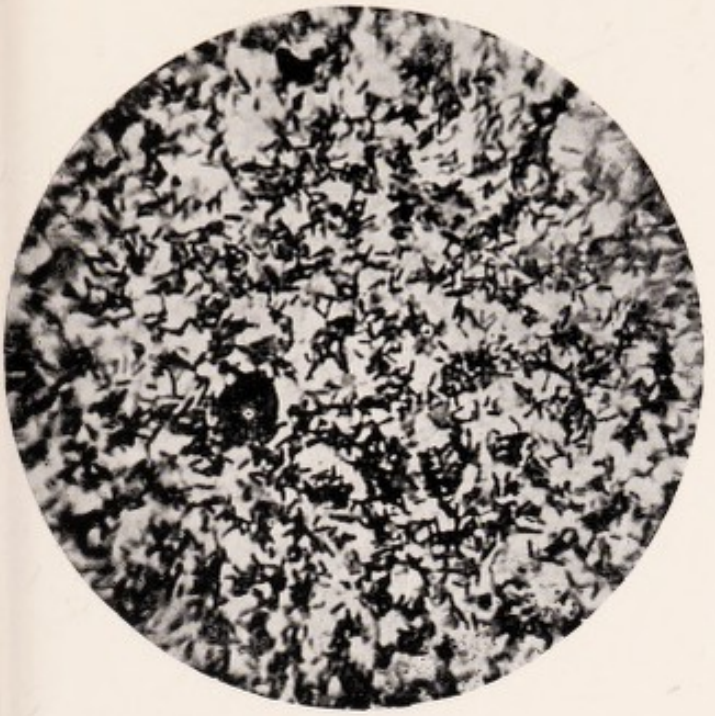
16

From blood cultures, human series, No. 9. Forms are mainly rods, mostly Gram positive, irregular in shape but more so in size.



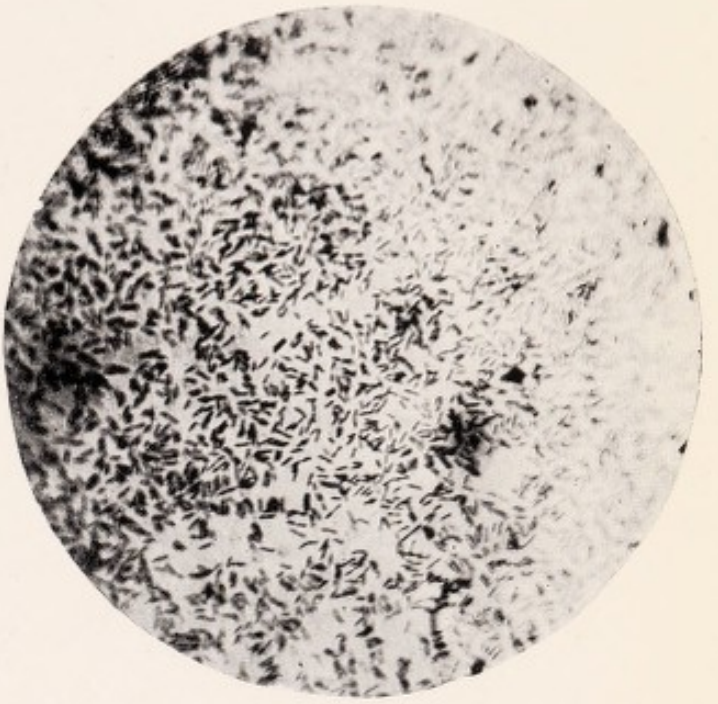
From blood cultures, human series, No. 5. Most of the forms are irregularly coccal. There are moderate sized, well formed rods, both Gram positive and Gram negative.

Three pictures showing the later stages of the metamorphosis taking place in human blood cultures. The forms in all are very mixed.



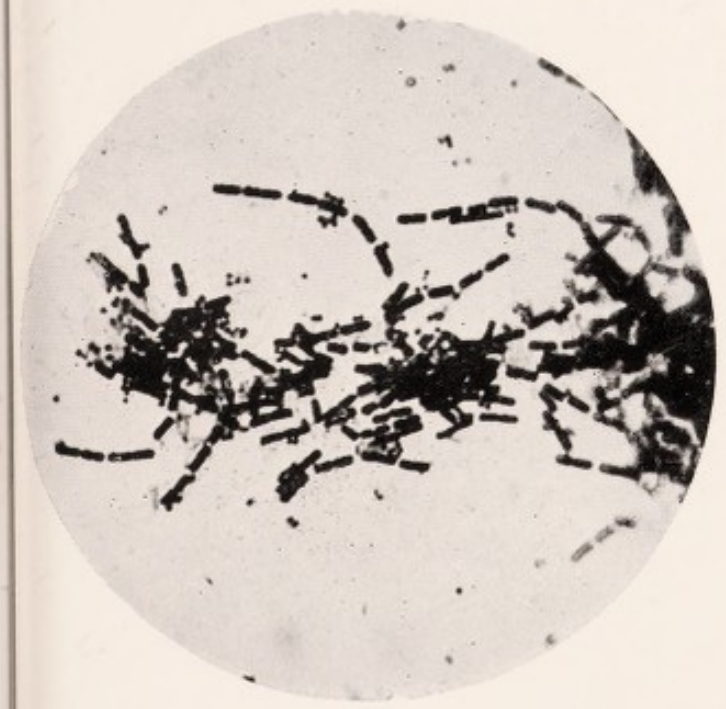
18

Gram negative, terminal rod forms. In the lung tissue of a mouse (a smear preparation). Many of the units are typical. There are also many coccal, diphtheroidal, and pear shaped forms. The picture corresponds to the very first mouse lung examined, (page 19) as well as to the lungs of mice injected with typical culture 1522.



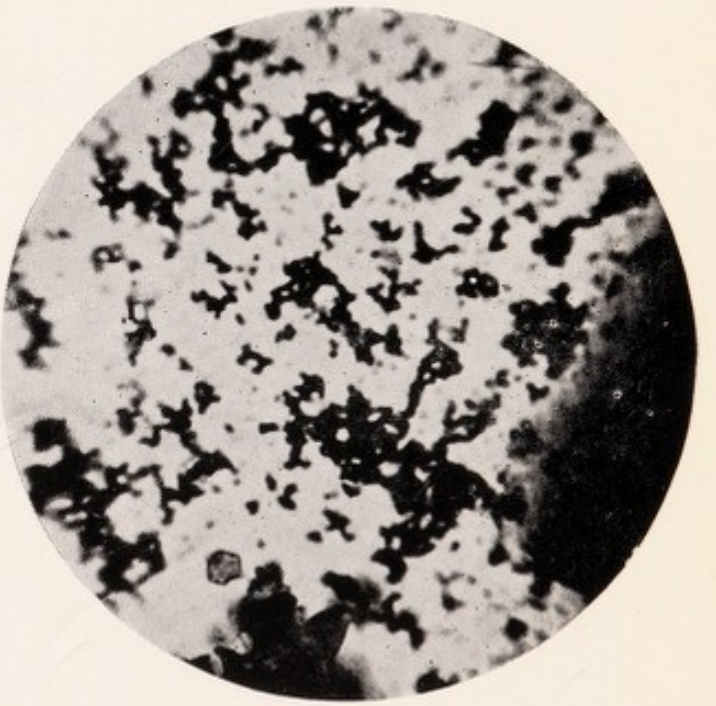
19

Fine Gram negative rods, seen in the smear of a culture in plain boullion, obtained from the lung of the first mouse shown in plate No. 18. The units are of an especial slimness. This organism was the first, from human sources, observed to cause green pigmentation.



20

This is the coarse Gram positive rod; the form frequently referred to, occurring in cultures from many sources. As will be observed, chains are formed of the bodies, slightly irregular in outline. A few of the spaces frequently referred to in the text will be seen. Cocci also are present; a frequent occurrence. Smear was from the blood culture of Case No. 9.



21

Amorphous-like units of bacterial protoplasm taking Gram positive stain, in the feces of a healthy parrot. There are only slight tendencies toward the assumption of rod and coccal shapes.

Chapter III

THE END RESULT OF CHANGE IN FORM AND FUNCTION. THE STUDY OF THAT COMMON STATE, THE SO-CALLED TYPICAL CULTURE, ASSUMED BY THE BACTERIAL PROTOPLASM FROM THE VARIOUS EPIDEMIC SOURCES, WHEN UNDER THE INFLUENCES OF A COMMON ENVIRONMENT, AND IN THE VARYING COURSE OF TIME

The Common Ground:

From all the sources of bacterial protoplasm indicated, if a slim Gram negative rod were obtained, when grown in plain bouillon, it so remained. Although colonies were "pure" on agar, unless smears of the bases were made, bouillon showed many aberrant individuals varying from diplobacilli to cocci. There were always units showing some tendency to be stained by Gram's method.

If other forms were observed at first, in time, whether left in the same container or passed through artificial media, more or less *typical Gram negative rods* finally appeared. This was frequently observed as a sudden change, but at times the transition was slow, many intermediate forms being seen.

Clothed in the full splendor of their role, these tiny delicate organisms emitted the odor of the swill barrel on a hot summer's day, and their digestive capacities were limitless. Injected into an animal, hemorrhages were especially concentrated in the lungs, diffuse, or restricted mainly to the intestinal tract. Apparently, balancing or complementing the hemorrhagic capacity is one for an increased intensity of putrefactive changes.

The parenchymatous organs exhibited, under the microscope especially, besides hemorrhagic changes, a destruction of the cytoplasm, while the nuclei remained remarkably distinct.

Thus far, most of the emphasis has been laid upon the many pathways leading to one spot. Not only have comparisons been drawn between those paths originating in parrots, and those having their source in human beings, but more in detail, all ways and branches have been followed for a view of the curves of changing forms and activities, the lengths, and the destinations. We may parallel the conditions at the sources. There were strong resemblances. The degree of possible variation was the same and the courses frequently varied similarly. The terminations of the courses were located within the space of a small circle. Before ending this, the first part of the investigation, the small circle where the pathways met, must be investigated. It was merely a state. In plain bouillon, certain facts were quite consistently true of such bacterial protoplasm. At all the sources there must have been, more or less latent, all the possibilities which became realities at the common ground of meeting.

Were the conditions reversible? Could the realities again become mere possibilities? Could the pictures at the sources be reassumed? Might the pathways of descent be reascended? Latency, of course, is only relative. What lay within the circle? To supply an answer, there was chosen the plain bouillon complex, reached quite deviously from the marrow of the second parrot. This is the source suggested for diagnostic material by Dieulafoy. Such a study, practically a dissection, could only be effected by subdivisions of the mass employed, with or without preliminary disturbances in the delicate balance instituted by the environmental adjustments.

When such a culture was first considered as a chemical whole, the idea was also conceived of that the real units were chemical. It was feared that if study was restricted to unit forms, the really active agents would be overlooked. Of course the first idea was to filter out the formed bodies of bacteria. While awaiting a filter, preliminary work was begun by centrifuging out the greater number of units. The almost equal destructive-

ness of the cleared and the richly populated preparations was most astonishing. The use of the centrifuge thus provided a special method for subdividing, or partitioning this culture. The results will be detailed under the second method of subdivision.

METHODS EMPLOYED:

After a description has been made of a Typical culture, the results will be given of the reactions in animal tissue of parts of the culture obtained by the following methods of division and partition:—

I. Simple, gross subdivision.

II. Subdivision by the use of the centrifuge.
   (a) Supernatant fluid versus suspended sediment.
   (b) Subdivision of sediment.
   (c) Subdivision of supernatant-contained units, by selective elimination through the use of various agents.

III. Subdivision naturally effected by the development of colonies on plate.

IV. Subdivision of the bacterial matter by segregation in animal tissues (lung areas employed) following the injection of the whole culture.

THE COMMON GROUND IN PARTICULAR:

Typical Culture No. 1522
Procured From a Sick Parrot During the Epidemic

HISTORY:

A glucose bouillon culture of the marrow of the second parrot, and which grew as a grey staphylococcus on blood agar, was injected into a mouse. From the mouse was obtained a rather coarse rod with air spaces which, upon agar, formed a dry, greenish, furry colony. This slowly became smooth, moist, and green, and the organisms were found to be fine, slim, *Gram negative rods.*

CHARACTERS:

Smears of a bouillon culture showed the majority of organisms as slim, Gram negative, single and double rods. There were also some Gram positive rods, also, coccal forms. The motility was of high degree. The organisms were usually agglutinable by normal human serum, in dilutions of 1–40; with some sera, only in a strength of 1–20; by occasional sera, they were not agglutinable. The organism was aerobic, facultative-anaerobic.

In plain bouillon of this type, the growth was very rapid, a high degree of richness being acquired in twelve hours, with a well marked pellicle, and a slight sediment. In time, the pellicle broke up, and, with the suspension, became transformed into a gelatinous mass, which has retained its vitality over many months. This bouillon, within twelve to thirty-six hours, emitted a most disgusting, putrefactive odor. This odor, less and less marked, finally disappeared. Indol was formed. A beautiful green pigment was formed. There were cultures in which these characters were absent. The pigment required an alkaline medium for its production, and free oxygen.

On blood agar, there was rapidly formed, a thick, slimy streak, shining and edged entire. It was typically green, and green haloed, in a wider zone of haemolysis.

The organism, typically, did not cause milk to coagulate, but, rather, liquefied it directly. It liquefied gelatin. It split saccharose and lactose and glucose, with the free evolution of gas and acid. On Russel's double sugar medium, it varied most frequently. The material was turned green after a preliminary decolorization.

RESULTS OF ANALYSIS, BY ANIMAL REACTION OF THE CULTURE, VARIOUSLY SUBDIVIDED

I. SIMPLE SUBDIVISION OF WHOLE CULTURE:

REACTIONS IN MICE:

No. 1547. .1 c.c. was injected, duplicating the results from the injection of the original culture.

No. 1548. .01 c.c.

DEATH:

48 hours.

AUTOPSY:

The organs retained their color fairly well. Lungs were congested, with large, dark areas. Intestine was slimy. Kidneys were large and pink. Liver congested and degenerated. Parenchyma degenerated. Splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Peritoneum: Many cocci as well as Gram negative rods.
Lung hemorrhages: Typical rods.

No. 1549. .001 c.c. failed to kill a mouse in a reasonable time. After some weeks the mouse escaped.

In the smears of No. 1548, there was seen a considerable assumption of round forms. No. 1549 was, apparently, negative. Otherwise, subdivision in these tiny animals was of little value in revealing the latent powers of the bacterial protoplasm.

REACTIONS IN RABBITS:

No. 87. Two c.c. original (3 month old) whole culture.

CLINICAL HISTORY:

Progressively ill.

DEATH:

4 days.

AUTOPSY:

Profound inflammation with hemorrhages of the testicle.

SMEARS:

Testicle: Gram negative rods, also irregular Gram positive forms.
Lungs: Cocci.
Chyme: Gram negative rods.
Spleen: Tiny Gram positive forms.
Nose: Tiny Gram positive forms.
Bile: Tiny Gram positive forms.
Heart: Tiny Gram positive forms.
Appendix: Tiny Gram positive forms.

CULTURES:

Plain bouillon: Gram negative rods, varying in form, but the characteristics of the cultures were typical of No. 1522 and practically all exactly alike.

A 24 hour culture showed the typical fluid pellicle, and very regular Gram negative rods, while the suspension beneath showed more variation, coccal forms and Gram positive rods.

Here, selection or subdivision, upon a basis of age, resulted in a very peculiar reaction, rather a slow death and unusual localization, but out of this the culture arose typical.

No. 1625. 5/12/1917. 2 c.c. twenty-four hour whole culture.

DEATH:

18 hours.

AUTOPSY:

Lungs showed large hemorrhages. Some congestion of the stomach. Intestine gassy, feces soft. Gall-bladder was distended. Brain was congested.

SMEAR:

Lungs: Gram negative rods, especially in the hemorrhagic areas.

Bronchus: Same.

Stomach: Same.

Heart blood: Same.

Spleen: Same.

CULTURE:

Gram negative rods, except that of the bronchus, which showed Gram positive rods.

No. 1733. 6/25/1917. .5 c.c. (6 days) whole culture.

DEATH:

12 days. (Dependent upon both subdivision and age).

AUTOPSY:

Abscess of the lungs. Parenchyma degenerated. Splenic tumor.

URINE ANALYSIS:

Indican: ++++

Albumin: +

SMEARS:

Lungs: Pus cells and Gram positive rods. Other areas were indefinite.

CULTURES:

Heart: Irregular Gram positive forms.

Bile: Same.

Lungs: Gram negative rods. After 2 months the heart culture on a plate showed a flat, grey growth. Smear showed Gram negative rods and cocci.

The heart culture No. 1733, demonstrated a metamorphosis.

No. 1550. 4/12/1917. .25 c.c. twenty-four hour whole culture.

DEATH:

6 weeks.

AUTOPSY:

Suppurative arthritis in left knee joint. Marked anemia and emaciation.

The parenchymatous organs, microscopically, showed scattered deposits of round cells.

BACTERIAL FINDINGS:

CULTURES:

Gram negative rods.

SUMMARY:

The relationship between the reaction following the injection of 2 c.c. of a plain bouillon culture and amounts below twenty-five per cent of this, exhibited a marked disproportion. The smaller amount induced only chronic disease. In the different pictures, form and function assumed those garments cast off in the courses of changes which took place and were exhibited in the human series previous to the assumption of the characters of such a state as culture No. 1522.

The results from the use of small amounts did not demonstrate the powers which must have existed in the tiny air born particles, which may be supposed to have caused the cases of disease during the epidemic.

II. SUBDIVISION BY THE USE OF THE CENTRIFUGE (Culture No. 1522):

This method was employed, as related, while awaiting the arrival of porcelain filters. The results provided facts of profound significance.

(A) SUPERNATANT FLUID VERSUS SUSPENDED SEDIMENT:

ANIMALS:

RABBITS:

No. 1642. 5/21/17. Two c.c. green supernatant, obtained by three hours centrifugation.

DEATH:

Twenty-four hours.

AUTOPSY:

Lungs congested, very markedly. Some enteritis. Parenchyma degenerated. Splenic tumor. Feces in colon.

BACTERIAL FINDINGS:

SMEARS:

Blood: Negative.
Lung: Negative.
Stomach: Few, irregular, Gram positive forms.
Spleen: Same.
Appendix: Same.

CULTURES:

Lung: Negative.
Gall-bladder: Gram positive diplococci.

No. 1641. (Pregnant, nearly at term). 5/21/17. Sediment of 2 c.c. of same culture as No. 1642.

DEATH:

Forty-eight hours.

AUTOPSY:

Hemorrhages of lungs, stomach, heart, and intestinal tract. Kidneys were pale and mottled. Parenchyma degenerated. Splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Blood: Plump Gram positive rods and cocci.
Heart: Same.
Intestinal hemorrhages: Same.
Lung: Slim, Gram positive-negative rods.

CULTURES:

Gram negative rods, colonies, grey, haemolizing.

No. 1653. (Pregnant). Two c.c. supernatant, obtained after same manner.

DEATH:

Under twelve hours. (Rapid reaction, as usual in pregnant animals). Further records lost.

No. 1666. 5/28/17. Two c.c. supernatant.

DEATH:

Under twelve hours.

AUTOPSY:

Right lung diffusely congested. Left lung showed what appeared to be beginning consolidation. Stomach showed some sub-mucous hemorrhages with splenic tumor. Rest of the organs were negative.

BACTERIAL FINDINGS:

SMEARS:

Lung: Gram positive rods.
Spleen: Gram positive rods and diplococci.

Sediment of this culture was used in the next series.

RABBIT:

No. 1669. 5/28/17. Sediment from two c.c.

DEATH:

Eight hours.

AUTOPSY:

No hemorrhages. General loss in outline and color differentiation, with destruction of parenchyma, and splenic tumor.

SMEARS:

Gram negative rods.

RABBIT:

No. 1698. 6/7/17. Two c.c. supernatant, *eight days old.*

DEATH:

Forty-eight hours.

AUTOPSY:

There were large hemorrhages of the left lung, a few hemorrhages of the appendix. Post mortem changes were very advanced.

SMEARS:

Lungs: Great number Gram negative rods.
Heart: Few Gram negative rods.

CULTURES:

Typical.

No. 1699. 6/7/17. Sediment two c.c. of same culture.

DEATH:

Forty-eight hours.

AUTOPSY:

Showed hemorrhages of the right optic thalamus with congestion of the brain. The lungs were slightly congested. Post mortem changes were quite advanced.

SMEARS:

Brain: Gram negative rods.

CULTURES:

Brain: Typical rods; motile, agglutinable. Produced green in cultures.
Heart: Typical rods; motile, agglutinable. Produced green in cultures.

No. 1730. 6/25/17. Two c.c. supernatant (six days old).

DEATH:

Thirty hours.

AUTOPSY:

Thymus showed hemorrhages. Heart showed hemorrhages. Lungs, one large hemorrhage. Stomach, appendix, and rectum, hemorrhages. Feces normal. Parenchyma degenerated. Splenic tumor.

SMEARS:

Heart blood: Irregular forms, from mere dust to cocci, and occasional Gram negative rods.
Intestinal hemorrhage: Same.
Spleen: Same.
Lung blood: Same.
Hemorrhagic areas: Same.
Normal area: Same.

CULTURES:

Gram negative rods.

More extended studies of the use of the supernatant fluid will be found in a later chapter. But the fact here was demonstrated that the bacterial units seen in the smears of the supernatant fluids, although present in such slight numbers as to cause frequently no clouding whatever, had a power equal, and often superior in mere force, to the great numbers in the whole sediment.

(B) SUBDIVISION OF SEDIMENT: (Measured according to the amount of the whole culture employed).

ANIMALS:

RABBITS:

No. 1671. (Pregnant rabbit). 5/28/17. One c.c.

DEATH:

Twenty-four hours.

AUTOPSY:

Masses of hemorrhage in small intestine, appendix, and rectum. Parenchyma degenerated. Splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Appendiceal hemorrhage: Gram positive-negative rods. The action of one c.c was enhanced, of course, by the condition of the host.

No. 1679. 6/4/17. .5 c.c.

DEATH:

Four days.

AUTOPSY:

Few hemorrhages of cæcum, congestion of lungs. Cholecystitis.

SUMMARY

By the methods of analysis described, this *Typical culture* was observed to be capable of causing the pneumonic, influenzal, and intestinal types of this general disease. The different stages in form and functional changes observed in the studies of the epidemic series leading to the development of this culture were reproducible, and metamorphosis and functional readjustment were observed.

However obtained, all units from this culture, whatever their initial form or reaction, in the end produced a *Typical culture.*

But at a given time there was demonstrated a distinct division of labor among the units, a remarkable difference in those functional capacities relating to disease production, not only in the absoluteness, but also in the preciseness of power. This was true equally between the units remaining in the supernatant fluid following centrifugation, and other units in general.

In the exhibitions by the supernatant fluid, we have suggested that excess of power in proportion to the associated mass of material, which must have characterized the protoplasm which floated upon the air at the time of the epidemic.

CONCLUSIONS

First, there appears to be a general increase in the power of all units representing the bacterial protoplasm. Then the power, or intensity of reaction, underlying the affinity for animal (including bird) tissues, is refined and segregated, as demonstrated by the study of the supernatant fluids of centrifuged *typical cultures.*

The effects of contact between the personal saprophytic bacterial protoplasm from the intestinal tracts of different birds, undergoing this stimulation at the same time, have not been studied.[1] Nor has there been investigated the effects upon the bacterial protoplasm of contact with parrot tissues. There must frequently have taken place such transfers of bacterial protoplasm from the intestinal tract, not only to the tissues belonging to the body of the same bird, but to the tissues of others, and from these been transmitted on through an unknown number of individuals. How much more intense the power became under such natural influences than it did under those which were artificial, can only be imagined.

Admitting these untested possibilities, the picture may be thus simply drawn. *The whole group of parrots, on the train en

[1] For the sake of simplicity, the normal respiratory flora of parrots has not been investigated beyond the point of the recognition that it resembled that in the intestines, but the same conception should hold good, also, for this region.

* EDITOR'S NOTE: This, and the following two paragraphs were questioned by the author but it has been thought best to leave them in.

route from the point where they were first chilled, and in the store, were one great culture. In the intestines of many birds, the bacterial protoplasm received the stimulus provided by an excess of exudates from the gut walls, practically, tissues in solution, natural preparations, imitated by artificially prepared plain bouillon. The "culture," comprised by the group of parrots, was then inoculated by this bacterial protoplasm, aroused to a state of activity already capable of demonstrating an actual affinity for living tissues. Acceleration of this activity of the bacterial protoplasm occurred in the tissues of the parrots, which formed, naturally, the principal component of the culture. This attack was made easy by the undermining of the birds' resistance, due to the chilling of their bodies. The effect of cold has the well known influence of reducing the resistance of higher organisms against bacterial infection. All about the parrots, dead animal material was deposited. Thus, there existed a state of affairs, to which that entity known as "the Typical culture of generalized bacterial protoplasm in plain bouillon," may roughly be compared.

The parallel between the two becomes more evident, if one considers that the factor provided by the bird bodies, in the parrot group culture, is represented in the Typical culture, by the bodies of the rabbits through which the bacterial protoplasm was passed, previous to the development of the Typical culture. In the latter, it has been shown that the power of the bacterial protoplasm, providing the affinity for animal tissues, was segregated in the "bodies" of certain units, which were proved to be the lightest of all the units in the culture by the fact that they remained in the supernatant fluid after the culture was centrifuged.

In the culture composed of the group of parrots, the action of the centrifuge was represented by the force of gravity. The more saprophytic portions of the protoplasm, clumped together, and therefore being heavy, remained upon the ground, while the units of disease-bearing power floated above and about the parrots, in the air, represented by the supernatant fluid of the *Typical culture*.

Thus, all bacterial protoplasm, as finally distributed to human beings, was in a high state of activity. Energy had flamed up and become disproportionate to its associated material mass, or we may say, every particle of material mass involved in the reactions when the epidemic was active among the parrots, was aflame with energy. In human beings, there occurred first a period during which the enormous energy carried in the tiny particles of matter was producing protoplasm, which was being diffused throughout the body. This is the period for which there has been no true, experimental analogue provided. Vaughan has explained it, especially in relation to the disease producing activities of members of the typhoid-colon group, as time consumed by reproduction on the part of the bacteria, and specific ferment mobilization on the part of the animal organism. He considers its termination as taking place at the moment when the body ferments begin to feed upon the bacterial substance. In the case of the generalized bacterial protoplasm under consideration, the only modification to this conception suggested by the investigation, is that the bacterial attack and the reaction of the body attack and the bacterial reaction, comprise a process which begins immediately, and which, very slowly, and gradually, generally, and diffusely develops. Specific ferments would seem undevelopable against such generalized bacterial matter (see agglutination tests). Such material was diffused in the zone about the parrots and alighted upon human tissue. Whatever the process of incubation, the disease, here, was general, with special exaggerations of signs and symptoms referring to the respiratory or gastrointestinal tracts, but variation in the clinical pictures was the rule.

The variations of the clinical picture, possible because of the lack of character fixation by the bacterial protoplasm, may be conceived of as dependent upon:

1. Accidents of initial deposit whether in the nose, or swallowed.

2. After the general distribution, upon the proportion between the total intensity of reaction-activity, and the bacterial mass supporting the energy represented.

Other things being equal, the pneumonic disease pictures indicated the greater reaction intensity on the part of the invading bacterial protoplasm.

A natural conclusion would be that the general lack of fixation in any one so-called character of the protoplasm, so frequently observed, must hold true also for the relationship between the protoplasm and this character of great activity in animal tissues. And indeed, there has been found experimentally, in the studies upon the supernatant fluids of *Typical cultures,* a dissolution of the relationship between the bacterial protoplasm and the degree of affinity it may exhibit for a rabbit's tissues, as far as the supernatant fluid units were concerned; i. e., a rapid dissipation of bacterial energy, per unit of mass after a sojourn in an animal, although plain bouillon rapidly reactivated. At any rate, in the first line of human bodies, as clinically reported, the epidemic faded, and great masses of bacterial protoplasm of a generalized character then lay about the human community with hardly more than saprophytic power. The epidemic was over.

It was this protoplasm that the author first procured; protoplasm capable of various degrees of activity in, and affinity for animal tissues; and, which of course, being generalized, under the influences of a common environment underwent metamorphosis and adjustment of function, until a common ground was reached, the *Typical Culture,* which developing in plain bouillon, provided such far reaching results.

PART II

INTRODUCTION

The next step was the search for the source from which this bacterial protoplasm could have originated. Naturally the first region investigated was that where there existed the most bacterial life under normal conditions closely associated with the parrot. This was of course the intestinal tract. In this and the two succeeding chapters, there will be described, first, healthy parrot feces taken as a whole, the variations in unit form, the interchangeabilities of form, at least between units. Also, there will be shown the great degrees of latency in functional capacities exhibited by subsistence in both living and dead matter, and the adjustments made to environment.

The artificial arousal of these latent powers will be related, and the gradual changes, step by step, associated with an increasing ability to live far from the natural habitat, the gastro-intestinal tract. The form will be shown, subordinated, irregular in the more definite cases of disease, fixed in a happy environment, but bearing little real connection to the physiology. In the course of the accretion of power, disease pictures comparable to human diseases during the epidemic will be exhibited, and the bacteriological studies upon the animals will be observed frequently to parallel those upon human and parrot material.

A common type of artificially produced culture will be exhibited, its relative stability established, and its character in particular compared to the specimen No. 1522, obtained from the epidemic parrot. It will be seen to be a state of adjustment to specimens from human and parrot epidemic sources, and experimentally obtained from healthy parrot feces. This will be studied generally, as a complex whole. From it will be reported the extraction by protoplasm, of a power in its comparative associated mass, almost supreme and quietly lying in the supernatant fluid, resulting from the centrifugation of a whole culture.

Chapter IV

NORMAL PARROT FECES

FORMS IN SMEAR

The smear of a parrot feces plate showed only indefinite forms, rather sparse, almost Gram positive, i. e., taking the opposite stain to that taken by the rod forms obtained as an end result in the epidemic cultures. Suspending it in salt solution and centrifuging provided more shapes to study, and there were seen many and bizarre units, some approaching rod and coccal types, and rarely a Gram negative form.

FORMS IN CULTURES

The first direct bouillon cultures from three separate specimens (three parrots) provided a very large variable rod, Gram positive, or Gram negative, motile or non-motile. In one case, in bouillon, in 48 hours there developed a Gram negative rod, growing typically upon a plate and in a sub-bouillon culture. Another specimen grew upon a plate in a homogeneous streak, showing forms varying in size, while sub-bouillon cultures showed more variation in form than in size. A third, with the same beginnings, grew as a staphylococcus on plates, a re-bouillon of this showed neither fine rods nor coarse cocci, but the original large rods and fine cocci. A fourth, large rod, causing coarse, dry, saprophytic types of colonies on a plate, and which showed profoundly aberrant forms, grew in a sub-bouillon culture as a slim rod.

FUNCTIONS IN ARTIFICIAL MEDIA

The results of direct inoculation of material from the feces into various media, may result in variations of function com-

parable to those of form. The digestive capacities are very generalized. In plain bouillon at first, there may be only a pellicle. Later, streams from this descend, and finally populate every nook and cranny. Indol may be formed. Glucose, lactose and saccharose can be split and acid and gas evolved. Milk may be coagulated, and gelatine liquified. The development of a green coloration is never observed, unless contact has been effected with animal tissues.

PARROT FECES (NO. 2)

FUNCTION IN LIVING TISSUES

The functions of the bacterial protoplasm in living animal tissue, far from the gut tube, may be demonstrated by an experiment upon a mouse.

Two c.c. 48 hour, direct culture of healthy parrot feces, in plain bouillon, was injected.

DEATH:

In 18 hours.

AUTOPSY:

Lungs, generally brownish, without hemorrhages. Peritoneal fluid, increased. Stomach, pale. Intestine, congested. Feces, normal. Liver, very soft and rather pale. Kidneys, swollen. Large dark splenic tumor.

BACTERIAL FINDINGS:

Peritoneum: Smears showed great numbers of large, single, Gram positive rods, rare mixed and Gram negative forms.

Cultures showed the same forms, also cocci.

Spleen, heart blood, and lungs: Smear and culture showed moderate numbers of the large Gram positive rods.

The same type of original culture, injected into the ear vein of rabbits, in about half of the cases, caused death with convulsions, in about one hour. Occasionally, where survival occurred, death supervened in a week, usually without any definite pathology.

PARROT FECES (NO. 3)

CHANGES IN FORM

ARTIFICIAL ENVIRONMENTAL VARIATION

Direct inoculation into:

PLAIN BOUILLON:

(a) Putridity, alkalinity, Gram negative rods. Slimy, colon-like streak on plate.

(b) Re-inoculation by single loop of bouillon into:
Plain bouillon: Same.
Glucose bouillon: Chained cocci, odorless.
Bile and Glucose: Chained cocci, Gram positive forms. Odorless.

GLUCOSE BOUILLON:

(a) Odorless. Acidity. Gram positive diplococci. On plate, slimy, colon-like streak.
(b) Re-inoculation into:
Plain: Moderately putrid. Mixed cocci and rods, in six days, slowly assuming Gram negative form.

BILE AND GLUCOSE BOUILLON:

(a) Odorless. Acidity. Gram positive, round, chained cocci. Slimy, colon-like streak on plate.
(b) Re-inoculation into:
Plain bouillon: Practically the same result as in case of glucose culture.

These results suggest that the form assumed depends upon the environment entered into, at least when the inoculation is made directly into a liquid.

PARROT FECES (No. 4)

PLATE COLONIES

Direct inoculation of the feces upon plates usually resulted in three forms of colonies. One caused by a rod having a tendency to moistness, transparency, flatness, colored a faint greenish or grey, or colorless. The second was the typhoid-colon type. The third was drier, more raised, breaking up light, hence usually white, and showing coccal forms.

RELATION OF FORM CHANGES TO ARTIFICIAL ENVIRONMENTAL VARIATION

Re-inoculation from plate colonies into plain bouillon, glucose bouillon, and bile and glucose bouillon, showed distinctly less submission in form variation to the new environment than was the case if inoculated directly from feces. That is, the coccal and transitional colonies showed more permanency in the form of units, only a few being rods among the cocci, more in the case of the transitional colonies.

VARIATIONS IN A GIVEN ENVIRONMENT

A series of six specimens was grown in plain bouillon. These were from bile and glucose cultures of the colon type of colony and of the staphyloid type; from plain cultures of an old staphyloid plate colony, transitional, and colon types, in bouillon, and an old bouillon of staphyloid type. These tubes were kept for 3 months, recultured twice in bouillon, grown upon plates, and for one period of 14 days were studied daily. A detailed study was certainly confusing, showing illimitable variations in form. But certain facts stood out: At any one time, when compared with another, the forms assumed in two different tubes might show a complete reversal.

Over a given period of time, certain tubes showed a great activity in form change, others a great quiescence. Certain tubes tended to a greater regularity than others. Always, in all tubes, some units were aberrant. Several times, the characteristics in plain bouillon were studied for the six cultures. These were, pellicle formation, suspension, sediment, odor and reaction. They tended towards a remarkable similarity in all the tubes.

Conclusions: Many interesting ideas were evolved from these observations, ideas already more than latent because of previous findings. Under normal conditions, parrot feces contain a common life represented to the eye in many minute units of matter varying in form. There appears a tendency to readjustments under variations in environment. There appears also a tendency to the reproduction of the adjusted form which tendency is enhanced by a close approximation of units somewhat resembling a crystallization. But, to characterize by the use of terms of form is useless.

The functions of this bacterial matter are general and comprehensive in relation to inert matter. Contact with more highly organized living matter results in the destruction of the latter. The commonly termed character of pathogenicity, is inherently latent, a fact demonstrated in the case of the mouse. The result was merely exaggerated by mass action. The study of functional variations under the influence of artificial adjustment of environment will be reserved for the next chapter.

Chapter V

THE EXPERIMENTAL INDUCTION OF DISEASE PRODUCING POWER IN BACTERIAL PROTOPLASM FROM THE FECES OF HEALTHY PARROTS

Although the heading of this chapter refers to the induction of pathogenicity, in reality, the subject, more broadly viewed, concerns the variations in the functional demonstrations exhibited by this protoplasm in living tissues. The capacity for growth in such material is inherently latent. The variations were studied both as they occur naturally, and as they are influenced by intentional changes of the bacterial environment. When these experiments were begun these facts were not fully realized and rather extensive methods were employed.

I. Classical Method:

Naturally, the first thought was to make use of mass action. In the beginning there was used 2 c.c. (a large dose) of a plain bouillon culture, directly inoculated from healthy parrot feces into the peritoneal cavity of a white mouse. This caused death in forty-eight hours. This result was paralleled with protoplasm from another parrot; 1 c.c. also caused death. Subcultures from so-killed mice, in 1 c.c. and .5 c.c. amounts caused death more rapidly still. After passing successively through two mice, the cultures showed a vast difference, even if differing by only one animal in the number through which they had passed. Thus, 25 c.c. might even cause immediate death, the toxicity being overwhelming after, but not before, the third mouse stage. But cultures varied through considerable ranges, a condition easily to be understood when the form and distribution variations, mentioned elsewhere, were considered.

Instead of searching for sharp lines of separation, it is better

to select five stages in the course of the rising power of the bacterium. Twenty-seven mice were used in checking and controlling the results. Finally .2 c.c. was, with fair consistency, deadly. This stage can be reached, probably, after either three, four, or five animal transfers. Paralleling this rise in the first and second steps at autopsy, there was seen first a marked gastro-intestinal dilatation of the viscera with a peritoneal and even a pleural exudate. There appeared no definite gross pathology, no spots of hemorrhage on the lungs. The unit forms, at first long rods, became shorter, but remained Gram positive. In the subsequent stages the autopsies showed organs which were generally darkened and slimy, especially following the use of the most toxic cultures; i. e., the activity of every individual unit was increased. Usually, in these small animals the general outlines and colors were obliterated; hemorrhages did occur, anywhere, before the most intense power was demonstrated. This power seemed to involve an actual putrefaction in life. In the later stages the rods were very short, of equal uniform size, and Gram negative. Green pigment occurred in cultures, the same as that seen in specimens in the epidemic series. The units showed great motility, and agglutinability by human sera, exactly as in cultures obtained during the epidemic.

This form occurred of course in bouillon from parrot feces, irrespective of animal inoculation, sometimes very soon, sometimes, as after blood-plate transfers, only with profound slowness. However, the green pigment was apparently associated with the mobilization and distribution of ferments following a sojourn in animal tissues.

PROTOCOL SERIES

I. Classical Method in the Enhancement of Disease Producing Power:

(a) Plain bouillon cultures injected into the peritoneal cavities of mice.

No. 1621. Two c.c. culture of feces of healthy parrot No. 2.
Death: In 48 hours.

No. 1622. Two c.c. culture of feces of health parrot No. 1.
Death: In 48 hours.

No. 1628. One c.c. culture of feces of healthy parrot No. 2.
DEATH: In 24 to 48 hours.

No. 1629. .5 c.c. culture of feces of healthy parrot No. 2.
RESULT: Survived.

AUTOPSIES:

Consistently showed very slow putrefaction, and pale organs. Lungs, congested and showed only rare hemorrhages. Duodenum especially dilated. Intestine, somewhat inflamed. Liver and kidneys swollen, soft, rather pale. Spleen, large acute tumor.

BACTERIAL FINDINGS:

Forms were widely distributed.

SMEARS:

Showed large, single, Gram positive rods. Coccal forms, especially in areas removed from point of injection.

No. 1630. 1. c.c. subculture of No. 1622. Result: Death.
No. 1631. .5 c.c. subculture of No. 1622. Result: Death.
No. 1659. .5 c.c. subculture of No. 1622. Result: Survival.
No. 1657. .3 c.c. subculture of No. 1622. Result: Survival.
No. 1658. .3 c.c. subculture of No. 1622. Result: Survival.

AUTOPSIES:

Lungs, somewhat more congested. More or less marked peritoneal exudate. Otherwise, little change.

BACTERIAL FINDINGS:

Smaller units: When 1 c.c. was used, these were Gram positive, short rods, oval diplococci, and round cocci. When .5 c.c. was used many of the units appeared as rather small Gram negative rods. This is suggestive. A diminution in numbers was followed by the development of the form which always exhibits the greatest power.

No. 1622. .5 c.c. subculture of No. 1630. Result: Death in 12 hours.
No. 1660. .3 c.c. subculture of No. 1630. Result: Death in several days.
No. 1661. .3 c.c. subculture of No. 1630. Result: Death in several days.

AUTOPSY (No. 1622):

Organs, generally darker. Lungs, discolored, brown. Liver, spleen, and kidneys were black and very soft. Stomach, dilated with gas. Intestine, slimy.

BACTERIAL FINDINGS:

SMEARS:

Short, Gram positive rod forms.

CULTURES:

Immediately developed a fine, Gram negative bacillus.

MICE:

No. 1664. .3 c.c. subculture of No. 1662. Result: Death in 12 hours.

No. 1663. .4 c.c. subculture of No. 1662. Result: Death in 12 hours.

AUTOPSIES:

Hemorrhages of skin and musculature. Lung, profoundly congested. Intestine, negative, feces firm. Liver, kidneys and spleen, very large, soft, and dark.

BACTERIAL FINDINGS:

SMEARS:

Marked tendencies toward uniformity. Short rods, and Gram negative staining.

CULTURES:

Fine, Gram negative, intensely motile rods, very agglutinable by normal human sera.

MICE:

No. 1686. .3 c.c. subculture of No. 1664. Result: Death in 18 hours.

No. 1687. .2 c.c. subculture of No. 1664. Result: Death in 18 hours.

No. 1688. .1 c.c. subculture of No. 1664. Result: Death in 24 hours.

No. 1689. .2 c.c. subculture of No. 1664. Result: Death in 18 hours.

The culture was so toxic that the first two, injected with .3 c.c. and .2 c.c. died almost immediately. Then .4 c.c. N/10 HCL was added in the case of No. 1689 and apparently caused a quicker recovery from the original depression than observed in No. 1686 and No. 1687.

AUTOPSIES:

Hemorrhages were lacking in these and were compensated for by a green discoloration of the intestines, with a foul smell and rapidly developing putrefaction.

BACTERIAL FINDINGS:

The *Typical Bacillus* form has been described above.

STUDIES CONTINUED IN LARGER ANIMALS.

(b) Plain bouillon cultures injected into the ear vein of rabbits.

No. 1700. Two c.c. of subcultures of No. 1687 and No. 1689

DEATH:

Seven days.

AUTOPSY:

Congestion of the lungs.

Parenchymatous degeneration.

No. 1722. Two c.c. of five day subculture of No. 1700.

DEATH:

12 hours.

AUTOPSY:

Hemorrhages of lungs, heart, stomach, jejunum, appendix. Liver, yellow. Gall-bladder distended. Kidneys, very pale. Splenic tumor. Feces, softened.

BACTERIAL FINDINGS:

SMEARS AND CULTURES:

Typical, green-pigment-producing motile, slim, Gram negative rods, indistinguishable from like forms seen during the epidemic.

No. 1755. Two c.c. of sediment of subculture of No. 1722.

DEATH:

12 hours.

AUTOPSY:

Beautiful picture of tiny hemorrhages scattered everywhere throughout the body.

SUMMARY:

The functional change was, evidently, merely an increase of a power, normally and naturally present. It was primarily aroused in bacterial units placed in meat extract bouillon. It was a mobilization.

RABBITS:

No. 1725. Two c.c. cold whole culture No. 1630. Results: Survived many days.

No. 1728. Two c.c. 24 hr. whole subculture No. 1630. Result: Death in 12 hours.

No. 1748. Two c.c. 24 hr. sediment No. 1630. Result: Death in 24 hours.

AUTOPSIES:

No. 1728 and No. 1748 showed remarkably few hemorrhages. Parenchymatous degeneration was especially marked.

BACTERIAL FINDINGS:

SMEARS:

Different areas revealed bacterial units lacking in uniformity and varying markedly in stain, size and shape. Hemorrhagic spots showed quite *typical Gram negative rods.*

CULTURES:

Were moderately typical of these types of animal reaction.

No. 1732. (Pregnant).

Two c.c. subculture of No. 1728.

DEATH:

In 15 hours.

AUTOPSY:

The lungs were brownish, not hemorrhagic. The heart showed hemorrhages, and the right ventricle was dilated. Gastrointestinal system showed inflammation and some hemorrhages. Parenchymatous organs were only moderately degenerated.

BACTERIAL FINDINGS:

Forms: More typical, Gram negative rods in form and culture, as described under studies of No. 1522.

All these efforts really only resulted in raising the general average of results. The limits of the range of variation were wide apart. After frequent and rapid mouse-passage, and when there were evidences of great power on the part of the bacterial protoplasm, the first encounter with rabbit tissues resulted in such a reaction as was seen following the inoculation of a rabbit with a plain bouillon culture of fresh parrot feces. Yet, a subculture of a mouse, representing only a second animal-passage, destroyed a rabbit in twelve hours. These are only examples from a solitary series, yet they are suggestive. It is not improbable that the effect of two mouse-passages was to reinforce and refine the bacterial protoplasmic reactions aroused by plain bouillon. Nor is it improbable that further mouse-passages set up a more and more specific chemical adjustment for mice, which rendered the protoplasm more and more temporarily inefficient in the tissues of other animals.

CONCLUSIONS:

These experiments demonstrate the possibility of obtaining from the feces of healthy parrots, bacterial protoplasm which, under the stimulating influences of artificial and natural media in like amounts, closely resembled in form and function bacterial protoplasm obtained from sick parrots and sick human beings, during the epidemic.

There appeared to exist, for a given speed of death, a balance between the intensity of hemorrhagic lesions, and, not only the rapidity of putrefactive post mortem change, but also antemortem changes,—very putrefactive in histologic appearance.

II. Special Method:

Employing the effects of mass but eliminating the artificial stimulation by plain bouillon. The effect of mass was exaggerated by undermining the host through fasting.

It was rather disturbing at first to observe the death of rabbits and mice following inoculation with ordinary bouillon—containing cultures, obtained directly from the fecal material of healthy parrots.

That a power for growth in animal tissue was latent in this protoplasm had to be admitted. It was also realized that meat substances constituted an unaccustomed food. These ideas agreed with the well known fact that animal foods tend to increase intestinal putrefaction.

Therefore to recapitulate: The immediate demonstration of considerable power in animal tissues by protoplasm developing only in plain bouillon, the influences of this medium as a standard environment of great potency, the production of foul odors and of forms profoundly differing in the characters evidenced in the normal habitat, were convincing proofs of the association of so-called pathogenicity and a meat substance diet.

After being convinced of this fact, a search was made for a medium in which the natural environment might be more nearly retained. A medium was selected, composed of equal parts of sterile ox bile and glucose bouillon. This is in itself, of course, very toxic. Therefore, after twenty-four to forty-eight hours incubation the cultures were centrifuged and the sediments suspended in normal salt solution for injection.

It was observed that following the inoculation of this medium with small particles of fresh, healthy parrot feces, the bacterial units were largely Gram positive, frequently irregular in form as in the direct fecal smears, or round or oval cocci, which might also be chained.

In this second series of experiments, very large amounts of bacterial protoplasm were employed in the initial steps. The centrifuged sediment of 14 c.c. of the cultures, in doses of 2 c.c. every 2 hours was used. Three lines of study were employed

by which two factors were compared; i. e., the influence of the variety of artificial pabulum employed, and the results following the previous fasting of the host. It may be thought that the complications of the stages and their number, were unnecessarily increased in order to arrive at the end result. If the only result desired were the induction of a pathogenicity, this would indeed be so. But, as will be observed, it is the transition stages, magnified by this method, which are most illuminating in the demonstration of disease-producing capacities, and the development of entities compared to those observed during the epidemic.

SECOND SERIES OF THE PROTOCOLS

SECOND METHOD IN THE INDUCTION OF PATHOGENESIS:

There was selected the arbitrary amount of 14 c.c. of a 24 hour-48 hour culture of fresh droppings of healthy parrots. Since bile is toxic, the sediments from both plain bouillon and ox bile and glucose bouillon were employed, after centrifuging to a practical supernatant clarity. The distribution of doses was 2 c.c. every two hours.

(1). SEDIMENT OF BILE AND GLUCOSE CULTURE:

(a) HEALTHY RABBIT:

No. 1809. Injected as described with the sediment of the above culture.

CLINICAL HISTORY:

8/ 8/17 Immediately very ill.
8/10/17 Better.
8/14/17 Worse, hind legs weak.
8/16/17 Diarrhœa.
8/17/17 Rhinorrhœa.
Alternately better and worse with increasing emaciation.

DEATH:

60 days.

AUTOPSY:

Gastrointestinal catarrh. Cholelithiasis. Cardiac hypertrophy and degeneration. Valves and aorta, negative. Adrenal hypertrophy. Kidneys, ? Splenic tumor. Urine: Albumin, negative. Sugar, negative. Indican, negative.

BACTERIAL FINDINGS:

SMEARS:

Bile: Gram negative rod.
Spleen: Negative.

CULTURES:

Heart: Alkaline. Indol ++ *Irregular Gram negative rods.*
Chyme: Acid. Irregular rods.
Urine: Alkaline. Rich slim, *Gram negative rods.*
Bile: Alkaline. Rich slim, *Gram negative rods.*
Kidneys: Acid. Indol + Irregular rods.
Milk and gelatin, not acted upon.

RABBIT:

No. 32. 8/17/17. Two c.c. of 7 day plain bouillon culture from nose of rabbit No. 1809, while alive, smears of which showed mixed forms.

DEATH:

Etherized in 24 hours; moribund.

AUTOPSY:

Hemorrhages of lungs, thymus, appendix, small intestine. Follicular splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Hemorrhagic areas: Slim, Gram negative rods.
Heart: Primary rods, short Gram positive rods.
Bile: Same.
Spleen: Same.

No. 44. 14 c.c. of a 24 hour bile and glucose subculture of the same plain culture as used in Rabbit No. 32. (Forms, irregular rods.)

DEATH:

108 days.

AUTOPSY:

Gastrointestinal catarrh, with cholecystitis. Adrenal hypertrophy.

(b) FASTING RABBIT (72 hours):

No. 11. Injected same as No. 1809.

CLINICAL HISTORY:

Alternately good and bad days. Progressively emaciated. Became filthy. Grew weaker, then helpless.

DEATH:

17 days.

AUTOPSY:

Rhinitis, passive congestion of lungs. Heart, degenerated, and dilated. Aorta, sclerosed. Stomach showed congestion and catarrh. Intestine showed many loops congested, with leukocytic infiltration and pink stained mucus with much gas; other loops were normal. Feces were formed. Sub-acute splenic tumor. Degeneration of liver and kidneys. Adrenal hypertrophy.

BACTERIAL FINDINGS:

SMEARS:

Nose: Gram positive diplococci.
Spleen: Gram positive diplococci.
Wall of gut: Negative.
Blood of normal gut areas: Gram negative rods.
Dark blood of diseased areas: Gram negative rods and primary rods.
Mucus: Gram negative rods.
Chyme: Gram negative rods.

CULTURES:

Nose: Gram negative rods.
Normal mesenteric blood: Gram positive rods.
Blood of diseased areas: Primary rods and Gram negative diplococci.
Contents of gut: Mixed forms.
Heart blood: At first, primary rods and Gram positive cocci; these slowly passed through stages as seen in human blood during the epidemic, and in two weeks had developed into Gram negative rods.

In bile and glucose, units from nose and mesenteric blood showed coccal forms.

Bouillon cultures were cloudy, with a pellicle. On blood plates, streaks were of coarse typhoid-colon type, but not green.

STUDY OF CULTURES OF RABBIT NO. 11 IN ANIMALS:

SOURCES OF CULTURES:

(A) INTESTINAL CONTENTS:

(a) CONGESTED AREAS:

RABBIT:

No. 49. Two c.c. of 72 hour plain bouillon culture, containing primary rods, cocci, and various coarse rods.

DEATH:

Three and one-half hours.

AUTOPSY:

In one hour, putrefaction rapid. Heart, somewhat dilated. Lungs, emphysematous. Intestine showed loops with vascular dilatation. Appendix showed beginning hemorrhages.

BACTERIAL FINDINGS:

SMEARS:

Lungs: Rods.
Chyme: Rods.
Hemorrhage of appendix: Rods.
Splanchnic blood: Negative.
Heart: Negative.
Spleen: Negative.

RABBIT:

No. 57. (Same experiment repeated.) One c.c. of 10 day ++ and 1 c.c. of 24 hour plain subculture in bouillon, containing mixed forms.

DEATH:

Sudden. 52 hours.

AUTOPSY:

(At once.) Heart, contracted. Lungs, pink with some small hemorrhages. Stomach, contracted. *Intestinal inflammation, mucus and gas.* Hemorrhages in appendix. Liver and kidneys, degenerated. Cholecystitis. Splenic tumor. *Urine:* Albumin, present ++. Indican, present +. Red and white blood cells present.

BACTERIAL FINDINGS:

SMEARS:

Bile: Mixed Gram positive cocci and rods.
Appendiceal hemorrhage: Rare, pale rods.
Splanchnic blood: Gram negative rods.
Chyme mucus: Rich in Gram negative forms.
Heart: Negative.
Lung: Negative.
Intestinal wall: Negative.

CULTURES:

Heart: Gram negative rods, with cocci.
Chyme mucus: Gram negative rods, with cocci.
Bile: Gram positive rods, with cocci.

(b) NORMAL AREA OF GUT:

RABBIT:

No. 50. 1.5 c.c. 96 hour plain bouillon. No reaction whatever.

(B) MESENTERIC BLOOD:

(a) BLOOD FROM CONGESTED AREA:

RABBIT:

No. 59. One c.c. of original + and 1 c.c. of 24 hour subculture in plain bouillon, containing mixed forms.

CLINICAL HISTORY:

Immediately ill, diarrhœa.

DEATH:

96 hours.

AUTOPSY:

Autopsy in three and one-half hours. Nose, dry. Heart, contracted, abscess-like areas on surface. Lungs, emphysematous. Stomach, negative. *Duodenum, appendix, and many*

areas of *small gut,* especially lymph patches, *showed vasodilatation, congestion,* and rare hemorrhages, with gas and mucus. Large acute splenic tumor. Liver and kidneys degenerated. Gall-bladder contained mucus and sand.

BACTERIAL FINDINGS:

SMEARS:

Bile: Gram negative rods.
Nose: Gram negative rods.
Appendiceal hemorrhage: Grain negative rods.
Duodenal contents: Gram negative rods.
Ileum wall and contents: Gram positive rods.
Heart, white masses: Negative.
Heart blood: Negative.
Liver: Negative.
Spleen: Negative.
Duodenal wall: Negative.

CULTURES:

Bile: Alkaline, of a putrefactive odor, and containing Gram negative rods.
Nose: Same.
Blood: Same.
Contents, ileum: Same.
Contents, duodenum: Same.
Heart blood: Showed a slow transition from variable cocci, in acid bouillon, to Gram *negative rods in typical culture.*

(b) BLOOD FROM NORMAL AREA:

RABBIT:

No. 58. One c.c. of original + 1 c.c. of 24 hour subculture in plain bouillon, containing mixed forms.

CLINICAL HISTORY:

Sick continuously, moist nose, diarrhoea at first; later, constipation, fever, anorexia.

DEATH:

In eight hours.

AUTOPSY:

Heart, contracted and degenerated. Stomach nearly empty. Duodenum, and many loops of small gut, cæcum, and appendix showed inflammation, rare hemorrhages and mucus. Mesenteric nodes were hemorrhagic. Acute splenic tumor. Liver and kidneys, degenerated. Cholecystitis. *Urine*: Albumin, ++.; Indican, trace; leukocytes, and granular casts.

(C) Nose:

Rabbit:

No. 56. One c.c. original + 1 c.c. of 24 hour subculture in plain bouillon, containing mixed forms.

Death:

One hour.

No. 60. Same dosage as No. 56.

Clinical History:

Not ill at first, then intense rhinorrhœa.

Death:

36 hours.

Autopsy:

(Autopsy in 12 hours.) Putrefaction advanced. *Pleuritis with suppuration.* Heart, pericardial hemorrhages. Lung, hemorrhagic. Gastrointestinal tract, and skeletal muscles, full of hemorrhages. Parenchymatous organs putrefied.

Bacterial Findings:

Smears:

Heart blood: Irregular Gram positive rods.
Lung hemorrhage: Irregular Gram positive rods.
Spleen: Irregular Gram positive rods.
Intestinal hemorrhage: Gram negative rods.
Muscle hemorrhage: Gram negative rods.
Peritoneal fluid: Gram negative rods.

Cultures:

Heart blood: Irregular rods.
Peritoneal fluid: Irregular rods.
Bile: Gram negative rods.

(D) Heart Blood:

This culture originally contained primary rod forms, which developed, later on as mentioned, into Gram negative rods.

Rabbit:

No. 112. Two c.c. of 72 hour subculture, of a seven day subculture, of a fifty day old original plain bouillon culture, containing Gram negative rods.

Clinical History:

Immediately ill, deep respirations. Leukocytes, 16400. High, continuous fever to the end.

Death:

48 hours.

Autopsy:

Pneumonia and *pleurisy,* with thick fibrin, especially on right side, on a basis of hemorrhagic inflammation. *En-*

teritis. Acute degeneration of liver and kidneys. Splenic tumor. Cholecystitis.

BACTERIAL FINDINGS:

Cultures foul, and form, *typical Gram negative rods.*

II. SEDIMENT OF PLAIN BOUILLON:

(a) IN HEALTHY RABBIT:

No. 27. 14 c.c. (2 c.c. every two hours) of 24 hour plain bouillon culture of feces of parrot No. 3.

DEATH:

24 hours.

AUTOPSY:

Hemorrhages of the thymus, heart, intestine, kidneys and muscles.

BACTERIAL FINDINGS:

SMEARS:

Lungs: Primary rods.

Heart blood: Primary rods, cocci, Gram negative forms.

Nose: Primary rods, cocci, Gram negative forms.

Spleen: Primary rods, Gram positive rods.

Appendix: Gram negative rods.

CULTURES:

Nose: Mixed cocci, and short rods.

Bile: Irregular Gram positive masses.

Heart blood: Showed good series of transition stages over a course of three weeks from primary rods to Gram negative rods.

Tubes, at first colorless, *became green.*

STUDY OF REACTION OF CULTURES OF RABBIT NO. 27 IN ANIMALS:

(A) BILE:

RABBIT:

No. 51. Two c.c. of 72 hour subculture in plain bouillon of eleven day plain bouillon culture, containing Gram positive rods of varying sizes.

No reaction.

(B) HEART BLOOD:

(a) IN PLAIN BOUILLON:

RABBIT:

No. 38. Two c.c. of 96 hour culture of heart blood.

DEATH:

77 days.

AUTOPSY:

Emaciation. *Chronic gastrointestinal catarrh. Acute* hemorrhages of stomach and duodenum, with secondary inflammation. Nose, heart, lungs, gall-bladder and ducts, liver and kidneys, all negative and did not show evidence of long toxemia.

BACTERIAL FINDINGS:

SMEARS:

Rectum: Gram positive rods.
Duodenum: Gram positive rods.
Ileum: Gram positive rods.
Bile: Negative.

CULTURES:

Rectum and ileum: Sterile.
Duodenum: Gram positive-negative rods.
Bile: Gram positive-negative rods.
Heart: Studied for 10 days, formless. In 13 days, streptococci appeared.

These results, especially the heart culture, and the lack of signs of chronic toxemia, might indicate a lack of connection between the organisms injected, and the streptococci presumbably causing death and the acute pathology.

No. 163. Two c.c. of same culture as used, No. 38, now 90 days old, re-cultured twenty-four hours in plain bouillon, containing Gram negative rods.

DEATH:

36 hours.

AUTOPSY:

Typical hemorrhages of the lungs, stomach, appendix, heart, kidneys, and muscles.

BACTERIAL FINDINGS:

Cultures showed *Typical Gram negative rods,* forming green pigment and growing like Bacillus paratyphosus.

(b) IN BILE AND GLUCOSE:

RABBIT:

No. 45. 8/31/18. 14 c.c. sediment of culture of heart blood of No. 27, containing cocci.

CLINICAL HISTORY:

Immediately, fever and diarrhoea, then, constipation, and improvement. Progressively weaker.

DEATH:

14 days.

AUTOPSY:

Emaciated. Appendix and cæcum, *inflamed*, some tiny hemorrhages. Cholecystitis. Heart, liver, and kidneys, degenerated. Adrenal hypertrophy. Small splenic tumor.

BACTERIAL FINDINGS:

SMEARS:

Nose: Primary rods and cocci.
Heart blood: Gram positive cocci.
Cæcum: Gram positive cocci.
Bile: Gram positive cocci.
Appendicular hemorrhage: Gram negative rods.
Lungs: Negative.
Spleen: Negative.

CULTURES:

Bile: Gram positive rods, typhoid-colon type of colonies on plate, putrefactive odor, alkaline.

Heart: Cocci which metamorphosed to *Gram negative rods* in about eight days.

STUDY OF CULTURES OF RABBIT NO. 45 IN ANIMALS:

(A) IN PLAIN BOUILLON:

RABBIT:

No. 54. Two c.c. of 8 day old culture taken from nose of rabbit No. 45 while it was alive. Contained Gram negative rods.

DEATH:

12 hours.

AUTOPSY:

(Autopsy in 12 hours.) Rapid putrefaction. Faint evidences of hemorrhages of the heart and appendix. Blood tinged peritoneal exudate. Parenchymatous organs, putrefied.

BACTERIAL FINDINGS:

Typical Gram negative rods.

(B) IN BILE AND GLUCOSE:

RABBIT:

No. 70. Six c.c. (three doses, 2 c.c. each) in culture of the bile of rabbit No. 45, in plain bouillon 13 days, on plate 24 hours, in bile and glucose 24 hours. Contained mixed rods and cocci.

CLINICAL HISTORY:

Immediately ill. Rhinorrhœa for four days, then improved. Alternately better and worse, nose always moist.

DEATH:

Rather sudden. 17 days.

AUTOPSY:

(Autopsy in three hours.) Putrefaction beginning generally; glassy appearance, purpling blood, discolored gut tube, darkening areas of kidneys and spleen. *Rhinitis, gastrointestinal catarrh,* with mucus and gas. Heart, atrophic, fatty degeneration of wall of right ventricle. Aorta, negative. Lungs, negative. Acute splenic tumor. Liver and kidneys, acutely degenerated. Gall-bladder acutely inflamed at tip, with thickened bile, mixed with mucus.

BACTERIAL FINDINGS:

SMEARS:

Chyme: Gram positive-negative rods.
Mesenteric blood: Gram positive-negative rods.
Heart blood: Negative.
Bile: Negative.
Spleen: Negative.

CULTURES:

Chyme: Gram negative rods.
Mesenteric blood: Gram positive rods.
Both were alkaline and foul in plain bouillon.

The chyme culture was green, showed indol, coagulated milk and liquefied gelatine immediately.

The mesenteric blood culture was not green. It did show indol but had no effect on milk and gelatine until after a long time.

Here we see the usual influences tending to coarseness and saprophytism due to sojourn in the lumen of the gut tube.

STUDY OF NO. 70, CULTURES IN ANIMALS:

I. CHYME CULTURES, various ages:

RABBIT:

No. 85. Two c.c. 24 hour subculture in plain bouillon of a 96 hour old original culture containing mixed rods and coccal forms, largely Gram positive.

DEATH:

Convulsions. 24 hours.

AUTOPSY:

(Pregnant.) Lungs showed a fine picture of hemorrhages, also the stomach, appendix, lymph, patches of the ileum and other areas of the small gut. The spleen was acutely tumefied. The liver and kidneys were acutely degenerated. Gall-bladder and muscles were negative.

URINE:

Negative.

SMEARS AND CULTURES:

These, taken from various regions especially of the hemorrhagic areas, were typical of the completely adjusted *Gram negative rod.*

RABBIT:

No. 119. Two c.c. of a 24 hour subculture in plain bouillon of culture used in No. 85.

CLINICAL HISTORY:

Leukocytes	12,000	Temperature	102
Leukocytes	6,400	Temperature	103
Leukocytes	6,500	Temperature	105
Leukocytes	5,600	Temperature	106
Leukocytes	4,500	Temperature	105
Leukocytes	14,300	Temperature	102.3

DEATH: 30 hours.

AUTOPSY:

Pneumonia in early stages.

CULTURES:

Heart blood: Light.

Lung and duodenal hemorrhages: Typical.

RABBIT:

No. 91. Two c.c. of the same culture used in No. 85 now 72 hours old.

CLINICAL HISTORY:

Anorexia, fever, progressively weaker. Conjunctivitis, rhinorrhoea and dyspnoea.

DEATH:

6 days.

AUTOPSY:

The brain was congested. *Rhinitis.* Lungs showed *pneumonia,* with grey areas and hemorrhages. Thick fibrinous *pleurisy.* Fibrinous *pericarditis.*

SMEARS:

Lungs: Hemorrhagic. Pneumonic fibrinous areas, all showed great numbers of small *Gram negative rods.*

Nose: The same.

Bile: Gram positive rods.

Heart blood: Negative.

Chyme: Negative.

CULTURES:

Lung: Typical, foul, alkaline, green, contained indol. Digested milk and gelatine, split lactose and saccharose, and contained *Gram negative rods.*

Nose: Same.

Bile: Same.

Chyme: Not so Typical, did not affect milk and digested gelatine slowly.

STUDY OF THE CULTURES OF RABBIT No. 91 IN ANIMALS:

A. CONTENTS OF SMALL GUT:

RABBIT:

No. 108. Two c.c. of a 24 hour subculture of a 6 day old tube, both in plain bouillon.

DEATH:

24 hours.

AUTOPSY:

Showed hemorrhages of the stomach, duodenum, appendix, and small intestine.

B. LUNGS:

RABBIT:

No. 109. Two c.c. of a 24 hour subculture of a 6 day old tube, both in plain bouillon.

DEATH:

24 hours.

AUTOPSY:

Hemorrhages of the stomach, appendix and small intestine.

CULTURES 108 AND 109:

All Typical and green.

II. SPLANCHNIC BLOOD:

RABBIT:

No. 86. Four c.c. of a 24 hour subculture in plain bouillon of a 96 hour old original culture.

No reaction. Alive 3 months later.

RABBIT:

No. 118. Two c.c. of a 24 hour subculture in plain bouillon of a 21 day old culture used in Rabbit No. 86.

No reaction. Alive 3 months later.

III. FECES:

RABBIT:

No. 120. Two c.c. of a 24 hour subculture in plain bouillon of the original culture now 25 days old.

DEATH:

12 hours.

AUTOPSY:

Typical hemorrhagic reaction.

SUMMARY

A Comparison of the Effects of the Artificial Media

The first rabbit injected upon a full stomach with 14 c.c. of the sediment of a bile and glucose bouillon culture died in 2 months. The autopsy showed great emaciation and very chronic disease, a low grade gastrointestinal catarrh and cholelithiasis. Cultures taken during life, 8 days after inoculation, from the nose and grown in plain bouillon caused rapid death with hemorrhages. The forms of the units were those of the *Typical Gram negative rods* so frequently described.

In the fasting rabbit previously described, death occurred in 18 days. There was chronic rhinitis, moderate but general parenchymatous degeneration, acute intestinal inflammation with vascular dilatation, and deposits of white blood cells. These were evident when the thin gut wall was examined under the low power of the microscope.

The forms of the organisms recovered, and their activities, compared very closely with the findings from human patients during the epidemic. The culture from the heart blood showed the primary rods which, after a period of time, developed into *Gram negative rods.* A subculture from this, after some months, caused death in 48 hours, with a beginning consolidation of the lungs, hemorrhages in the intestine, acute cholecystitis and rapid putrefaction fairly comparable to a *typical culture.* This phenomenon occurred again and again with cultures from human and parrot sources during the epidemic.

No. I. Opposed to these results was this striking fact that when the same dosage (14 c.c. of sediment suspended in salt solution) was supplied by an organism grown for 24 hours in plain bouillon, death occurred within 24 hours.

The post mortem revealed hemorrhages distributed generally. The organisms were irregular and largely Gram positive. It would appear that the mass employed, because of the influence of the plain bouillon, provided a functional power equal to that

mobilized in 2 c.c. of the so-called Typical culture. But there was no parallel in the forms assumed by the units.

After 3 months in plain bouillon, a subculture in the same medium of the heart culture mentioned as ineffectual, had adjusted itself to nearly the standard type, killing in 36 hours, with hemorrhages of the lungs, stomach, appendix, muscles, heart and kidneys. The organisms and cultures were *Typical Gram negative rods* and grew as paratyphoid organisms on Russel's double sugar medium. Here we have the remarkable course from healthy parrot feces, 24 hours in plain bouillon, 24 hours in a rabbit, 3 months in plain bouillon, and a nearly *typical (Standard) culture resulting.*

No. II. Results of the employment of the mass action of the bacterial protoplasm without the preliminary effects of plain bouillon, an action exaggerated by previous fasting of the host. Rabbit No. 11.

Subcultures of the fasting rabbit studied separately, and through stages by means of animal injections.

Of the two cultures of the nose in plain bouillon, one killed in one and one-half hours and the other in thirty-six hours. This latter reaction was of the hemorrhagic, rapidly putrefying type. This also paralleled the findings in parrots, human beings, and other rabbits. Organisms passed through an animal, and finding lodgment amid saprophytic surroundings, often acted immediately, as the individuals obtained in the blood or at disease points did only after a sojourn in plain bouillon.

From the gastrointestinal tract, two cultures were made of the mesenteric blood, and two from the duodenal or jejunal contents. The organisms were moderately large, Gram positive and negative rods. The culture from the contents of a congested area of the gut, in plain bouillon, killed, in one case, in three and one-half hours, again, in fifty-two hours, with acute gastroenteritis and a few scattered hemorrhages in the lungs, adrenals, etc. The culture of the heart-blood, a *fine Gram negative rod,* was typical of cultures of the bacterium when acting in force, variation being unnecessary; the form was uniform.

The specimen from the blood of the same area of gut, killed

in ninety-six hours. Here, at autopsy, there was revealed a more delicate gastroenteritis, parenchymatous degeneration, and splenic tumor. The cultures of the heart blood showed cocci slowly developing to *Typical Gram negative rods.* These heart cultures were acid. Odorless at first, they later became (usually best observed in subcultures) alkaline, and foul; all characters changed synchronously.

The culture of the blood from a pale area of the gut, killed in eight days. Here was shown enteritis, mesenteric adenitis, following a clinical course (after the primary injection-depression) of progressive illness, rising fever; first diarrhoea then constipation; a febrile period, and, finally, death. Albumin was present in the urine.

The heart specimen, 8 days in plain bouillon, then recultured in bile and glucose bouillon, centrifuged, suspended in salt solution and injected in 2 c.c. amounts every 2 hours for 7 doses (or 14 c.c.) as usual, required 8 days to cause death. The clinical course was: Preliminary fever and diarrhoea, then a return to normal, followed by progressive illness and death.

This rabbit dying in 8 days showed at autopsy emaciation, inflammation of the appendix and cæcum, cholecystitis, parenchymatous degeneration. The organisms slowly assumed the Gram negative rod form. The unit in the heart blood culture metamorphosed slowly from an irregular coccal form to that of *Gram negative rods,* exactly as in the human specimens.

A nose culture, taken during life, in plain bouillon developed rod forms, which killed in 12 hours with especially marked putrefaction.

A heart culture, thirteen days in plain bouillon, one on a blood agar plate and one in glucose bouillon and bile, in three 2 c.c. doses (6 c.c. in all) required 17 days to kill. Here appeared again an evidence of the demobilization or retrogression of power in this medium. The post mortem showed enteritis with gas and mucus, cholecystitis, atrophy of the heart, parenchymatous degeneration and splenic tumor.

Cultures and subcultures in plain bouillon of the chyme from this rabbit were made. One 24 hour reculture of a 96 hour

culture caused death in 24 hours with hemorrhages, especially of the lungs. The organisms were fairly typical, but the animal was pregnant. A 72 hour culture of the same, killed in 6 days. (No. 91) There was observed, clinically, conjunctivitis, rhinorrhoea and dyspnoea. The autopsy showed suppurative pneumonia with pleuritis and pericarditis and congestion of the brain. In all the smears and cultures typical rods were immediately found.

Three weeks later, a 24 hour subculture of the same culture caused death in 30 hours. There was a suggestion of pneumonic consolidation, but no fibrin. Putrefaction was rapid. Smears and cultures were typical. (It must be realized, as in cases under No. 1522, that this apparent increase in virulence, so-called, has taken place in test tubes.)

Cultures from the splanchnic blood caused in two rabbits only temporary illness. Subcultures from the rabbit which died in 6 days (No. 91) were studied. Both a 24 hour reculture in plain bouillon of a 6 day original culture of the intestinal contents, and one of equal age from the lung, killed in 24 hours with typical hemorrhages, mainly of the stomach and bowel. Cultures were *Typically green* and contained *Gram negative rods.*

GENERAL SUMMARY. CHAPTERS IV AND V

A demobilized but latent power of adjustment to new environments of course exists in the protoplasm sojourning in a saprophytic mood in the intestinal tracts of parrots. This low form of life is represented to the eye by irregular units of mass. Under the influence of artificially prepared meat substances or living tissues or both, the mobilization of these powers is shown by stages. Thus may be observed an adjustment sufficient only for life in tissues close to the accustomed stamping ground, with very moderate effects upon the host. Then step by step develops a more intense affinity for these intestinal regions, then a capacity to live in regions further removed, and finally an indiscriminate power; so intense a general affinity that, allowed to operate in

the tissues of an animal, it is soon incompatible with life in the host.

Paralleling this adjustment in function to environment there is morphologically a more or less gradual assumption by the bacterial protoplasm of forms most suited to the delegation of superior power, and more, a growing tendency to regularity and uniformity in mass subdivision.

The predominating influence of plain bouillon is shown again and again, an influence apparently far surpassing that wielded by prolonged existence in the tissues of an animal.

The clinical and post mortem pictures described, imitate in many ways those observed in human beings who were associated also with comparable bacteriological findings. The more acute cases of rhinitis, pneumonia and typhoid-like intestinal disease resembled the epidemic cases; the more chronic pictures paralleling those seen in many patients, especially since the epidemic. These latter will be detailed in another publication.

The metamorphosis of the units in cultures, especially of the heart bloods of the rabbit chronically and subacutely diseased, compared closely with that described in Parts I and II.

The more rapid exhibition of power in cultures from the nose and feces as compared to those from the heart, corresponded to the findings in the human series.

But it would appear that in the case of neither series was the adjustment at these points in correspondence with that right at the seat of disease. Such a relatively unchanged adjustment appeared to be exhibited by the cultures from the splanchnic blood of the fasting rabbit, No. 11. The same condition appeared to be true of the sputum culture in the case of No. 12.

Certainly that *common state of adjustment,* the *typical culture,* described especially in the case of No. 1522, was reached by many and devious paths, as it was in the epidemic series. This result was accomplished thus intentionally in imitation of that observed while the epidemic sources were being investigated. But the sources were then sick parrots and sick human beings. Here the single original source was the intestinal tract of healthy parrots.

Chapter VI

THE END RESULT OF CHANGE, THE STUDY OF THAT COMMON STATE, THE SO-CALLED TYPICAL CULTURE, ASSUMED BY THE BACTERIAL PROTOPLASM, FROM A SINGLE SOURCE, HEALTHY PARROT FECES WHEN UNDER A COMMON ENVIRONMENT, AND IN THE VARYING COURSE OF TIME

Up to this point, the purely experimental studies had revealed the fact that in the intestinal tracts of healthy parrots existed a low form of living protoplasm having generalized characters. They had also established for this protoplasm in the form of bacteria, a function inherent and latent which was capable, when aroused and intensified, of the institution in the tissues of animals of disease entities. These were represented by pictures, comparable to those caused by similar protoplasm, obtained from sick parrots and sick human beings, during the epidemic common to both.

This protoplasm in the form of bacteria, obtained from all these experimental secondary sources, showed various similar "stages" in their unit forms and general functions, and under the influence of the common environment provided by plain bouillon. All showed changes, until in time a terminal stage or state occurred common to all, represented in an experimentally produced *Gram negative rod culture.*

This has been partially analyzed, in the case of a specimen, No. 1522, acquired from an "Epidemic Parrot." It will now be described in greater detail and the naturally arising and experimentally produced states or Typical Cultures compared.

First, relative reality will be further established, under the heading of "Stability."

Then an example of an artificially induced culture will be especially investigated and compared in character to culture No. 1522, to the study of which Chapter III was devoted.

THE EXPERIMENTALLY INDUCED COMMON GROUND IN GENERAL

Its Stability:

The relative reality or stability of the *typical culture,* when it is used as a whole was investigated. The method employed was the injection of 2 c.c. of a plain bouillon culture into the ear vein of a rabbit. Comparisons in reactions were made in regard to the age of the culture, and the influence of repeated animal transfers. Cultures of the heart blood were invariably employed. The individual specimen selected for use was an old subculture of rabbit No. 1755, the first typical hemorrhagic rabbit reaction, experimentally induced. This was developed in the experiments in the production of pathogenesis by the first method.

The culture was 6 weeks old when the initial injection was made. As a result of the injection death occurred within 48 hours. Hemorrhages were present, and fairly general, but there were distinct signs of inflammation in the gastrointestinal tract, and the kidneys. This slowing up in the reaction time has been found true for cultures of corresponding forms from other sources.

The following key will show at a glance just how age and transfers were checked.

No. 22. 24 hour No. 1755.
  No. 29. 24 hour No. 22.
  No. 35. 48 hour No. 22.
  No. 36. 72 hour No. 22.
No. 20. 48 hour No. 1755.
  No. 23. 24 hour No. 20.
  No. 28. 72 hour No. 20.
  No. 34. 96 hour No. 20.

No. 19. 6 weeks old No. 1755.
No. 21. 24 hour No. 19.
No. 24. 24 hour No. 21.
No. 31. 24 hour No. 24.
No. 30. 48 hour No. 21.
No. 25. 72 hour No. 19.

PROTOCOLS

No. 1755. Last case in the Pathogenesis Induction Experiments.
DEATH: 12 hours.
AUTOPSY: Hemorrhages distributed everywhere.
CULTURES: *Typical and green.*

ANIMALS:

RABBIT:
No. 22. Two c.c. 24 hour reculture of a 6 weeks old No. 1755.
DEATH:
12 hours.
AUTOPSY:
12 hours later. Putrefaction negative. Hemorrhages of the thymus, lungs, heart, small intestine, appendix, colon, rectum, kidneys and ureters.
CULTURES:
Typical and green.

RABBIT:
No. 29. Two c.c. 24 hour old culture of No. 22.
DEATH:
18 hours.
AUTOPSY:
12 hours later. Putrefaction moderate.
Hemorrhages: Slight in the lungs, thymus, appendix and cæcum.
CULTURES:
Typical and green.

RABBIT:
No. 35. Two c.c. 48 hour old culture of No. 22.
DEATH:
12 hours.
AUTOPSY:
Hemorrhages of the lungs marked. Few of the small intestine.
CULTURES:
Typical and not green.

RABBIT:

No. 36. Two c.c. of a 72 hour culture of No. 22.

DEATH:

14 hours.

AUTOPSY:

Eight hours later. Putrefaction moderate. Hemorrhages moderate in the lungs, thymus, stomach, appendix and small intestine; marked in the duodenum and large intestine.

CULTURES:

Typical and not green.

RABBIT:

No. 20. Two c.c. of a 48 hour reculture of a 6 weeks old culture of No. 1755.

DEATH:

12 hours.

AUTOPSY:

12 hours later. Putrefaction negative.

Hemorrhages: Moderate of the lungs, appendix, mesenteric nodes and adrenals; marked in the heart and kidneys.

CULTURES:

Typical and green.

RABBIT:

No. 23. c.c. of a 24 hour culture of No. 20. (Incomplete in MS.—Editor.)

DEATH:

12 hours.

AUTOPSY:

12 hours later. Putrefaction marked. Rhinorrhoea.

Hemorrhages: Large of the lungs, many small ones of the appendix.

CULTURES:

Typical and green.

RABBIT:

No. 28. c.c. of a 72 hour old culture of No. 20. (Incomplete in MS.—Editor.)

DEATH:

12 hours.

AUTOPSY:

12 hours later. Putrefaction marked. Rhinorrhoea.

Hemorrhages: Slight, of the stomach, appendix, cæcum and colon.

CULTURES:

Typical and green.

RABBIT:

No. 34. Two c.c. of a 96 hour old reculture of No. 20.

DEATH:

12 hours.

AUTOPSY:

4 hours later. Putrefaction slight.

Hemorrhages: Moderate of the lungs, marked of the heart, intense of the entire gastrointestinal tract.

CULTURES:

Typical.

RABBIT:

No. 19. Two c.c. of a 6 weeks old culture of No. 1755.

DEATH:

48 hours.

AUTOPSY:

2 hours later. Putrefaction beginning. (Diarrhoea.)

Hemorrhages: Slight, of the heart; moderate, of the stomach. Congestion of the small intestine and appendix. Single hemorrhages of the colon and psoas muscle.

CULTURES:

Typical and green.

RABBIT:

No. 21. Two c.c. of a 24 hour old culture of No. 19.

DEATH:

12 hours.

AUTOPSY:

12 hours later. Putrefaction moderate.

Hemorrhages: Large, of the lungs, few, of the heart, mediastinum, many, of the kidneys, stomach and duodenum, especially of the caput, small intestine, appendix, colon and rectum.

CULTURES:

Typical and green.

RABBIT:

No. 24. Two c.c. of a 24 hour old culture of No. 21.

DEATH:

12 hours.

AUTOPSY:

12 hours later. Putrefaction very marked. Rhinorrhoea (green).

Hemorrhages: Few, of the lungs, moderate, of the heart, small intestine and stomach. Large, of the appendix, also in the fascia and muscles.

CULTURES:
Typical and green.

RABBIT:
No. 31. Two c.c. of a 24 hour culture of No. 24.
DEATH:
18 hours.
AUTOPSY:
12 hours later. Beginning putrefaction.
Hemorrhages: Were moderate of the nose, thymus, lungs, stomach, intestine, appendix and uterus.
CULTURES:
Typical and not green.

RABBIT:
No. 30. Two c.c. of a 48 hour culture of No. 21.
DEATH:
15 hours.
AUTOPSY:
Performed at once.
Hemorrhages: Of the thymus, lungs, appendix and adrenals.
CULTURES:
Typical and green.

RABBIT:
No. 25. Two c.c. of a 72 hour culture of No. 19.
DEATH:
12 hours.
AUTOPSY:
12 hours later. Putrefaction beginning.
Hemorrhages: Fairly marked of the nose, heart, lungs, stomach and especially of the duodenum, small intestine, cecum and rectum.
CULTURES:
Typical and green.

SUMMARY

These experiments show a remarkable uniformity in the functional capacity of the culture under consideration. Death, with hemorrhages not restricted to any one organ or system, occurred regularly in a little over half a day and this was not affected by the age of the culture (up to 4 days), or by animal passage.

Variability did occur in the number of the hemorrhages, and

the rapidity of the onset of putrefaction. These two phenomena seem to bear that relationship to one another, frequently observed, that they were rarely both marked in the same animal. This is a fact strongly impressed upon the mind of the investigator.

The locations of the hemorrhages varied. There appeared to be four poorly differentiated pictures; large lung hemorrhages, heart and kidney hemorrhages, gastrointestinal hemorrhages (these especially of the stomach, caput duodeni, appendix and rectum), and entirely general hemorrhages.

In general all autopsies showed acute splenic tumors and degeneration of the liver, kidneys and heart, with especially marked destruction of the cytoplasm.

The smears studied from the hemorrhagic areas, and the bile and spleen, showed either great variability in form or, more rarely, quite regular *Gram negative rods* larger than grow in bouillon. Frequently at first the units showed a tendency to take the Gram positive stain. It appears likely that this variability was due to a greater flexibility, in turn due to the relative recentness of environmental change. The possibilities of the effect of the time factor upon the freedom of chemical interactions, have a very important bearing upon the suddenness and intensity of the reactions at the moment of an epidemic.

Chapter VII

THE STUDY OF AN EXPERIMENTALLY INDUCED TYPICAL CULTURE IN PARTICULAR NO. 24 ITS CHARACTERIZATION. ITS ANALYSIS

Having established the functional stability of a standard amount of the typical cultures in general, the more fascinating search was made for the hidden powers lurking within its confines; the potential energy stored in this half teaspoon of a suspension of bacterial protoplasm in plain soup.

For this work there was chosen the culture of the heart blood of rabbit No. 24, in plain bouillon. This was one of the series just described and its position may be seen as indicated in the outline.

First a description of its characters will be given. Growths of this bacterial protoplasm were instituted in liquid suspension and massed upon a solid, in both cases under the influence of peptone and meat extract nutrient. (For the sake of uniformity all the studies involving No. 24 were made with subcultures from one culture tube.)

CHARACTERS PER SE

In bouillon, the impressive characteristic is the overwhelmingly foul and putrid odor, permeating far beyond the confines of the tube, and arising within 12 hours after inoculation. Within the same time, a profound clouding has occurred, and a delicate, slightly bluish, iridescent pellicle lies upon the surface. In 12 hours more, usually, in the presence of plenty of oxygen and brought out by shaking, the already described exquisite, green color appears. The liquid is now intensely alkaline. Upon adding acid the color disappears.

After 48 hours the odor becomes less intense, the pellicle is

disrupted with ease, and floats in large masses at various levels in the liquid. Gradually the heaviness of the cloud grows less, and the green color loses its brilliancy. Then the odor disappears. Finally the cloud is represented by a gelatinous core, and the whole culture is quite transparent. The green color very slowly fades to a dirty brown.

The functional capacities of this type of culture, as exhibited in artificial media other than plain bouillon, appear very general. Indol is produced. Milk may be completely liquefied, especially in the chromogenic cultures, and a peculiar violet, semi-transparent liquid is the end result. Some cultures merely coagulate the casein with more or less acid production. Glucose saccharose and lactose are disrupted, with the evolution of gas and acid. Recultures from these, inoculated into plain bouillon, exhibit a return of the usual typical characters observed in that medium. Upon Russel's double sugar medium, a growth frequently takes place which shows merely white, rather dry colonies, without gas or acid production. But variations are seen, with pictures resembling those due to various fixed strains of organisms of the typhoid-colon group.

The one fact stands out; the more pathogenic and disease producing the individuals of a culture, the less is the activity upon these media, especially in regard to indol, alkali production, and the digestion of milk and gelatine. This will be well shown in the case of the supernatant fluid-born organisms of high pathogenic power which do not digest these substances until the cultures have resumed a more saprophytic state. Such a difference of course is observed between the activities of Bacillus typhosus and Bacillus coli communis, but not to any such marked degree.

THE UNITS OF MATTER

The microscopical appearance of the cloud is that of finely subdivided matter. The units in the great majority of instances are single, short, slim, rather pointed *Gram negative rods,* exhibiting a most intense motility. There are units which

are double, some plumper, or longer, and some Gram positive. Some units are not rod shaped at all, but round.

The development of these units was studied, but the exact course was very hard to determine since the changes in a single piece of protoplasm could not be observed without serious effects, dependent upon the methods of observation. There may be mentioned the use of a series of small culture tubes, drawn out at the butts, so that at regular intervals they could be broken and the bottom levels of all compared chronologically. But of course such cultures did not develop synchronously.

The same culture tubes, studied hourly for 12 hours and daily for one or two weeks, repeatedly provided a distinct impression of certain definite stages, arbitrarily separated and outlined. Actually, the stages so widely overlapped that an exact demonstration was impossible and any series as exhibited by the relatively immense platinum loop pictures, only confused, if merely one set were compared. Dilution and centrifugation were more selective, but naturally there could be no assurance as to the individuals studied.

UNIT DEVELOPMENT

Roughly, there appear to be about seven stages in the course of unit development following the inoculation of plain bouillon:

1. In the first few hours the forms decrease in number and definiteness of outline.

2. Then increasing numbers appear, largely Gram positive, and more or less irregular.

3. The next definite stage is mainly characterized by irregular masses varying in size, and in the response to Gram's stain, but having a special tendency to respond negatively.

4. These masses appear to crystallize into the fine Gram negative units. This process becomes intense between the 6th and 12th hours.

5. The relation between coarse masses and finely formed units of matter, appears to swing back and forth, the forms

diffusing time and again into the masses, which for a time appear in ever increasing size.

6. The intensity of reaction grows less after some days until entire quiescence ensues.

7. In this stage of hibernation, form is again irregular and poorly developed.

NOTE: In all likelihood these changes represented the metamorphosis so frequently referred to, finely marked out by a temporary exaggeration of the "Stages."

COMPARISON OF UNITS AT DIFFERENT POINTS IN A CULTURE

At a single period, let us say after 12 hours incubation, there was a great difference in form between the units lying upon the surface and those in suspension. In the case of the latter, irregularities were quite common both in shape and staining reaction. A common picture was that of a large Gram positive amorphous globule, attached to several rather large, Gram negative rods. This region was presumably that of intense chemical activity. On the other hand the pellicle was characterized by great uniformity in all unit characters.

The careful selection of individuals at the various levels of a liquid culture failed to show any differences in their colony producing characteristics.

STUDY OF MASSED UNITS

Upon the surface of a solid, composed of a mixture of agar and blood, in the proportions of 4:1, a homogeneous streak rapidly formed. This was well developed in 12 hours and continued to broaden out for 12 to 36 hours more. At times a delicate runner spread over the entire surface. The colony was a fused, transparent, shiny, moist mass, somewhat elevated in the center. The edges were entire. In many cases a green pigment was formed, which spread in a zone extending from 2 to 4 mm. into the surrounding medium. This frequently was associated with a still wider halo of haemolysis.

The study of the individual units revealed a state of affairs practically paralleling that existing in a liquid. The individuals nearest the surface of the medium tended to be larger than at the top levels and less uniform in all morphological characters. At the summit were the closely packed, typical, uniform individuals.

ANALYSIS OF LATENT CAPACITIES

Further study of this material involved analysis by animal inoculation, with partitions resulting from partition and subdivision, either by mechanical, chemical, or other means, directly, or by offering an opportunity for selective development. The methods employed were the following:

1. Simple subdivision of a whole bouillon culture.
2. Centrifugation.
3. Filtration.
4. Separation by the inherent powers of unit-motility in a liquid.
5. Heating.
6. Drying.
7. Ageing.
8. Anaerobiosis.
9. Variations in food supply other than oxygen.

PROTOCOLS IN THE STUDY OF SUBDIVIDED STANDARD CULTURE NO. 24

(Rabbit alone used)

(Comparisons of amount are calculated on a basis of cloudiness in suspension).

I. Subdivision, Whole Culture, Plain Bouillon:

Rabbit:

No. 140. 1 c.c. of a 24 hour old culture.

Death:

8 hours.

Autopsy:

Hemorrhagic reaction typical of 2 c.c. of this culture. (rabbit pregnant).

No. 141. Five c.c. same.

CLINICAL HISTORY:

Three rabbits born at term during first 24 hours.

DEATH:

53 hours.

AUTOPSY:

Performed immediately. Putrefaction beginning about fecal masses.

Brain negative. *Rhinitis.* Right side of heart dilated, white nodules and hemorrhages into a leaflet of the tricuspid valve. Lungs were congested and oedematous. Parenchymatous organs were degenerated. Splenic tumor. Gastrointestinal system showed only beginning putrefaction.

CULTURES:

Injected: Gram negative rods.

Lung: Gram negative rods.

Even in the pregnant rabbit used, .5 c.c. did not give a typical reaction at all, but provided about the only case of endocardial disease observed in any of the series.

2. SUBDIVISION BY CENTRIFUGATION:

A. USE OF SUPERNATANT FLUID:

Cultures centrifuged for 3 to 5 hours. Supernatant fluid at times was perfectly clear and at times showed a slight suspension. Upon standing, the supernatant fluid always rapidly developed a secondary growth in richness equal to the original.

RABBIT:

No. 132. Two c.c. Fluid clear.

CLINICAL HISTORY:

Very weak. Breathing labored.

DEATH:

40 hours.

AUTOPSY:

Lungs: The right was dark and in the first stages of *consolidation.* The left was paler, but mottled. The remainder of the organs were merely degenerated. Intestinal catarrh was present.

CULTURES:

Forms were variable in smears.

Plain bouillon: Typical culture.

RABBIT:

No. 144. Two c.c.

CLINICAL HISTORY:

For the first 18 hours there was no change. Appetite good. Became rapidly worse for 6 hours, rapid breathing.

DEATH:
24 hours.

AUTOPSY:
Rhinitis. Lungs showed *pneumonia* with fibrin and fluid in the pleural cavity, in the primary hemorrhagic stages. The remainder of the organs showed only degeneration.

URINE:
A trace of indican was the only abnormality.

CULTURES:
Irregular as to unit forms, but digested milk, gelatine, saccharose and lactose; were foul in bouillon.

RABBIT:
No. 145. Parallel to No. 144: After 5 hours centrifugation fluid was shaken 10 minutes with kaolin, and then recentrifuged for 15 minutes.

CLINICAL HISTORY:
Exactly like No. 144, only it lived longer.

DEATH:
36 hours.

AUTOPSY:
Like No. 144 in every particular, only pathology was more advanced, with a suppurative *pericarditis.*

URINE:
Albumin, trace.
Indican, positive.

CULTURES:
Much more regular in form than those of No. 144 but exactly resembled them in effects, in milk, gelatine, sugar media and plain bouillon.

Variation in the use of kaolin cultures shaken *first* with kaolin for 10 minutes, then centrifuged for 15 minutes, shaken again for 10 minutes and centrifuged 10 minutes more.

RABBIT:
No. 147. Two c.c.

CLINICAL HISTORY:
Immediately ill.

DEATH:
36 hours.

AUTOPSY:
Lungs showed a few hemorrhages.
Appendix showed a few hemorrhages.
Parenchymatous degeneration.

CULTURES:
Not unusual.

RABBIT:

No. 149. 1 c.c.

DEATH:

After 3 months.

AUTOPSY:

Chronic disease.

RABBIT:

No. 150. 1.5 c.c.

DEATH:

After 4 months. Suddenly.

AUTOPSY:

Acute *rhinitis.* Gastroenteritis upon a basis of chronic suppurative *appendicitis* and *cholecystitis, cholelithiasis, hepatitis* and mesenteric *adenitis.*

SMEARS:

Pus and acutely formed mucus: Typical Gram negative rods.

CULTURES:

Rather slow in developing, but typical.

Mice were used in an effort to differentiate the cultures of the chronically diseased areas from those of the acute lesions.

.5 c.c. Heart culture.
.5 c.c. Bile culture.

Same results. Death within 20 hours. Stage autopsy primary. (First Method, induction pathogenesis.)

Apparently this slight variation in the kaolinized preparation lacked all the refinement of centrifugation and merely allowed units of less intense power to remain, localizing in the intestinal tract, with or without sufficient power to cause rapid death.

SUPERNATANT STRAIN

An attempt was made to develop cultures in which all the individual units of the bacterial protoplasm would exhibit, at least in some degree, the power in animal tissues exhibited by the supernatant-fluid-borne units. The resulting cultures have been designated as the supernatant strain.

DEVELOPMENT OF THE SUPERNATANT STRAIN

12/3/17. Reculture of No. 24 in plain bouillon.
Unit Motility: In Drop: Very active and generally so.
Slide test: 2nd position grew richly in 24 hours.

12/4/17. First supernatant fluid. Prepared by centrifuging this culture for 5 hours.

Cloudiness: About as usual (after 5 hours).

First reculture: Prepared by inoculation of plain bouillon from the supernatant fluid.

Rich suspension.

Pellicle.

12/5/17. Second supernatant fluid. Prepared like first from first reculture.

Cloudiness.

Second reculture: Prepared as above.

Moderate suspension.

Pellicle.

12/6/17. Third supernatant fluid. Prepared like the first from the second reculture.

Cloudiness.

Third reculture: *Slight* suspension.

Pellicle.

12/7/17. Fourth supernatant fluid. Prepared like the first from the third reculture.

Cloudiness.

Fourth reculture: *Moderate* suspension.

Pellicle rather heavy.

12/8/17. Fifth supernant fluid. (Used for animal injection).

Cloudiness.

Reculture of the fifth supernatant fluid. (Called the supernatant strain and used for animal injection)

Artificial media: In plain: After about 48 hours the pellicle, cloud and odor were all typical.

In milk: Very slow digestion.

In gelatine: Very slow digestion.

In saccharose and in glucose bouillon: Rapid gas and acid formation.

Unit motility: In drop: Very slight.

Slide test: Positions No. 2 and No. 3 rich. Position No. 4 negative.

PROTOCOLS

ANIMAL REACTIONS

A. Supernatant of 4th Reculture:

Rabbit:

No. 164. Two c.c.

Clinical History:

Acutely ill, and rapidly weakening.

DEATH:

72 hours.

AUTOPSY:

Brain: *Pus* in the ventricles and beneath the arachnoid. Hemorrhages of the large nuclei. Kidneys showed pus near the pelvis, hemorrhages of the cortex, no pyelitis. Heart, swollen and *inflamed.* Lesions, and rare hemorrhages of the gastrointestinal tract. Pus in the gall-bladder. Parenchymatous degeneration. Splenic tumor.

URINE:

Albumin present.

CULTURES:

Gram positive and negative rods.

RABBIT:

No. 165. One c.c.

CLINICAL HISTORY:

Acutely ill, then improved, but head was twisted to one side.

DEATH:

168 hours.

AUTOPSY:

Brain: Intense *congestion,* and fine hemorrhages in the stem. The kidneys were acutely inflamed. The heart was acutely degenerated, but pale; clots were antemortem. Adrenal hypertrophy. Few hemorrhages of the small gut. Parenchymatous degeneration. Splenic tumor. Gall-bladder, negative.

SMEARS:

Spinal fluid: Leukocytes, and bunches of primary rods.

B. FIFTH RECULTURE. WHOLE CULTURE:

RABBITS:

No. 169. Two c.c.

DEATH:

36 hours.

AUTOPSY:

Rhinitis: Slight *pneumonitis* and *pleuritis.* Hemorrhages of the stomach, intestine, and kidneys. Parenchymatous degeneration. Splenic tumor.

URINE:

Albumin positive.

RABBIT:

No. 167. One c.c.

DEATH:

48 hours.

AUTOPSY:

Hemorrhages of the stomach. The remainder of the organs were remarkably free of disease.

RABBIT:

No. 168. .5 c.c.

CLINICAL HISTORY:

Head high and pneumonic breathing.

DEATH:

36 hours.

AUTOPSY:

Slight *pneumonia* of the base of right lung.
Serofibrinous *pleurisy* marked.
Gastrointestinal tract negative.
Parenchyma degenerated. Splenic tumor.

RABBIT:

No. 170. .25 c.c.

CLINICAL HISTORY:

Weak for a long time.

DEATH:

3 weeks.

AUTOPSY:

Gastrointestinal catarrh. Adrenal hypertrophy. Kidney, suppurative lesions. Parenchyma degenerated. Splenic tumor.

URINE:

Albumin positive.

RABBIT:

No. 171. .1 c.c.

DEATH:

36 hours.

AUTOPSY:

Lungs were congested, with a suggestion of fibrin over the pleura.

Gastrointestinal system negative. Parenchymatous degeneration and congestion. Splenic tumor.

CHECK BY ORIGINAL WHOLE CULTURES (24 hours):

RABBIT:

No. 173. Two c.c.

DEATH:

24 hours.

AUTOPSY:

Hemorrhages of the stomach, intestine and muscles.

RABBIT:

No. 174. Two c.c.

DEATH:

24 hours.

AUTOPSY:

Hemorrhages of the thymus, heart, stomach and intestine.

It is likely that we have here demonstrated the highest and finest degree of unit power attained by any method of experiment included in this investigation. There was first an unusual uniformity between the effects of the different amounts of culture. There was the very rare involvement of the brain, and the unusual attack upon the heart and kidneys, with a most striking degree of reaction and resulting lesion development. Death occurred slowly, not rapidly as in the cases where there were associated units of low grade saprophytic power in a toxic manner, but rather associated with finely pictured anatomical faults.

Regrowth in bouillon of the active 5th supernatant fluid showed an increase in bacterial-unit-affinity for animal tissues, being at least equal in disease producing power to .5 c.c. of the original culture, and also more specific in acting. But there was evidently a dissipation as well as restriction in this power due to the necessary development of saprophytic units under the influence of artificial media.

CHECK: To investigate the temporary effects of an animal's tissue upon the segregation of this power in the centrifuge-resisting-units of this bacterial protoplasm.

RABBIT:

No. 223. Two c.c. of the supernatant fluid prepared as usual from an original typical culture.

CLINICAL HISTORY:

Pneumonic breathing.

DEATH:

24 hours.

AUTOPSY:

Trachitis: The upper lobe of the right lung was solidified, with suppurative *bronchitis.*

Hemorrhages of the stomach, duodenum, small bowel, appendix and kidney. Parenchymatous degeneration. Splenic tumor.

RABBIT:

No. 233A. Two c.c. of the supernatant fluid after 3 hours centrifugation of the heart blood culture of No. 233.

RESULTS:

Negative.

Temporarily, at least, the refined power segregated in the supernatant born units, appeared dispersed in the animal passage, where use was made in the first animal of all the units of the culture. This had an interesting bearing upon the clinically established fact of the non-transmissibility of the disease between human beings, during the epidemic.

B. USE OF SEDIMENT:

Comparison of units subdivided by the manner of sedimentation, with those remaining in the free fluid. Bacterial forms suspended in plain bouillon and standardized against a 24 hour whole culture according to the degree of cloudiness.

Sediment after 15 minutes centrifugation

RABBIT:

No. 142. One c.c. Standard. (Comp. 140.)

DEATH:

8 hours.

AUTOPSY:

Hemorrhagic reaction.

Thus comparable to whole culture.

RABBIT:

No. 143. .5 c.c. Standard. (Comp. 141.)

CLINICALLY:

Ill for first 24 hours, then improved. 72 hours, weakening.

DEATH:

96 hours, with convulsions.

AUTOPSY:

Lungs: Large black rimmed *abscesses* of both, full of pus. *Pericarditis.* Pus in the liver, gall-bladder and kidneys. Spleen hardly affected.

CULTURES:

Typical, generally, in plain bouillon and in form, but tended to a Gram positiveness of units.

1. As will be observed by reference to the protocols, various amounts of 24 hour plain bouillon cultures were employed in the study of the reaction in animals. Of course the smaller the dose the less rapidly did death occur. The rapidity of death and the degree of development of pathological lesions bore an inverse ratio to one another, up to a certain point. Further, the more delicate adjustments, as indicated by slight pneumonic lesions, at times were demonstrated. But this ratio was very limited, occurring more or less evenly in subdivisions involving amounts from 1 c.c. down to .3 c.c Below this amount it appeared impossible to show gradations following the use of different amounts of protoplasm. The units adjusted themselves, living in localities varying in their presumed environmental adaptiveness, and symbiosis lasted for months.

It seemed likely that in these small amounts there was an increasing chance for variety in the units obtained by subdivision, depending upon temporary abnormal inequalities in the functional adjustments of the various portions of the whole culture. But apparently by this method, chance never permitted the acquirement of protoplasm in which there was any great concentration of the powers of adjustment to animal tissues.

2. A subdivision by centrifugation and the comparison of the reaction following the injection of different amounts of the supernatant fluid and the sediments, provided a method by which a real segregation in functional power was recognizable, just as in the case of culture No. 1522.

After 3 to 5 hours of centrifugation the practically clear supernatant fluids, in 2 c.c. amounts, quite uniformly killed rabbits in 24 hours. The amount of material remaining suspended was roughly calculated frequently to equal only that in .002 c.c. of the original.

Not only did death ensue with astonishing rapidity, but the degree of pathological development, the ocular effects of the chemical interaction between the invading bacterial protoplasm and the essential tissues of the host, were frequently remarkably advanced.

Especially distinct, was the experiment in which kaolin was employed to further clarify the supernatant fluid. This experiment was controlled, to check the possibility of the introduction of anything by means of the kaolin.

Unassociated with shock from the injection and an incubation period, there was a clinical picture of pneumonia, death resulting in 36 hours. At autopsy there was found a most typical fibrinous pneumonia, with associated pleurisy and pericarditis. Such a clean cut result was never observed except in these two rabbits.

The supernatant fluid did rarely fail to kill. The pathological lesions at times were restricted to hemorrhages variously located. Centrifugation segregated the protoplasm according to its functional capacity. The supernatant-borne-units showed the most intense power, but of course this power varied in degree. The separation, therefore, probably was not restricted to the functional factor.

It was interesting to observe that a smear of the supernatant fluid showed plump Gram positive single units, resembling the forms in cultures from natural sources of disease which had not retrogressed and were active in causing real pathology. Rabbits injected with the supernatant fluid, showed in a subculture, at first, these same rather irregular, Gram positive forms. These forms, apparently, cannot be thrown down by force, i. e., presumably only bunches of the adhering masses of the more saprophytic reproducing units gain sufficient weight to overcome their surface resistance and be cast down. This makes one believe that the non-centrifugable forms, probably, do little towards reproduction in artificial media. After they are developed they lie quiescent. They are less active in bouillon.

A. A supernatant "strain" was developed. On 5 successive days, recultures, made each from the supernatant fluids of the day before, were centrifuged, and finally the 5th supernatant fluid was injected. It required 72 hours to kill, and there were profound *abscesses* of the brain, heart and kidneys. One c.c. or one-half of the amount, required 168 hours to kill. Lesions

were found in exactly the same localities, but were all less marked.

It will be seen, that in the case of the supernatant fluid, 1 c.c. never resulted in any definite reaction. Also, in the case of the original cultures, the more slowly acting preparations generally showed the more intense lesion-production. It appeared that selection by repeated centrifugation might have caused a more even distribution of functional power; a relatively definite relationship between mass and the functional factor for disease.

B. The 6th bouillon inoculated from the 5th supernatant fluid was allowed to develop, and did so richly, thus constituting a relatively definite strain. Different amounts, from 2 c.c. to .1 c.c., were inoculated into rabbits and there was a distribution of the factor controlling the function of disease-production shown. In general, there occurred more frequently the production of definite disease; usually pneumonia was induced to some degree. One-tenth c.c. of this whole culture showed a far different and more impressive capacity than .25 c.c. of the original whole culture of 1522. (Page 123)

C. The cultures obtained from a rabbit, killed within 48 hours by a supernatant fluid, provided a supernatant fluid which had no such effect whatever in an animal.

D. Direct inoculation of milk and gelatine, from the supernatant fluid, resulted at first in no evidence of digestion. Yet after standing some days the fluid was found to be full of units. Apparently these were about as easily developed as in the first place, and re-inoculation of the above two media resulted in complete digestion.

E. While using the supernatant fluids, the sediments were studied. "Whole sediments" in various amounts, were injected. The centrifugable units were also segregated by centrifuging for 15 minutes, the first sediment being compared to that resulting from equal amounts of a whole culture. The sediments did not appear to be weakened in function through the absence of the supernatant organisms. In fairly small amounts they caused a quite definite pathology. Indeed there is no intention of conveying the idea that, by any means, all specific disease

producing units lie uncentrifugable. There is no sharp dividing line, but centrifugation leaves behind in the upper reaches of the fluid, the material of the most intense, relative power. The second sediment appeared a trifle more like the supernatant fluid than the first.

3. Filtration

Experiments were made with filters, American made, of the Berkefeld type. Cultures No. 24 were sucked either slowly through by water power or rapidly by an electric pump.

A. Study of a Non-Incubated Filtrate:

I. Slow Filtration. A. 40 hour culture; clear filtrate. Sterile.

Rabbit:

No. 115. Two c.c.

Result:

Alive 8 months later.

B. 96 hour culture; clear filtrate. Developed in artificial media a *typical culture.*

Rabbit:

No. 95. Two c.c.

Death:

Two months.

Autopsy:

Intense hemorrhagic lesions of muscles, fascia, skin of fore-legs and breast, i. e., a secondary disease in a carrier.

II. Rapid Filtration: 8 day old culture; filtrate, cloudy. *Smear:* Irregular Gram positive and small masses?

Rabbit:

No. 151. Two c.c.

Result:

Alive 9 months later.

Associated experiments, with three filtrates of a culture from another source gave similar results; continued life, or death after an interval of months, with chronic or secondary disease from bacterial protoplasm. This resembled that of the original culture, and was obtainable from no other known source than through the needle used in injecting the filtrate.

B. FILTRATION: Followed by cultivation in plain bouillon of filterable matter. Culture rich and typical, injected into a rabbit.

RABBIT:

No. 96. Two c.c. of culture as described above.

DEATH:

12 hours.

AUTOPSY:

Typical hemorrhagic reaction.

This proves the filterability of this bacterial protoplasm in a form capable of complete development, but not in a state of development, or structural arrangement, adjusted to any but feeble functional demonstrations in organized living material.

Filtration of bouillon cultures, as described, provided interesting results. At one time the filtrate of a 24 hour culture, after incubation in artifical media, exhibited a rich growth, the units developing after the typical manner. The 96 hour culture of the same provided a filtrate which remained sterile. A subculture of this allowed the passage of protoplasm only after incubation for 96 hours.

A filtrate produced by inoculation developed a living protoplasm; this injected before artificial incubation, induced merely a chronic, gastrointestinal catarrh, (Carrier).

A sterile filtrate excited no reaction whatever.

Thus there is an absolute variability of filterability. There may occur cycles, at certain stages of which the bacterial protoplasm undergoes an adjustment between the material and its functions, which permits of viability in the minute amounts which pass through the pores of a porcelain filter.

It was thought possible that perhaps the study of the most motile units might prove them to be closely related to those not centrifugable.

To do this, layers of bouillon were spread on sterile slides and these were stood in the incubator for 45 minutes, covered to prevent drying. Then by smear and culture, various stations were examined for those individuals which had moved furthest

from the point of inoculation. This was repeated 5 times, each time with a culture of the most active units obtained. The cultures grew lighter in population. Finally a comparison was made with the original.

The individuals were Gram positive, large rods and cocci, and not motile after incubation. They formed no indol, acted neither on milk nor gelatine, but disrupted sacchrose and lactose. After the culture became again rich, the units assumed a Gram negative form.

In a rabbit, a standard amount of culture induced a peculiarly localized adrenal hemorrhage, but did not compare in power to the supernatant fluids.

CONCLUSION: Motility was a necessity in the original rich culture; variations in unit powers were necessary for distribution. Richness depended upon a division of labor. Various capacities for labor were inherent, all more or less latent according to their distribution. Hence in higher cultures, those resulting from a selection induced as here shown, there was less necessity to go far for food, so a condition of non-motility developed. As in the case of the supernatant strain we merely got a diffusion of function. Evidently there was no hereditary tendency shown in this loss of the complicated differentiations and groupings seen in the more populous colonies.

4. STUDY IN MOTILITY

From bottle 11/28/17 (from old tube), 12/17/17. Tube inoculated; 24 hour growth used. 3 mm. platinum loop used, 2 loops of culture applied to bouillon on slide as described and 5 equal divisions marked off.

CHANGES BY HOURS

First Test.

Position.	24	48	72	96
No. 2	+	++ more pellicle	+++	++++
No. 3	+	++ more pellicle	+++ green	++++
No. 4	+	++	+++ green	++++
No. 5	−			

Second Test. For inoculation from previous cultures by position No. 4.

Position.	24	48	72	96
No. 2	+	+	++ green	+++
No. 3	+	+	+	++
No. 4	—			
No. 5	—			

Third Test. By No. 3.

Position.	24	48	72	96
No. 2	+	+	+	++
No. 3	+	+	+	++
No. 4	—			
No. 5	—			

Fourth Test. By No. 3.

Position.	24	48	72	96
No. 2	+	Equal to about	+	+
No. 3	+	1/16 cloudi-	+	+
No. 4	+	ness original	+	+
No. 5	—	culture.		

Fifth Test. By No. 4.

Position.	24	48	72	96
No. 2	+	+	+	+
No. 3	+	+	+	+
No. 4	—			Forms very irregular.
No. 5	—			Motility— Indol—

Position No. 3, first 24 hour growth:

Plain bouillon: Form, fairly regular.
Size *increased.*
Motility negative.
Fair growth.
Indol negative.

Gelatine: Negative.
Milk: Negative.
Glucose bouillon: 60% gas.
Saccharose: 60% gas.

PROTOCOL MOTILITY EXPERIMENTS

The effects of a culture developed from the most motile units.

RABBIT:

No. 146. Injected with mixed cultures, 48 hours old, made from positions 4 and 5, on a slide after 45 minutes incubation. The density was equal to .5 c.c. of the original, in 1.5 c.c. of a plain bouillon.

CLINICAL HISTORY:
19 hours well.
11 hours ill.

DEATH:
30 hours.

AUTOPSY:
The brain was congested and showed slight oedema. The heart was dilated with blood. The lungs were congested and oedematous. There was a gastrointestinal catarrh, parenchymatous degeneration, a splenic tumor.
Adrenals: Intensely hemorrhagic.

SMEARS:
Adrenals showed great numbers of fine *Gram negative rods.*
Spleen showed the same forms.

CULTURES:
Heart blood culture was light. Forms were very fine and delicate *Gram negative rods.*

5. HEATING

Whole bouillon cultures, 24 and 96 hours old, were sealed in glass capsules, which were immersed and heated in a water bottle to the two different degrees of temperature, 60° and 80° centigrade. Heat was continued for 1½ hours. The capsules were then opened, and studied daily for 96 hours, and more.

Inoculations made into plain bouillon every 24 hours usually gave negative results for the first 48 hours. In the case of the 24 hour cultures 60° C. destroyed all life. Older cultures survived 80°. The resulting cultures were typical in form. In a rabbit a culture so prepared induced the quick hemorrhagic reaction of the typical, untreated culture.

6. DRYING

Drying as it occurred in the bottom of cotton plugged test tubes has been withstood for at least three months. Cultures made from such material were frequently slow of development, but might develop typically. No animal injections of such cultures were performed.

7. Ageing

Age was studied for its effect with regard to both variability and function. The oldest and hence the most valuable cultures for this purpose were employed, those of No. 1522. After 1 year variability was intact. After 6 months a culture, when recultured, provided a typical reaction in a rabbit; 12 to 24 hour death, with indiscriminately located hemorrhages.

8. Removal of Free Oxygen (Anaerobiosis)

Anaerobic cultivation of this culture was performed in connection with a like study of No. 1522. Subcultures were made in each case from very old cultures (3–6 mos).

Two methods were employed.

No. 1. Small tubes of previously boiled bouillon were inoculated, and placed upon pyrogallic acid crystals, immersed in 10% NaOH. A vacuum was produced through a narrow drawn out test tube, sealed in the cork. This was sealed tightly with a flame, while the aspiration was taking place. After 72 hours these tubes were studied. Both cultures showed pellicles and rich clouds, with moderate sedimentation. The odor of both was faintly putrefactive. The reactions were faintly alkaline.

The smears showed two pictures. The pellicles were composed of *typical Gram negative rods,* the suspensions were remarkable for the diversity of form and the varying relationships to the Gram stain.

No. 2. Tubes drawn out, were filled with plain bouillon, boiled, cooled, then inoculated. After the air had been driven out by mild heat, they were sealed. After 72 hours these were studied. They both showed light growths, largely in the form of coarse particles in the suspension. There were no pellicles. The reaction of the bouillon was nearly neutral.

The smears disclosed the interesting fact that the forms developed were Gram positive, delicate, oval diplococci; but no more exclusively than was usual. No. 1522 showed only a very

few, and short, mostly Gram positive, rods. No. 24 showed more rod forms, and some were Gram negative.

This latter method certainly excluded to a far greater degree the possibility of free oxygen, irrespective of mechanical faults.

Further study of the above anaerobically growing cocci revealed a surprising tendency to fixation. The growth in the original anaerobic tube remained the same, and a recultured growth appeared unchanged. In 10 days on blood agar the original bouillon culture grew as a delicate streptococcal-like streak, composed of cocci, mostly rounded, but irregular in form and stain. Reculture of another more staphyloid streak showed much greater irregularity in the staining reactions. A subculture from this streak in bouillon still showed cocci. A reculture of this tube on blood agar, showed a whitish, staphyloid colony, grossly resembling the first streak, but a smear showed a profound tendency to protoplasmic fusion, with formlessness, and alternate areas differing sharply in their response to the gram stain.

Unhappily time has not sufficed for a study of the effects of anaerobiosis, upon the reaction induced by contact with animal tissues.

The profound effect upon the form and mass production in bouillon would indicate upon the basis of a comparison with other experiments, that interesting possibilities may well be along such a line of study.

9. Variations in Food Supply Other than Oxygen

The most important of all such influences has already been described. But a summary gives a more comprehensive idea.

The influence of the media used upon the flora concerned is well known. This was considerably disconcerting and it was realized that meat was presumably a new substance to the fecal bacterial protoplasm of the parrots. It caused special adjustments, best demonstrated by form; the fine Gram negative bacillus and a toxicity, presumed because of the severe evidences of shock after leading to death within 2 hours, as already mentioned.

A COMPARISON BETWEEN EFFECTS OF BILE AND GLUCOSE AND PLAIN BOUILLON

I. Living Tissues

Rabbit No. 1809, injected with 14 c.c. of the sediment of a culture of a healthy parrot feces, grown in ox bile and glucose bouillon, administered in 2 c.c. amounts, every two hours for 7 doses, lived 2 months. Plain bouillon cultures from the nose taken during life in 2 c.c. amounts, caused death with an acute hemorrhagic reaction.

Rabbit No. 27 injected with 14 c.c. of the sediment of a healthy parrot feces, grown in plain bouillon, in 2 c.c. amounts, every two hours for 7 doses, died within 24 hours, with hemorrhages. Subcultures from this rabbit, in glucose bouillon and bile, injected in the same amount (2 c.c. for 7 doses) caused death only after 8 days. Subcultures of this rabbit so injected, taken while alive, in plain bouillon, caused a rapid (12 hour) death.

These results seem to indicate that the same life material was used in inoculating both media, although this was unselected matter from fresh parrot feces.

Upon the other hand a culture was chosen, No. 24, repeatedly grown in plain bouillon, and causing death with hemorrhages, in 12 to 18 hours, many times and consistently. Bacterial protoplasm from No. 24 grown in glucose bouillon and ox bile for 24 hours, showed no loss of power (in sediment), but after 6 days, even following the use of three times the usual dosage, rabbits were hardly affected and lived indefinitely. Recultures into plain bouillon, even after living 3 months in glucose bouillon and ox bile, when injected, killed rabbits in 12 hours with hemorrhages. Like results were obtained with other strains. In ox bile and glucose bouillon cultures, Gram positiveness supervened, with usually an irregularity in form or even an assumption of coccal shapes, although a more or less fixation of form represented in a persistency of rods, may have proved an

exception. This was found to be the case in a large number of individual cultures tested.

II. Dead Tissues

In dead animal tissue there were shown almost as striking differences in reaction between specimens of protoplasm artificially influenced in this way.

The following simple experiment was performed. Four equal-sized rabbits were chosen. Three of these were injected with 2 c.c. of 24 hour cultures. Thus:

No. 1. With a culture of healthy parrot feces in ox bile and glucose bouillon.

No. 2. With a culture of healthy parrot feces in plain bouillon.

No. 3. With a culture of a typical culture No. 24.

No. 4. Control rabbit was not injected.

These were killed after one half hour, sufficient time to allow for free circulation of material. They were put upon the ice and examined for intervals of 3 hours. The rabbit injected with the culture of parrot feces in plain bouillon (No. 2) showed a most rapid and profound putrefaction. The one injected with the culture of parrot feces in ox bile and glucose bouillon showed almost no putrefaction. No. 3 showed only a moderate putrefaction and the control rabbit exhibited considerably less than No. 3.

Although included under the general heading, these effects of food substances should not be considered as resulting in a subdivision of a culture, except that by proper manipulation, there may be observed the capacity for simple saprophytism, held latent.

The attention undoubtedly will be focused on the principal fact frequently discussed, that like a match to a fire or a jar to powder, plain bouillon sets off chemical activities in the bacterial protoplasm. Like a dash of water, ox bile and glucose bouillon quenches to an innocuous sizzling.

DIRECT COMPARISON OF CULTURES NO. 1522 AND NO. 24

No. 1522, a bacterial specimen obtained from the marrow of a parrot which died during the epidemic. No. 24, induced by artificial means.

Both specimens after lying for months in closed bottles, in their original bouillon, were re-grown in plain bouillon. They both clouded the bouillon and formed well marked pellicles. They both evolved a foul putrefactive odor and formed a green pigment.

Smears from four levels, including the pellicles and at intervals to the bottoms, compared exactly as to unit form. These showed almost exclusively slim, short *Gram negative rods.* But both also showed scattered irregular forms, cocci and larger rods and units which were Gram positive. This was always the case with all specimens and the degree of variation differed widely.

On blood agar plates they looked exactly alike, streaks edged entire, smooth, shiny, slimy; of a beautiful rather dark green, of some variation in shade, and a wide halo of haemolysis.

SUMMARY CHAPTERS VI AND VII

1. Following the establishment of a relative stability for the so-called "Typical Culture" and its reactions, a stability resisting the influences of time up to 96 hours, and animal passages, a specimen developed experimentally (No. 24) was analyzed after the manner employed in Part I, Chapter III, but more thoroughly.

2. As in the former case the generalized bacterial protoplasm was most interesting as viewed concretely. It developed in plain bouillon, as the typical culture with its factor for disease production activated, and was kept active by the bouillon.

The points relative to form in the culture and the functions in artificial media have been described.

The separation of the cultures by various methods into very small portions, demonstrated:

(a) Flexibility and relativeness of any fixation.

1. Whatever the appearance and demonstrated functions of the small portions, in time, in plain bouillon environment, they provided *typical cultures.*

2. The preparation of a series of cultures by inoculation with the most motile units did not provide a culture showing great motility, but on the contrary, as it was lightly populated at first, the units had lost their motility. There was thus nothing binding between this and other characters.

3. Filterability through porcelain was observed at times.

4. Some cultures withstood heat up to 80 degrees C., others were killed at 60 degrees C.

5. Drying under ordinary conditions in a test tube was withstood indefinitely.

6. Ageing up to 1 year had no effects upon the viability, but the reaction produced in animals grew slowly less intense.

7. Two methods of anaerobic cultivation provided rapid growths, in one case of unchanged forms, on one the forms changed to cocci. The latter changed so slowly in returning to the state of the *typical culture,* that the course was not followed up for the want of time.

8. The principal variation in the face of pabulum changes was the adoption of the coccal form and the diminution in the activity of the factor for disease production when grown in ox bile and glucose bouillon.

(b) A division of labor in the culture and a temporary association between form and function. The factor for disease production was found largely segregated in non-productive, Gram positive, large non-filterable units which were discovered remaining in the supernatant after the typical culture was centrifuged.

3. It was found to be an entity practically like the culture No. 1522, obtained from the tissues of a parrot which was ill and died during the course of the epidemic.

4. In animals, portions of the culture in 1 c.c. amounts and less, induced a general disease, localized in the gastrointestinal

tract, respiratory tract, or both, as well as frequently in many other regions; acute, subacute and very chronic disease.

Amounts of less than 20% of the usual 2 c.c. dose did not provide bacterial matter with noticeably more definite or clear cut disease-producing power. Very commonly, the smaller doses, and in all cases, .1 and .2 c.c., induced only chronic disease.

5. By the use of the centrifuge, an even finer concentration of power for a given mass of protoplasm, represented by the studies upon the Supernatant Fluid, was observed during the experiments upon No. 24.

SUMMARY PART II

Bacterial protoplasm of healthy parrot feces was found to resemble, exactly, that bacterial protoplasm in the epidemically diseased parrot and in human tissues. Under the stimulating influences of artificially prepared meat substances and of living tissues, reactions, metamorphoses, and functional adjustments were induced similar to those observed in the study of the epidemic bacterial protoplasm. A concentration of disease-producing power in, or affinity for, animal tissues was observed. It could be artificially aroused, to such a degree, and in association with sufficiently minute particles of protoplasm, as to provide in the imagination an existing entity, resembling the wind-borne virus, conceived of as the vehicle of the epidemic.

CONCLUSION PART II

It is reasonable to conclude, therefore, that at least one natural, original source of the bacterial protoplasm concerned in the production of the epidemic cases of the disease in parrots and human beings, was the healthy intestinal tracts of parrots, where the bacterial protoplasm normally resided in the role of a saprophyte.

CONCLUSIONS PARTS I AND II

CONCLUSIONS FROM THE OBSERVATIONS UPON THE BACTERIAL PROTOPLASM RESIDING IN THE INTESTINES OF HEALTHY PARROTS

In this area, aside from the presence of animalcules, primitive life appears to be represented in material which is abstractly generalized in regard to the demonstration of those characters commonly employed for the classification of bacteria.

The bacterial forms are amorphous, irregular, regularly rounded, or rod shaped, thick and thin, long and short, homogeneous in density, or stippled.

The bacterial functions, executed for the incorporation of surrounding matter, seem universal.

Quick readjustment is one of the chief characteristics of this material.

It can pass through the finest porcelain filters now obtainable. (American; non-standardized).

It can withstand at least 80 degrees Centigrade of heat.

It appears to be oblivious to the passage of time.

It almost seems to survive, during periods of "hibernation," upon that energy resulting from the feeble interchanges in food and water molecules, which remain within the confines of an unchanging mass.

PART III

INTRODUCTION

Thus far there has been considered a disease of parrots and human beings. The bacterial factor was generalized bacterial protoplasm, its source the intestinal canals of the parrots. The capacity for the production of disease in rabbits, representing entities encountered in the epidemic series, has been experimentally induced in the bacterial protoplasm. In order to understand the train of circumstances which led this protoplasm from the intestines of parrots to human beings, it is necessary to consider primarily the generalization of the bacterial protoplasm more abstractly.

This has been named, "generalized." The mechanisms or determinants of characters are all flexible. That involved in disease production in animals is the one of prime interest. It has been shown to be simple, easily arousable, and intensifiable, by a substance composed of organized material to which the protoplasm is not accustomed. Therefore, first will be considered the method of producing disease in the bacterial protoplasm.

For the sake of simplicity the group of rabbits will be considered to represent the parrots and human beings. There must be investigated the disease set up by diseased bacterial protoplasm in animal organisms, not only as judged by the end results, but how these are brought about. The method or mechanism by which such disease ensues, including the manner of contact, will be the second undertaking.

Finally there will be summarized the end results of these two processes.

Chapter VIII

GENERAL SUMMARY AND CONCLUSIONS CONCERNING THE AROUSAL AND DEVELOPMENT IN THIS TYPE OF BACTERIAL PROTOPLASM OF THE POWER* OF GROWTH IN, AND AFFINITY FOR, ANIMAL TISSUES, TOGETHER WITH THE MORE THEORETICAL CONSIDERATION OF THE MECHANISM OF THE EPIDEMIC

The investigations of the supernatant fluids, and the "supernatant strain" involved the highest point reached in the volitional control of the living chemistry represented by the bacterial protoplasm under consideration. The demonstration of such hidden power was of course easier than the clear realization of just what constituted the steps involved.

The bacterial protoplasm sojourning amid the food debris in healthy parrot intestines, contained, latent, the capacity for the digestion of almost any organized material, including that demonstrating life energies. Within 24 hours, meat substance could arouse the most complicated of these capacities, into very demonstrable activity. At this stage, under the influence of artificial media alone, there appeared to be a transitory increase in activity which included all portions of the protoplasm of a culture, on a more or less even basis. This increase for all units was sufficient to permit of existence throughout an animal's tissues, and

*Note: This term "power," so closely related to the conception of an adjustment to an environment, will be explained for this form of bacterial protoplasm, in Chapter XI, under the description of the theory of catalysts as applied to bacterial protoplasm-animal tissue affinities.

in the case of the 2 c.c. amounts employed, in time (8–10 days) a general reaction might result, effecting the death of the host.

The next stage, definitely higher in the demonstrations of power, was that of the *Typical Culture,* so frequently referred to. This had arisen apparently only after a sojourn in animal tissues. But that the length of time required for this contact may be less than a day, was proved in the case of the heart blood culture of No. 27. (Induced Pathogenic Series, No. 11 Method, Page 91). This *Typical Culture* was characterized, in the main, by the causation of the death of the host in 12 to 24 hours. Such a short course imposed a condition of hyperactivity upon any lesions present. This death was usually associated with hemorrhages.*

Gross subdivision of the material at this stage did not indicate that there had been a general increase in power. The study, first of the relationship of the number of lesions to the degree of putrefactive changes, second, of the results of the partition of a culture by the use of the centrifuge, indicated that in the main, the difference between these two principal stages lay rather in the distribution of power among the units than in refinements or intensification of material so segregated.

That the pathway to power may be unquestionably short, as just described, was also indicated by the quick changes in this power demonstrated in the series included under Rabbit No. 11. These were brought about at will by changes in the artificial media employed. The demonstrations of facility have a profoundly important bearing upon our ideas as to the mechanism of the epidemic. Certainly it would appear that the time required for its origin could, conceivably, be very short.

Considerable intensity of activity developed in the mobilization of this power as actually observed in the specialized portions of the protoplasm suspended in the supernatant fluid remaining after the centrifugation of a bouillon culture. The stage exhibiting these phenomena could not be altered as regards relation to a

*Note: See also results following the injection of supernatant fluid. I, when of a youthful culture. II. Later: Supernatant Strain Controls. III, Still Later: Chapter VII, interactions.
III. Still later: Chapter VII, interactions.

general increase in power, by the usual methods of animal passage, that is, after a *Typical culture* was developed.

But although a refined power was demonstrated, it was realized that this might result from great rapidity of change and animal passage, upon these specialized units.

On the other hand, whole cultures, while retaining their capacity to cause quick death in animals, showed, in time, a tendency towards the production of less generally located hemorrhages. The supernatant fluids, as months passed, produced pneumonia with less regularity, or not at all, and even slower death. (Part II, Chapter VII.)

Thereby, only after the intensity in the activities was diminishing, could degrees of refinement in the animal-tissue affinity of the special units of bacterial protoplasm be established, as was their relationship to one important factor, *time*.

One step further was advanced in volitional control. The development of cultures, more and more purely of supernatant-borne units, ("Supernatant Strain"), at least added perspective. The clinical picture producible was drawn out (No. 164), with a longer course, and more extensive lesions existed. The power to cause disease seemed to be distributed throughout more of the bacterial matter in the culture than it was in the case of the ordinary supernatant preparation. Thus more tissues became involved, and the disease was more than ever a general one, clinically and at autopsy, as were the cases seen during the epidemic. It seemed likely that the actual amount of power per unit-mass was somewhat decreased. The use of half the amount of material, or 1 c.c. of the supernatant fluid of the 4th reculture, of the cultures providing the so-called "Supernatant Strain," resulted in just about half as much disease, so to speak, as 2 c.c. of the same fluid. Apparently by these manipulations, in some way the relationship between mass and its related function became, at least temporarily, to some degree a fixed factor or quantity. The units resembled infectious units, the more inflexible variety seen in the case of sporadically diseased Parrot No. 3.

The sojourn in artificial media, as shown by the use of whole cultures of this supernatant strain, demonstrated a diffusion of

power (degree of functional activity). The diffusion was well shown, as represented in the loss of power by the supernatant units, in a subculture of a rabbit which had just been killed with an ordinary supernatant fluid, not from the supernatant strain cultures.

Just as in the human bodies, bacterial epidemic power faded and the force of impact was taken up like the wave upon the beach, so this experimentally instituted, feeble initiation of an epidemic, was observed to fade. The evanescent character of such phenomena seemed a little illuminated by these observations.

To recapitulate: A start was made with entirely generalized, healthy, saprophytic, bacterial protoplasm. This was living matter not fixed as to any irritability-reaction, flexible in the adjustment-responses to all factors in its environment. Such a manner of meeting and dealing with all things without discrimination, alone could perpetuate generalization of character.

An artificial imitation of animal tissues, (plain bouillon), by contact with such bacterial life, sets up a reaction response which is antagonistic, a special evolution of chemical energy which breaks down the structure of the exciting material. The results of replacement of the artificial extract by living animal tissues, even under experimental conditions, seem to indicate that to the bacterial protoplasm the two substances are closely related, for the activities aroused in the bacterial protoplasm become merely intensified. In the last analysis, such contact seems to result in an organized tissue-replacement process. The bacterial protoplasm is temporarily specialized, and of course its life, primarily a generalized one, is thus endangered. Therefore it may be considered as diseased. The activity in living tissues results in a counter effect, an antagonistic process resulting from activities of the host's tissues. To some extent these are the result of the exercise of primitive functions, at least upon the part of many specialized, fixed cells. Disease is induced in the animal organism.

Reculture of the bacterial protoplasm from these tissues into plain bouillon merely provides much temporary general saprophytic activity upon the part of the bacterial protoplasm. But

the power of affinity for living tissues already developed is not allowed to be interfered with, being segregated in special non-reproducing, non-vegetative units of matter.

It appears that the development of all this power in the bacterial protoplasm is rapid, an arousal tissue-contact in one animal. Power, rapidly accumulated, proceeds by its own inertia up to a certain point, experimentally limited, as demonstrated by the Supernatant Fluids of the freshly developed Typical cultures. It then slowly diminishes.

Its use does not seem to depend so much upon the amount of living tissue or the time, but upon the suddenness of the arousal of a tremendously sensitive, flexible, reacting mechanism, which becomes intensified on a basis of its own kinetic energy.

It does seem to require both plain bouillon and animal tissues, but only a small amount of each seems sufficient. Moreover, it requires, first plain bouillon and then animal tissues. Injection of the protoplasm unaroused in ox bile and glucose bouillon results in a very slow process. In the face of living tissues inactivated bacterial protoplasm is at a disadvantage.

This mercurial production-process could be impeded. Buried suddenly in animal organism, especially in one to which it is unaccustomed, whatever the initial distinctive effects, momentum is lost; energy is absorbed in the surroundings and the bacterial protoplasm exhibits more or less diminution in power. (This must not be considered in conjunction with the mass effects of the *Whole Typical Cultures*).

Before leaving this subject, necessarily at present so full of conjecture, let us conclude any discussion of the mechanism of an epidemic, such as occurred in association with the parrots, justification for which, extending from this point on a theoretical basis, may be found in the investigation taken as a whole.

In the first place, it may have seemed paradoxical to assert that the effect upon the function of disease production of bacterial protoplasm, the meat substance in the artificial media, was greater than life in animal tissues, themselves. The effects of the former were certainly far more easily demonstrated however much the results of its unsupported action lacked in

fineness. Naturally, the effect produced by the appearance of something, where there was for all practical purposes nothing, was more easily observed than those effects dependent upon further increments exhibited in animals, of a power already in evident existence. Thus, the underlying phenomenon of all the experiments in the artificial production of disease may be said to be a quick arousal of latent powers on the part of this bacterial protoplasm, by means of a simple stimulus of meat substance in bouillon.

The key phenomenon is one measured and to be conceived of in terms of energy.

What a match to start the mighty conflagration of this primitive form of an epidemic!

The next step, that of living-tissue contact in general, the fuel for the epidemic, has been considered in the case of the rabbit organisms as substituted in the experimental studies.

But, from this point, difficulties lie in the way of a further understanding. Unhappily, one of the most interesting possibilities has not been investigated at all.

There are, perhaps, three main possibilities. First, arguing from a single fact, in a number of cases, the disease, in its epidemic form, seemed as if it must have been transmitted to human beings from the parrots, after the removal of the latter to the homes of the former. Thus, it is possible that the epidemic picture, drawn in the store, was merely a simple multiplication of effects, capable of being produced by the material from each parrot, as represented experimentally in rabbits. This would at least presume a most intense form of individual disease, which latter was unquestionably possible and very likely, under the effects of the severe chilling of all the birds.

The other possibilities are concerned with two theoretically positive factors, both of course dependent upon a special increment of their affinity for animal tissues, experimentally aroused and viewed in concentration, in the supernatant fluids of *typical cultures*. This increment might be added in one of two conceivable ways:

(a) By the passage of protoplasm, already aroused, from the

intestinal tract of one parrot, through the organisms of one or more other parrots. Certainly, no such dispersion of power would occur as that which is effected by a change in animals and which limited the human phase of the epidemic. It seems probable that in such an epidemic the focal points are multitudinous, originating in many intestines. Unquestionably, such a passage of protoplasm from the intestines to the tissues of the same and other individuals, and from tissues to tissues, must have occurred, and presumably, an intensity in power, not experimentally observed, thereby accrued. Whether there is, also, a factor constituted by parrot tissues, as such, is a question. Whether a certain degree of intensity of power may thus, alone, accumulate from any parrot tissue-contacts, and thus reach that degree imaginable at the moment of the epidemic, is not yet known. The only experiments really bearing upon this point are those involved in the first method employed in the artificial induction of pathogenesis. There, with a slowness of course incomparable to the time employed for the epidemic changes, bacterial protoplasm from birds was passed through mice and showed an increasing affinity for animal tissues. Then, in poor imitation of the natural epidemic method, this was transferred to another animal, not differing as to class, but only as to genus. Temporarily, the power did seem to decrease, as it may have been supposed to do in human beings during the epidemic, exhibiting the nine day, human incubation periods, and a lack of further transmissibility of the epidemic. Perhaps in larger measure, the differences between the experimental and natural methods lie in the intensity and rapidity and regularity in accumulation of the reaction characteristic of the initial stages of the activity involved. Quantity, not quality, is demanded, and an uninterrupted sweep must be essential.

(b) The other idea depends upon a summation of power, not by contacts of tissue, but by those of similarly aroused particles of bacterial protoplasm.

Perhaps all these factors served to intensify the natural process of arousal of that activity in the bacterial protoplasm which

resulted in affinity for animal tissues, and which has been experimentally developed and already outlined above.

Protoplasm in such an active and abnormal state was transmitted from the parrots to the human beings at the time of the epidemic.

In the next chapter a theory will be worked out as to the mechanism whereby the complicated disease pictures arise in the animal.

Chapter IX

THE MODE OF DISEASE PRODUCTION IN THE ANIMAL ORGANISM BY DISEASED GENERALIZED BACTERIAL PROTOPLASM

The state called "Common," represented by a typical culture in plain bouillon, was at first by the force of circumstances, later by choice, the ground from which the bacterial protoplasm was viewed. The forms it assumed, and their development, have been described as observed in both dead and living organized material.

The functions of which it was capable have been studied, principally, by their end products, the most important entities of all, the disease pictures. It remained to study the functioning more closely; to investigate how and why these results were obtained. In other words, there still was left an empty gap, hardly touched. On one hand there has been described bacterial protoplasm, upon the other the end results, viewed in the autopsy pictures and following upon the introduction of this bacterial protoplasm into the blood stream of animals. What took place between the events of inoculation and death had been thus far left in the dark. Unhappily, much of it will have to be allowed to remain there.

Throughout the work, with the exception of some white mice, the rabbit has been the animal employed for the study of artificially induced disease. But one route of inoculation was used, that by way of the ear vein. For the lack of any other fixed points from which to work, the receiving organism, or host, was assumed as a fixed quantity, its death a fixed point in time. For this reason also, it was obligatory to investigate in general this artificial equation, the interaction and resulting reactions, inducible between the high and low forms of life herein represented.

This was a very difficult undertaking. But, in the experiments some effort was made to determine what the various particles of this bacterial protoplasm did after they entered the vein, where they went, and, if possible, why they went, and what occurred after they had arrived at their destination. On the other hand, the causes of the variation in and the location of the reactive lesions in the receptive, more complex organism, may be suggested.

The first two experiments were only preliminary, the third was fairly complete, so far as it went. The fourth was largely a control. Perhaps, after many such efforts have been made, using this variety or form of bacterial protoplasm, definite ideas of bacterial localization and animal reaction may be acquired, and upon, apparently, a very abstract basis.

EXPERIMENT I

7/3/18. A moderate sized, healthy rabbit was etherized, the abdomen opened, and covered with hot towels. There was injected 2 c.c. of a plain bouillon culture of culture No. 24, ninety-six hours old, and green in color.

RESULTS:

(A) ANTEMORTEM:

Examinations were frequent and smears were studied at frequent intervals, for four hours, at which point death occurred.

No organisms were found in the blood from the leg, breast, bladder, intestinal wall vessels, or mesenteric arteries. About four drops of the heart blood was laked and centrifuged, but no forms were seen. After one and one-half hours, the appendix became profoundly engorged, but showed no hemorrhages. It did not appear twisted upon its vascular stem. The stomach was never touched, but showed several sub-mucous hemorrhages after death. The duodenum appeared continuously dilated, while the remainder of the small bowel was not dilated. Perhaps, this difference may partially account for the frequent location of hemorrhages at this point.

(B) POST MORTEM:

Smears of the tissues of the various areas of the apparent lesions were made after death, and where lesions usually had been found. The stomach, mesentery, bladder, small intestine, and duodenum were thus studied. None of these areas showed bacterial forms. The liver showed great numbers of rather large, Gram positive, round masses.

EXPERIMENT II

7/5/18. A rather small rabbit was obtained from a barn yard, where several had been ill. Its leukocyte count was 17,000, hence, it was probably not perfectly normal.

The carotid arteries were first dissected out. Injection was made with a forty-eight hour culture of the heart blood of the rabbit used for the first experiment. One hour later, it was bled to death, as completely as possible, from both carotids. The blood was caught in distilled water, laked, and centrifuged. Parts of the lung were removed, under sterile conditions, and ground up with sand, washed with normal saline, and the resulting fluid centrifuged. Part of the spleen was pressed in Rosenow's press, and the fluid was centrifuged. The organs, lungs, spleen, heart, liver, and kidney, were removed under sterile precautions, placed in a petri dish and set in the incubator for eighteen hours.

NOTE: Fibrous nodes in the liver were found, perhaps, the cause of the leukocytosis.

RESULTS:

Of the mixture of blood and distilled water, about 300 c.c. in all, 260 c.c. was centrifuged for thirty minutes. About twenty per cent of the fibrin cast obtained in the top of the tube was smeared and examined. Very few bacterial forms were seen, most fields were empty, some showed a few bacteria. The forms seen, varied from regular rods to aberrant, bizarre, and poorly stained units.

In the fresh pieces of the lung and the spleen, ground up and smeared, no forms were seen.

After incubation, all the organs were moist and had a sour odor, but not a putrefactive stench.

The smears showed:

Lung: Fine rods to cocci.
Heart: Much coarser rods.
Kidney: Same.
Liver: Mostly cocci.
Spleen: All cocci.

HISTOLOGIC EXAMINATION:

The heart, liver, and spleen showed cytolysis, especially marked in the liver.

EXPERIMENT II-A*

A moderate sized rabbit was chosen, immediately after it had been received from perfectly healthy surroundings. Its leukocytes, nevertheless, were 14,000 in number. The method of procedure was the same as in experiment II; the rabbit was bled to death, one hour after it had received 2 c.c. of a thirty-six hour plain bouillon culture of No. 24.

The blood, prepared as in experiment II, was smeared, as were small

* See chart at end of chapter.

pieces of the lung, liver, spleen, appendix, and mesenteric nodes. These organs were all cultured in duplicate, (by the use of small portions) in plain bouillon, litmus milk, and gelatine.

The same organs, plus the heart and kidney, were then incubated in a dish for twenty-four hours. Small portions were again smeared, and inoculated into the same media. The milk and gelatin cultures were studied at 18, 48, and 72 hour intervals.

RESULTS:

Before incubation the blood showed a very few bacteria, and the smears of the organs, no forms at all. Following incubation, smears of all the organs were rich in forms, which varied somewhat in each one and greatly between different smears.

FORMS:

The lung showed coarse Gram positive rods.
The kidney showed *Typical Gram negative rods.*
The heart showed Gram positive cocci.
The appendix showed irregular Gram negative forms.
The liver showed irregular Gram positive-negative forms.
The spleen showed same as liver.

In the artificial media, especially in the milk, the variety of pictures was remarkable.

CULTURES: In the milk, the liver showed the earliest coagulation and digestion. In the liver, lung, and spleen cultures the most uniform degrees of digestion resulted. The mesenteric nodes caused somewhat less intense results. The blood and appendix caused slow, but watery digestion in one of each pair of tubes. In gelatine the results were practically parallel to those observed in the milk.

After incubation the results were more rapidly induced and were more uniform in speed and type.

The forms assumed in the milk were mostly Gram positive rods and cocci. In the gelatine all were *Gram negative rods,* but they were irregular in the liver.

The appendix was an exception in the size of the units. In one tube, these were great, Gram positive rods, which, as the later experiment IV (Page 158) indicates, were probably not related to the organisms injected. The other appendix culture tube compared very closely with the positive blood culture tube.

EXPERIMENT III*

This was a study of the localization, reactions, general and local, in primarily both living and dead tissues, and in tissues first alive, then dead, of bacterial organisms, both of whole bouillon cultures and of the supernatant fluid.

Four nearly equal sized rabbits, all healthy, were selected. (Three had a leukocyte count of 7,000, one, that injected with the *whole culture* and allowed to live, had 16,000.)

* See chart at end of chapter.

Fifteen c.c. of plain bouillon was inoculated with a reculture of No. 1522. Half of this was centrifuged for 3 hours until only a faint cloud remained. Two rabbits were injected with 2 c.c. each of the supernatant fluid, and two with 2 c.c. each of the *whole culture*. One of each of these pairs was allowed to live through the course of the disease. One of each was bled to death in one hour, from the two carotid arteries.

A common method was employed for study. Blocks of the heart, lung, liver, spleen, kidney, and skeletal muscle (thigh) were removed under sterile conditions. The appendix wall was sectioned, and a piece dipped in alcohol, and then flamed several times over. These blocks of tissue were then in part sectioned into 1 to 2 mm. pieces, one piece being directly smeared, and the others placed in 2 test tubes each of plain bouillon, litmus milk, and nutrient gelatine. The original blocks were then placed in a petri dish and incubated, as were the inoculated media.

After 24 hours the blocks were removed, sectioned, and the smears and cultures repeated. The degree of putrefactive change in the different organs was noted.

The rabbit inoculated with the supernatant fluid and allowed to live, was found to be in a dying condition after 24 hours. The whole culture caused death in 20 hours. The organs of these two rabbits were studied, not only as described, but pieces also were fixed in formalin, sectioned, stained, and compared as hereafter described.

RESULTS:

I. Demonstration of bacterial forms by direct study of tissues.

(A) One hour after the injection of,

1. WHOLE CULTURE:

(a) Before incubation. Forms were very rare, except in the appendix which showed a moderate number, and in the thigh muscle, a few.

(b) After incubation. Immense numbers in the appendix, muscle, and spleen, and these were fairly regular, but both Gram positive and negative. In the liver there were considerably less units and all were Gram positive. In the kidney, lung, and heart, there were great numbers, regular in the first, very irregular in the latter two organs.

2. SUPERNATANT FLUID:

(a) Before incubation. Parallel to the above.

(b) After incubation. There were great numbers in the muscle, appendix, and spleen. In the rest the numbers were moderate. All units tended toward uniformity.

(B) Twenty to twenty-four hours after the injection of,

1. WHOLE CULTURE:

(a) Before incubation. In the lung, and heart, there were many units. In the appendix, spleen and liver, there were less.

They were rare in the kidney and muscle, and none were present in the blood.

(b) After incubation. There were great numbers in all the regions but in the blood, which showed moderate numbers. The forms were fairly regular in the muscle and the blood. The most bizarre units were in the lung. Elsewhere they were irregular.

2. SUPERNATANT FLUID:

(a) Before incubation. Forms were rare except in the appendix.

(b) After incubation. Forms were extraordinary for their numbers except in the kidney. As in the other pair this experiment showed marked uniformity of the units.

II. Forms demonstrated by culturing the organs in artificial media:

These may be briefly summarized, in general. After one hour, and before incubation, the light supernatant fluid caused the lung, liver, and spleen to richly inoculate plain bouillon. The effects upon milk and gelatin were always marked in the case of these same organs; much less marked as a result of inoculation with portions of the blood and appendix.

III. Location of lesions. A parallel between the effects of the use of the whole culture and the supernatant fluid.

Whole Culture		*Supernatant.*
18 hours.	*Death*	Etherized in 20 hours.
Dry.	*Nose*	Dry.
Large hemorrhages.	*Lungs*	Negative.
Negative.	*Heart*	Negative.
Few small hemorrhages.	*Stomach*	Few small hemorrhages.
Hemorrhagic.	*Caput duodeni*	Negative.
A few areas, also paresis, mucus and gas.	*Ileum*	Negative.
Hemorrhagic.	*Appendix*	Hemorrhagic.
Dark and soft.	*Kidney*	Rather light.
Soft.	*Liver*	Glassy.
Acute tumor.	*Spleen*	Practically negative.

Thus, differing practically by billions in the numbers of bacteria by which they were attacked, these rabbits resembled one another in the results of death and the presence of appendiceal hemorrhages.

Therefore, considering the fairly established fact that the greatest disease producing power remains in the supernatant fluid, one is inclined to explain the production of the more intense lesions by the *whole culture* on the ground of a mass action; the effect of mere numbers.

IV. The comparison of the histological effects seen in the organs preserved in formalin, of the two rabbits allowed to complete the reaction to the point of death, after receiving respectively the *whole culture* and the *supernatant fluid.*

The organs studied were the heart, liver and kidney. Comparisons were drawn between the degrees of congestion, nuclear obscurity, cytoplasmic granulation or destruction, and the intensity of glomerulitis as evidenced, mainly, by the increase of nuclei.

The difference in the effects of these two preparations was very marked, though the duration of illness was similar. The *whole culture* caused death in about 20 hours. The *supernatant fluid* placed the animal in a dying condition in 24 hours at which time it was killed.

The *whole culture* caused by far the greater degree of congestion, except in the liver. The nuclei were generally marked by their distinctness. The cytoplasm was, in general, either in good condition or it was completely destroyed. Glomerulitis was intense and much more marked in this case.

The most marked result of the reaction to the supernatant fluid was the general tendency towards obscuration of the nuclei everywhere, associated also with areas of more or less complete destruction of the cytoplasm.

Thus we have in the case of the latter, changes more definitely associated with well known disease entities, a diffuse chemical reaction. On the other hand, the reaction of the great number of units of the whole culture was, apparently, concentrated in spots. This result might be conceived of as due to too many actions, taking place too rapidly to permit of an even diffusion.

V. Variations in the development of macroscopically evident putrefaction, following the injection of the two preparations, both with and without an intervening period of life on the part of the host.

1. Whole Culture in Living:
   General profound softening, moistening, and production of foul odors.
2. Supernatant in Living:
   Less marked changes.
3. Whole Culture in Dead:
   Much less moisture. Odors foul but more sour. All changes much less marked.
4. Supernatant in Dead:
   Practically negative. Much drying. Odor a trifle sour.

By smears:

Comparison was made according to the calculated microscopic structure, and the organs were roughly ranged according to the percentage of elements remaining. (Paralleling the two extremes):

1. Whole Culture in Living.		2. Supernatant in Dead.
Appendix	40	20
Liver	100	20
Spleen	50	20
Kidney	80	10

	1. Whole Culture in Living.	2. Supernatant in Dead.
Heart	100	Trace
Lung	50	0
Blood	50	0
Muscle	100	100
(Control normal).		

EXPERIMENT IV

I. Performed practically for the control of certain facts seeming to arise from the previous three experiments.

Four healthy rabbits were selected from the country, all having normal leukocyte counts.

A 36 hour culture of No. 1522 was prepared in these amounts; 15 c.c. in a corked 30 c.c. flask, 3 c.c. in a cotton plugged test tube. Bacterial growth appeared to about an equal degree but green coloration occurred only in the tube. Both cultures were centrifuged for 5 hours, with the result that only the faintest cloud remained in the supernatant fluids.

The following procedures were entered upon:

.2 c.c. of the supernatant from the flask were injected into the ear vein of a rabbit. In one hour the rabbit was bled to death, and the blood expressed from the body. The blood was segregated in two portions to determine the difference in content of bacteria, between the more actively and less actively circulating blood.

Portions of the organs were removed under sterile conditions, separately incubated for 24 hours, and then smeared for the study of the degree of bacterial unit formation.

II. An uninjected rabbit was bled to death in the same manner. The organs were also removed and cultured, immediately. The appendix was smeared. All the organs were separately incubated for twenty-four hours and then smeared.

In all the experiments, the portion of the appendix wall used, was washed in water, then dipped in alcohol, and flamed for a few seconds. This flaming was repeated three times. The same procedure was used to separate the blood from the heart muscle. It was supposed that deep in the normal appendix wall, bacterial protoplasm would likely be found.

III and IV. Into each of two rabbits, 2 c.c. of the supernatant fluids were injected from the flask, in order to compare the activities of the supernatant fluid from the culture in which the whole amount was involved.

RESULTS: NORMAL RABBIT BLED AFTER ONE HOUR:

1. In the case of the organs of the normal rabbit, the appendix immediately upon removal showed in a smear of its walls very little, if any, bacterial protoplasm.

As in the preceding experiment, the organs removed, besides the appendix, were portions of the lung, heart, kidney, liver, spleen, and thigh muscle.

Cultures taken immediately into plain bouillon, litmus milk, and gelatine, were all sterile, except that of the appendix wall.

After 24 hours of separate incubation the organs were all dry and odorless except for a faint suggestion of sourness.

Smears showed in all but the appendix, few bacterial forms, none in the spleen and kidney, one or two in the heart muscle, and a few in the lungs. In the appendix no forms were seen before incubation, but there were considerable numbers in the cultures, as might be expected. In character these forms were large, coarse, strongly Gram positive rods. (See experiment II., page 153).

RABBIT INJECTED WITH SUPERNATANT FLUID AND BLED ONE HOUR LATER:

II. The same organs were selected for incubation. Cultures were not taken, since few differential points appeared to arise from their study. But after 24 hours the organs were examined and found to be definitely in the early stages of putrefactive change and more or less moist. The odors of the appendix, heart, and liver were foul. The kidney was very dark and moist. The spleen was dark and dry, and the lung was dry. All showed great numbers of distinct bacterial forms, especially the heart muscle. These forms were regular small rods, associated with round, and some longer forms. All were more or less Gram negative.

The blood specimens, drawn at two stages, that is at the beginning of bleeding and towards the end, showed, immediately, few bacterial forms. After 24 hours the cultures, and the specimens of blood, showed great numbers of the same type of organism as seen in the other organs. But no difference of any kind could be detected between the two specimens.

RABBITS INJECTED WITH THE SUPERNATANT FLUIDS AND ALLOWED TO LIVE:

The rabbits injected with the supernatant fluids both lived seventy-two hours. At autopsy, neither one showed pneumonia, but intestinal hemorrhages, as in the preceding experiments.

NOTE:

Here, three times the supernatant fluids of this culture No. 1522, had failed to show the fine, disease producing powers which formerly were frequently observed to induce definite pneumonic changes. This power was best exhibited by culture No. 24 when it was fresh, and presumably most facile. The slow weakening, if this term can be used, of No. 1522, seemed very baffling at that time because this culture was obtained during the epidemic.

BRIEF TABULATION OF THE RESULTS OF ALL EXPERIMENTS

SUMMARY:

In 2 c.c. of the whole cultures used there were probably, roughly, 10 billion units. These must all have passed through the capillaries of the

lungs before reaching the general circulation. If completely distributed, each gram of rabbit should have received about 50 million. As about .05 grams of the organs were smeared these should have contained over 2 million units.

The following results seem to be deducible from the preceding experiments.

I. One hour after injection, formed bacterial units were present in extraordinarily slight numbers.

II. By incubating whole organs, bacterial protoplasm was found to be generally distributed and present in great amounts. It appears reasonable to conclude that it represented the material artificially introduced.

III. The various areas showed moderately distinct pictures.

A. The blood under all conditions showed the least number of formed units. These in turn exhibited in even less degree the powers indicative of saprophytism.

B. The lung, liver, and spleen showed in general the greatest number of units, and these appeared to be the most saprophytic in type of all. Presumably this was due, in the case of the lungs, to the original large size of the dosage. The liver showed an increase in forms with the prolongation of life, probably due to a persistent portal supply. The spleen appeared as a sac for the reception of great numbers of units, but showed no evidences of increase with the passage of time. The kidney appeared to free itself of some units after the first overloading.

C. The appendix, where it was possible to determine this point, was the principal seat of disease all through the experiments.

D. There appeared to be some segregation in the capacity for bacterial unit formation in living tissues. In the rabbit injected with the whole culture, after twenty hours of life, before the organs were incubated, forms were seen in the lungs and heart in considerable numbers, and in the appendix as well. Forms were otherwise rare, and yet the protoplasm was found to be generally diffused.

E. In general, there were great variations in form between the bacterial units in the different organs.

IV. A much greater regularity in bacterial unit outlines was observed after the injection of the *supernatant fluids* than resulted from the introduction of the *whole culture*.

V. The presence of this bacterial protoplasm enhanced putrefaction. This phenomenon was more intense, following a prolongation in the life of the receptive organism. Thus life in living tissues apparently enhanced capacities for life in dead tissues, just as vice versa.

VI. Diffuseness of interaction seemed to be the governing principle. There was remarkably little reduction of the reaction during a life period in the host. The diffuseness in reaction was more absolute following the use of fewer units of more definite disease producing powers, i. e. greater after the injection of the *supernatant fluid* than by the use of the mass of units contained in the *whole culture*.

CONCLUSIONS:

One, only, is clear. It is that following injection into an animal, there is a complete diffusion of this type of bacterial protoplasm, so complete as to establish relationships between the body tissues and the molecules, units, of course, far smaller than the visible bacterial bodies. This conception arose from the observation of the primary loss in forms; the increase in forms in all tissues upon incubation, and the variations in these, not only in comparison to one another, but to the forms injected. This diffuse contact exists especially throughout the fixed tissues. Probably all primary changes and reactions take place, largely, outside of the blood stream. The formation of lesions must be a secondary consideration. In the case of such generalized protoplasm, capacities for adjustment to living and dead tissues appear to be separated by a very small gap.

RECAPITULATION:

Experiments were performed whereby the fate of injected bacterial units was studied in the various tissues of an animal. The *whole culture* and the *supernatant fluid* were compared. The effects in one hour were compared with the course of disease, completed naturally, in twelve to twenty-four hours. The organs were incubated as a whole, and smears of the tissues were studied before and after. Cultures were made from various visceral points, as well as the blood and skeletal muscles; litmus milk, gelatine, and plain bouillon.

There was found evidence that the bacterial protoplasm penetrated everywhere, remained generally distributed irrespective of the lesions found, but was not generally represented by bacterial unit forms during the animal-life periods. If a great mass were employed, a general spottiness tended to be exhibited in the tissue changes, while the slight numbers in the supernatant fluid, no less generally distributed, caused a much more delicate diffuseness in the ocular evidences of reaction. (Especially well seen, of course, in parenchymatous organs.)

The blood showed the least evidences of involvement, a fact of interest when viewed in the light of the slow metamorphoses seen in the cultures of the bloods of the human patients, evidencing the presence of very slight amounts of relatively inactive bacterial protoplasm. The blood, and areas where lesions were caused, showed units having the least generalized digestive power.

Putrefactive changes were in direct proportion to the mass of bacterial protoplasm present in the tissue, a fact of common knowledge.

These facts must be correlated with the following, which were observed under other circumstances:

(a). The inoculation of a tube of plain bouillon with material from a *typical culture,* resulted first, in a diminution in the numbers of the formed bacterial units.

(b). In the form of *typical culture* studied, there was a vast difference in the distribution throughout the suspended bacterial protoplasm of the power of growth in animal tissues. Many of the units were relative saprophytes. Yet it appeared a fact that all the parts of a culture had the power, whether latent or not, of developing a fresh culture, complete in all its characters.

(c). The location, near or far from the gut tube, bore a relationship to the degree of power for growth in living tissues.

(d). In such a culture, pathogenesis, in general, appeared to be a relative condition.

(e). Although in a culture, and causing no visible effects following its injection into an animal, the bacterial protoplasm was almost invariably found in or about the intestinal tract. Its life appeared to be practically immortal.

With these facts in mind, some explanation may be evolved which will throw light upon the various disease processes which have been induced by this protoplasm.

The experimental disease pictures comprise these possibilities:

Lesions:

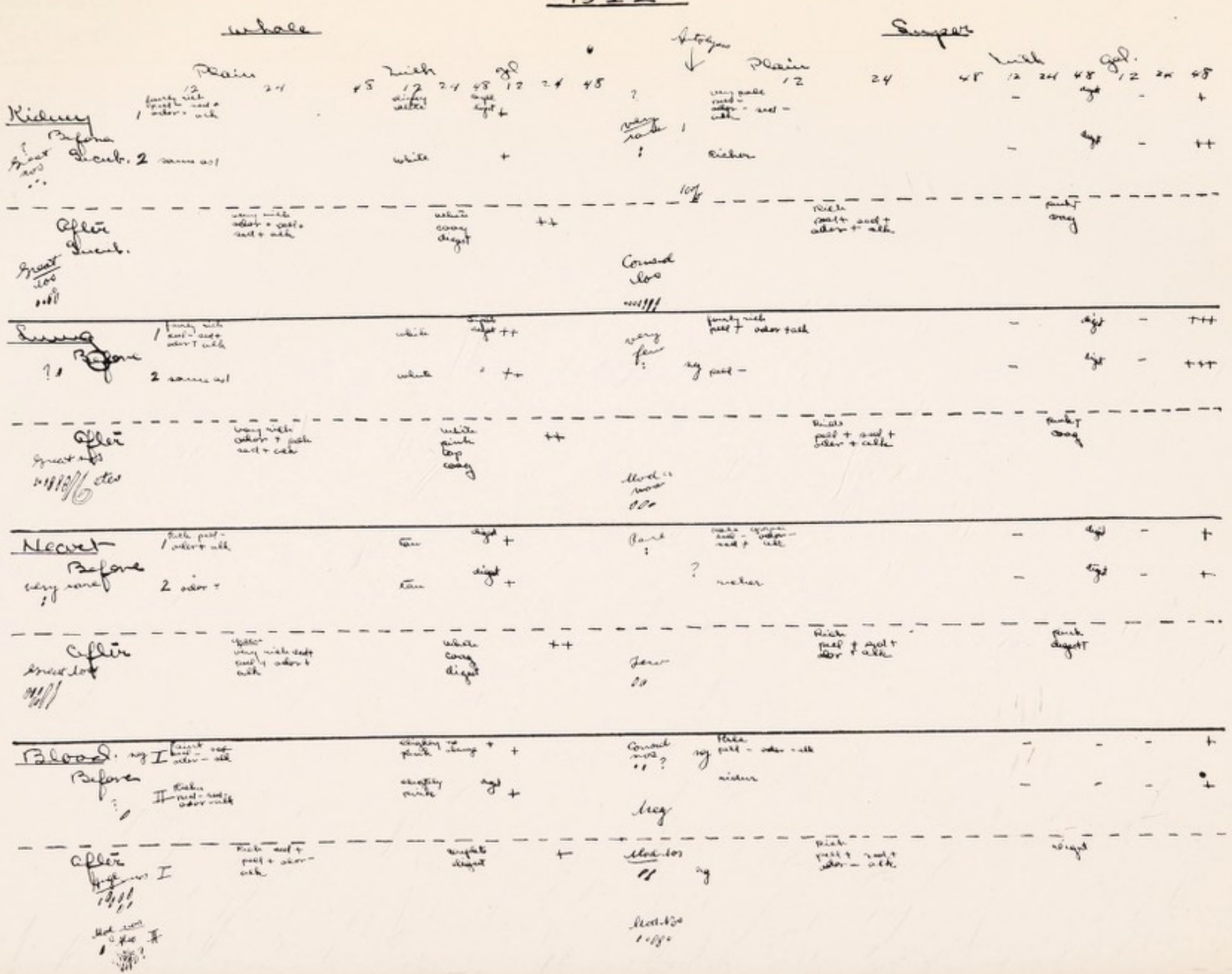
1. Location, grouped roughly in:
   a. Gastrointestinal tract.
   b. Lungs.
   c. Heart and kidneys.
   d. General.

Death:

1. Irrespective of lesions.
2. Largely dependent upon lesions, and more or less low in incidence.

1522

		Whole: Plain 12	Plain 24	Plain 48	Milk 12	Milk 24	Milk 48	Sug 12	Sug 24	Sug 48	Autogenous		12	Super: 24	48	12	24	48	12	24	48
Muscle Before Incub.	1	Pale pinkish red - odor - ack	Pinkish red + odor -		-	complete + digest		+			Consid. loss		very pale red - sed - odor - ack			-	-	-	++		
	2	same as 1			-	same +		++				100	same as 1			-	-	-	++		
After Incub. (Enormous loss)		Rich red + pept + odor + ack			white soap				++		Great loss			Rich pept + ack sed + odor -			white soap pept.				
Spleen (Appendix?) Before (Mod. loss)	1	fairly rich pept - sed - odor - ack			slight acid	curd soap		+			Heavy loss	20	very pale pept - odor - sed - ack			-	-	-	+		
	2	same as 1			"	"		+					heavy digest sed - odor ack			-	sig	-	+		
After (Huge loss)		very rich odor + pept + sed + ack			white soap				++		Huge loss			Rich pept + odor + sed + ack			Rich soap				
Spleen Before	1	Rich pept + odor + ack sed +			white	complete + C		++			neg	20	Rich ++ pept + sed + ack odor +			-	digest	-	+++		
	2	same as 1			"	+		++					same as 1			-	dig	-	+++		
After (Huge loss)		very rich odor + pept + sed + ack green			white soap digest				+++		Great loss			Rich pept + odor + sed + ack			white soap digest +				
Liver Before	1	Rich pept + sed + odor + ack			white soap digest	digest + C		+++			Fair loss?	24	same as spleen			heavy sed + white	digest	-	+		
	2	same as 1 odor +			"	+		++					" " "			"	digest	-	++		
After (Consid. loss)		very rich odor + pept + sed + ack			white soap				+++		Consid. loss			very rich odor ++ sed + ack pept +			white soap				


whole
Plain
Milk
Antigen
Serum
Kidney
Before
After
Blood
Liver

1522

	Whole Siwing Plain 24	Milk 24	Agl 24		Super Siwing Plain 24	Milk 24	Agl 24
Muscle 1	Rich peel + seal + odor + alk	white part top, soay + digest	+	Not found	1 fairly rich peel + seal – odor – alk	1 —	+
2	same as 1	" "	+	Immense no.	2 same as 1	2 white slight soag	+
Gizzard 1	Very rich peel + seal + odor + alk	white part top digest	+	Mod. nos.	1 Rich peel + seal + odor – alk	white beginning soay	+
2	same but + green	all white not as much digest	+	Immense nos.	2 same as 1	"	+
Spleen 1	Rich ++ peel + seal + odor + alk	white tracing pink digest	++	Some	1 Rich peel ++ seal + odor – alk	complete digt	+
2	same as 1	" "	++	Great no.	2 same as 1	white soay	+
Liver 1	Very rich peel + seal + odor + alk	white tracing pink digest	+++	?	1 fairly rich peel + seal + odor + alk	digest	+
2	same as 1	" "	++	Huge no.	2 same as 1	"	+
Kidney 1	very rich peel + seal + odor + alk	complete digt	++	?	1 Rich peel + seal + odor – alk	yellow digt	+
2	same as 1	" "	++	Rather few	2 same but seal +	slightly pink	+
Lung 1	Rich peel + seal + odor – alk	yellow digest	++	A few	1 fairly rich peel + seal + odor – alk	gray digest	+
2	" " " odor + "	red "	++	Great nos.	2	Tan	+
Heart 1	Rich peel + seal + odor – alk	almost complete digest	+	?	1 Very rich peel + seal + odor – alk	white soay	+
2		" "	+	Great nos.	2 +	—	+
Blood 1	Rich [illegible] + odor + alk	white soay digest	+	Rather Rare	1 fairly rich peel + seal – odor – alk	—	T+
Before Sacrif. 2	" " " "		+	"	"	—	T+
After				Many			

Injected 2cc 36 hr cult. 1 hr later blood removed. Smears | for aerobic + anaerobic

(Own cult 14000) as shown set in incubator 24 hrs odor not putrefactive

Smears cultures made from pieces of organs. Blood citrated portions

1/10		Plain anaerobic	Milk 12	48	72	Gel 12	48			Plain 24	After 24 hrs Gel	Milk	
Blood full centrifuged only 4	I		12 trace grey	48 Purplish	72 Purple watery	12 –	48 Green	+		Fairly Rich odor – Red + Sed + Alk	– +	White coag digest	like liver
	II		Gave grey	Pinkish coag	Soft coag pink water	– neg	–	–					
Lung?	I	:	Grey	complete digestion	complete yellowish	T	+++	"		Fairly Rich Red + Sed + odor? Alk	– +	White coag	coag + digestion
	II		white	"	"	–	+						
Liver:	I		white Red	"	"	T	+++			Rich Red + Sed T odor – Alk	+ +	"	digestion
	II		white slight digest	"	"	+	+++						
Spleen!	I		white	· purple		–	++			Rich Red + Sed + odor? Alk	– +	white	coag pink purple digestion
	II		white	" purple	"	–	++						
Appendix	I		–	trace of pink	Just off blue	– ?	–	–		Rich Red +++ Sed + odor? Alk	– +	Pink + white	Pink coag slight digest
	II		–	–	complete purple	–	+						
Mesentery	I		Pinkish white coag 25%	digestion purple	Partial	– ?	++			(++) Rich Red ++ Sed + odor +	– –	White coag digest	like liver + pink
	II		Grey	"	complete yellow	T	++			Green Alk Reading same	Green – +	Pink & white coag slight digest	like heart

Intensity:

a. Apparently relative.
   The *hemorrhagic* type appeared to have almost no chemotactic properties.
b. Chemotactic type, varying, as usual, from an acute exudate to pus, with an exaggeration of either the fluid blood or cell factors.

Putrefaction:

1. Most rapid and intense in the cases in which the rapid death appeared to bear little relationship to lesion production.
2. Appeared complementary to the degree of lesion development.

Chapter X

HYPOTHESIS REGARDING DISEASE OF THE ANIMAL ORGANISM

The simplest hypothesis, and the one which proved of most practical value, is the following: For the bacterial protoplasm under consideration, the real unit is a chemical molecule, in itself invisible. It is mainly to be conceived of in terms of energy. The evolution of this energy naturally depends upon the action of catalysts. The results of employing elements from the surroundings, must be a reproduction of like chemical units. The rapidity and completeness with which the surroundings are consumed, depends upon the intensity of the reaction, i. e. the heat of the flame, and the suitability of the fuel. With sufficient massing of these, visible forms reappear. The surrounding elements are thus transferred from one living fabric to the other, which thereby increases in extent. Organized tissue acts as a catalyst, and the resulting reaction operates best in the presence of the same catalyst.

Organized tissues may be graded thus:

1. Broken up, partially digested, as in the gut tube, and in artificial media.
2. Dead tissue, as yet untouched. (Post mortem.)
3. Living tissue, saturated with tissue food material, ready for assimilation, represented by the intestinal system.
   (a). The lungs, derived from the primitive gut tube, appear intermediate between three and four.
4. Living tissue, unconnected with digestion, represented by the heart, and kidneys, skeletal muscles, central nervous system, etc.

Let us assume that the so-called disease-producing power of this bacterial protoplasm is dependent upon the theoretical mechanism just described.

In general, an abstractness in the chemical reactions in bacterial protoplasm resulted in animal tissue affinities and disease producing power. In the processes involved in the experimental induction of pathogenesis, it was assumed that the plain bouillon used, acted in the role of the initial catalyst. The magnitude of the result was considered due principally to the irritability and flexibility of the bacterial protoplasm, and the fact of the sudden change in the type of catalyst from that presented in the routine of life. Following the use of this substance, the bacterial protoplasm encountered living animal tissues; these seemed to increase the intensity of the reaction, begun under the influence of the bouillon. Also, a readjustment occurred in the cultures, and the most intense and exacting of the reactions thus aroused were exhibited only by certain units of the bacterial protoplasm (supernatant borne). In the table, organized tissue is shown graded directly, according to the intensity of the life processes, and inversely, according to the amount of inactive material present. The reaction conceived of as aroused and taking place in the bacterial protoplasm, adjusted first to bouillon catalysis, then to animal catalysis, in general may be conceived of as growing more specifically refined by the action of the more completely alive tissues, and in their presence, more intense. It appeared likely that the intensity of such a reaction varied in regular waves. Through frequent artificial subcultures, it was re-stimulated by the bouillon. Yet as time passed, it appeared very slowly to decrease. Catalysis by dead tissue must provide bacterial protoplasm with an intensity of reaction-power, capable of life in the tissues of, and of the destruction of an animal. Distributed everywhere, the amount of chemical robbery, so to speak, is incompatible with life, but at no point may the adjustment between the reaction and the catalizer be fine enough to induce sufficient intensity to provide the halo of destruction called a macroscopic lesion. Of course mere quantity may imitate quality. A perfect adjustment (catalyzee-catalyzer) between the bacterial-unit-chemistry (catalyzee) and the most intensely and completely living of animal tissues (catalyzer), should provide the most perfect picture of disease.

Thus the degree of intensity in energy evolution varies greatly in different regions of the cultural protoplasm. (Compare Supernatant Fluid and Sediment.) Also, the sum of all the powers represented in a culture varies considerably. (Effects of 2 c.c. of Typical Whole Cultures varied in effects following animal inoculation, at different times.)

We have seen that in the *typical reaction,* death is the only unqualified fact. This may well represent the minimum of the culture's power. When death is thus a solitary fact, there is associated with it a great intensity of putrefactive change. This form of death would appear to be due to the presence throughout all the host's tissues, of chemical units belonging to the bacterial protoplasm under consideration. These would seem to be the most closely related of all units, in their capacities, to the saprophytes. Above this minimum, in the rapid reactions, greater intensity in chemical reaction may exist in relation to a few or many chemical units. This may be due to finer or more recently aroused adjustments between the catalyzee and the catalyzer. Thus, tissue hemorrhages occur as the result of these especially intense bacterial reactions, and may take place in small or great numbers. They would be localized in the intestinal, pulmonary, cardiac, renal, or muscle regions, somewhat in the order given, in direct proportion to the degree of intensity of the chemical reaction in the active units. Unquestionably, the commonest location of hemorrhage was shown to be the gastrointestinal tract. Wherever else hemorrhages might exist, this tract was practically never free. A unit-reaction-intensity, sufficient to induce a gastrointestinal hemorrhage, would, according to our hypothesis, act in other tissues, relatively, in a more saprophytic manner and probably not cause a lesion. That is to say, they would retain their viability, and en masse destroy, but cause about themselves no halo of special individual destructiveness.

In the use of the *supernatant fluid,* more truly than with any other preparation, reactions were proved to be concerned, at least in the main, with the more intensely energetic lesion-producing units. Following the injection of such fluids, there was

typically observed in an animal three more or less distinct reaction pictures.

1. The use of the youngest, freshest, most recently developed cultures, resulted in the quickest death, in the case of several different specimens, and caused the greatest number of and the most generally located hemorrhages.

2. Certainly, as time passed, these fluids from the same culture caused a fatal result less rapidly (24 hours, or 100% and more, prolongation), with fewer associated hemorrhages, and these were in the gastrointestinal tract.

3. Between these two extremes lay the various degrees of pneumonic involvement, from acute inflammatory congestion to a true pneumonia, associated with more or less gastrointestinal hemorrhages. The most localized and defined of all the pictures of disease artificially induced was the fibrinous pneumonitis, following the inoculation of a kaolinized *supernatant fluid*. It seemed fair to conclude that in that case the action of the centrifuge was reinforced and it removed practically all of the units of less perfect adjustment; the lower grade, more saprophytic ones, clumped in more vegetative activity. In general, the number of supernatant reactions was rather slight to pass upon. But the variations in them described, might have been due to:

(a). A cycle in the life phenomena of the bacterial protoplasm in the test tube cultures.

(b). Accident.

(c). A definite trend. As judged by the investigator, best able of course to sense the effects of time, there seemed to be a very gradual fading in the energies demonstrated by the cultures (see Chap. VIII.) and, inversely, comparable to the more rapid change well observed in the rising or growing power of adjustability to living tissue, artificially aroused in the efforts towards the induction of disease producing powers.

If these observations, and the resulting line of reasoning, be correct, there is evidence of the relationship between the intensity of the power for disease production, the points of lesion development, and the capacity of the bacterial protoplasm to

survive at a distance from the gastrointestinal tract. Thus, with this conception, what was found to be true might have been foreseen, viz., that gastrointestinal hemorrhages were the most common and the adjustments to the catalyzers here found were more nearly related to those in the saprophytic state. Hence, a relatively greater bacterial energy must always be present in this region than could be exhibited elsewhere. The more units adjusted to the finer catalysts of the more essential tissues, or acting purely by their intensity of reaction, the greater will be the number of extra-intestinal hemorrhages. Indeed, it is conceivable that by the centrifuge almost all the units of lower catalytic adjustability might be thrown out. Once, as already mentioned, they appeared to be, with the help of kaolin, and there occurred the purest case of pneumonia observed.

The mere numbers of chemical units must also play an important role at times, and quantity must compensate in some measure for quality, whereby, lesions, so to speak out of order, might occur.

This relationship between virulence and location has been brought out in other publications, especially by Rosenow, by more direct experiment in relation to areas of the gastrointestinal tract.

So much for the cases of eight to twenty-four hour deaths with hemorrhages.

Those cases in which death occurred, apparently as a result of lesion production, may in general be explained upon the ground of a great disproportion in the segregation of the elements providing the capacities for disease production throughout the areas of bacterial protoplasm. Thus, even in a culture of Gram negative rods, most of the protoplasm could be purely saprophytic, while the remainder might be only very delicately catalizable. The result would be localized and slowly developing disease; the extreme cases would be those showing pus formation.

CLINICAL OBSERVATIONS:

If the results of clinical observations were to be a basis of all determinations, rabbits would prove disappointing. The

periods of their life courses, subsequent to their inoculation, proved not to be as valuable in phenomena as were the other relationships. Rabbits may eat at the very edge of the abyss; they may be mortally ill and hardly appear so; they may die, like a candle blown out by a gust of wind.

The ruination of the clinical picture is the unnatural stage of depression, illness and shock, following the intravenous injection of the large doses of foreign protein usually employed. In the two cases (No. 144 and No. 145), injected with kaolinized and unkaolinized supernatant fluids, this effect appeared to be most distinctly circumvented. Although the clinical courses were short, the first two-thirds were certainly associated with a quiet and in many ways natural period of incubation. The units of course reached the lungs more quickly than if received through the nose.

The most ideal clinical courses were demonstrated by rabbits Nos. 1610 and 1611. Injected with the fluids from separate culture tubes from the sputum of human case No. 12, after four days incubation, one developed a picture of pneumonia, with rhinorrhoea, labored breathing, weakness, etc. The autopsy revealed a true picture of fibrinous pneumonia simulating that in the human host. The other, in the same length of time, showed only a few hemorrhagic spots in the lungs. But what process other than such simple hemorrhages could more ideally explain the peculiar respiratory triad in the human cases, with the very indefinite lung signs, namely, pulmonary dullness, indefinite and wandering, moist râles, bloody sputum. In the latter rabbit, inflammation also existed in the stomach and intestine. Rabbit No. 1572, after recovering from pneumonia, suffered a distinct relapse two months later, and died of pneumonia.

The clinical sign of rhinorrhœa, often associated with simple or suppurative conjunctivitis, was very common, irrespective of pneumonic associations, and provided the principal foundation upon which to establish the fact that the epidemic influenza-like cases had their animal counter-parts.

Definitely labored breathing, especially when associated with wheezing, might indicate a pneumonia, but the initial shock

was associated with vibratory respiratory movements, though only for a short time. Cardiac decompensation, and severe terminal toxemia (bacteremia), both frequently imitated pneumonic breathing.

The enteric cases were as obscure as in human beings. Diagnosis, if it had been attempted, could only have been accomplished by exclusion. Diarrhœa frequently occurred within an hour after injection. It might be of short duration, or persistent. Just as in the human epidemic cases, constipation was the more frequent symptom of the two. This was evidenced principally by a diminution and variation in the size of the fecal balls.

Of the nervous system, the most interesting symptom was nystagmus, with a drawing of the head to the side, and incoördination of locomotion. Several such cases were observed, with acute or chronic lesions, in two cases, in the corpora quadrigemina.

Fever was universally present, but the courses were usually erratic, though, this was to be expected, as it was observed also in the human cases. There will be observed recorded in the case of some rabbits, definite initial rises with sustainment and more or less definite crisis. Practical values were attained occasionally by the discovery of fever, when it was the single sign of disease. Also, the development of a subnormal temperature, with or without a preceding rise, constituted the commonly accepted indication of approaching death.

The leukocytes were counted, not by any means consistently, but often enough to establish a few rules which corresponded with those already commonly accepted.

The injections were followed, in an hour or so, by a leukopœnia, a fall occurring often to below thirty-three per cent of the normal (7000). Frequently they did not increase again, or they might do so only slightly, death occurring before a day had gone by. They frequently rose, only to fall in a somewhat longer course. They frequently rose and remained about double the normal, especially in the severe enteric cases, death occurring after some days. In the pneumonic cases, this sign was very grave. If there was a great increase, and very great ones were

observed (80,000) in the presence of pus, the animal did not die, at least for a long time. The definite, sustained leukopœnia, associated with only moderately severe rather prolonged disease courses, observed in the epidemic human cases, could not be considered to have been imitated.

Anemia was observed in some instances, especially pronounced in No. 1550.

The urines were frequently examined, being removed from the bladders after death. Indican was the commonest abnormality, of course, representing the indol formed in the bouillon of the *typical cultures* (artificial). Albumin was frequently found, at times appearing within thirty-six hours. (See Nos. 144 and 146). Heavy clouds, associated with blood, other elements from inflammatory exudates, and casts, were correlated with cases of nephritis. A desquamation of genito-urinary cells, from all levels of the tract, was most commonly evidenced in the sediments.

Gross Pathology:

In the study of the gross pathology, produced experimentally by the use of the bacterial protoplasm under consideration, the key note was the primitive, hemorrhagic lesion. The mechanism of its production, common points of occurrence, and relationships to the phenomena of death and unnatural putrefactive changes in the host, have been suggested in preceding pages of this chapter. No investigation has been made of a specific haemolytic substance.

Factors in the Production of Inflammatory Reactions:

Inflammatory phenomena in the very simplest form are of course dependent upon activities on the part of the tissues of the host. But the intensity and complexity of such processes depend not alone upon the inherent capacities of those tissues. Three other important factors are of course the intensity of stimulation upon the part of the bacterial units, known in general as chemotaxis; the time permitted, both for this factor and that of reaction, to operate; the freedom of assisting tissues,

other than those directly involved, from similar disease influences leading to depression. There were examples among the protocols of various complexes comprising different proportionments of these factors in which the relationships were especially apparent.

Following the introduction of 2 c.c. of a *whole culture,* rabbits which died in about eighteen hours, and revealed hemorrhages, presented conditions in which the only inflammatory reaction was at times an outpouring of mucus in the lumen of the intestine.

Following the use of the kaolinized *supernatant fluid,* No. 145 was a case in which, in just twice as long a time, bacterial units were capable of exciting a phenomena which advanced even to suppuration. The general clouding and depressing effect of the enormous mass of units in the *whole culture,* rather than the duration of time allowed, would seem to explain these divergent results.

Many substantiating experiments occurred, viz., in which .3 c.c. to 1 c.c. of a *whole culture* or sediment were injected, and induced very frequently pneumonia in its early stages.

In parallel cases, Nos. 1610 and 1611, rabbits injected with equal amounts of cultures from separate tubes, inoculated from the same sputum, appeared to show a difference in the chemotactic capacities of the units. After eight days, fibrinous pneumonia had developed in one, but in the other the lungs revealed only a few hemorrhages. Such a discrepancy could hardly be due to variations in the hosts.

No. 143, in which .5 c.c. of a standard amount of the material first thrown down by centrifugation caused abscesses of the lungs, seemed to illustrate well the influence of the time factor. Ninety-six hours was an unusually long course for a reaction following the use of .5 c.c. of whole culture, or sediment. The units contained in these preparations, in general, had been found to be the less chemotactic. Forty-eight hours has been required for the development of even the early stages of pneumonia (1707). Time would seem, here, to have been an important factor in causing the abscesses.

DEGREE OF INFLAMMATORY REACTIONS:

As stated under another heading, beginning with the simple hemorrhage, there have been observed, experimentally induced, the primary pure exudates in which the proportions of fluid, blood, leukocytes, and fibrin varied between extreme limits; and the secondary productive processes, characterized principally by round cell deposits. These later stages were rather unusual in the intensity of peculiar hyaline changes.

SYSTEM DISEASE:

It has been frequently pointed out that this bacterial protoplasm executes a most general contact, under experimental conditions, with an animal's tissues. The disease equation is general; the degree of generalization, diffuseness, completeness of interaction in an animal, ocularly demonstrable, naturally varies. Even at post mortem, clinically of course, many cases appear only as those of a local disease. Hemorrhages have occurred most commonly in the gastrointestinal tract. Real pictures of disease have certainly most commonly involved the lungs.

RESPIRATORY TRACT:

Congestion of the mucous membrane of the nose, with or without hemorrhages, was to some degree present in the hemorrhagic cases, the subject dying in between eight and twenty-four hours. In the series involved in the experimental induction of pathogenesis, the inoculation of rabbits with cultures of this protoplasm frequently failed to produce evidences of reaction for long periods. Yet inoculation by the bacterial protoplasm was proved to result in an intranasal as well as intestinal localization. Actual epistaxis was observed in animals in a number of instances. Intense suppurative rhinitis accompanied many of the most typical cases of pneumonia. Ulceration about the nares, associated with considerable bleeding, was also seen in one or two cases.

Pharyngitis, trachitis, and bronchitis were, when present, usually associated with rhinitis above, and pneumonitis below.

Occasionally, local delimitation occurred, especially embracing that area included in the tissues of the trachea and bronchi.

The pneumonias involved a combined pneumonitis, and pleuritis. The hemorrhagic basis for even the complex fibrinous processes, was suggested by the extensive and frequently occurring lung hemorrhages in the *typical reactions.* By a long series of examples, scattered through the protocols, well graded steps in increasing fibrinous deposits and pneumonic consolidation may be grouped, and in imagination viewed in chronological procession. The significant specimen of croupous-like pneumonia from rabbit No. 1611, showed beneath the pleuritic fibrin the brown remnants of massive hemorrhages. The outlines of these, irrespective of the lobar boundaries, roughly comprised circles. Bloody (cellular) and haemoglobinized exudates were the rule.

Some degree of pericarditis was far more commonly associated with pleuritis, than was the case in human beings. More localized processes in the humans were represented by abscesses, both acutely purulent, and healed. Fine, chronic pleuritic adhesions were rarely well developed.

GASTROINTESTINAL TRACT:

In experimentally induced disease of this system, by far the most interesting results were the points or regions involved. These have been frequently indicated. In the case of this unspecialized bacterial matter, and in instances where the possibility of environmental or special adjustment may be excluded, the duodenum and the vermiform appendix* were the areas most often and most intensely attacked. Other regions to which at times disease was restricted were the gastric mucosa and the rectum. Frequently no discrimination was observed between adenoid and non-adenoid tissue. At times, a distinct preference was shown for the former type. True inflammatory stages were frequent enough, but rarely exhibited much complexity or intensity; rarely indicated a refined chemotaxis. In

* One of the most important observations recorded by the Author. (Editorial Note).

the stomach, there were instances of acute diffuse gastritis. Of great importance was the frequent discovery of small round depressed areas, black in color because of the action of HCL upon the escaped blood. These were most commonly present in association with chronic gastric catarrh. These stomachs normally, loaded, were half empty and more or less stiffly contracted. The contents were moist, and mixed with mucus, a condition never seen normally.

At the sharp curve of the duodenum, just beyond the pylorus, the hemorrhages frequently fused. Ulceration was rarely observed, and once, there was revealed an adhesion, pyloric obstruction and hypertrophy.* Enteritis was almost universally present; it developed chiefly in the rabbits exhibiting no results for months after inoculation. Acute vascular dilatation was very common, even in the case of a *typical reaction.* Mucus was almost always suspended in a yellow or brown, haemoglobinized liquid. Sub-acutely, an unusually fine, diffuse, capillary dilatation resulted. In the chronic cases, one or more single loops of small gut would, alone of all tissues, exhibit an abnormality.

These loops suffered apparently muscular paralysis, though it may have been merely post mortem relaxation from the weight of the contained localized liquid. Thus, a quantity of reddish-brown fluid, full of gas bubbles, lay in a loop. The underlying mucosa was delicately pink, from capillary distention. These cases took their position in the general scheme as carriers, not so much of a definite form of bacterium, as of the last lingering remnants of an equation, which usually had its beginnings in preceding hosts.

Distinct pictures of appendicitis were rare, and never typical. It appeared to be the case, that the evolutionary refinements, which were certainly inherent in this bacterial protoplasm, naturally led to a constant seeking for more favorable environment. In the acute and intense pictures, if the intestinal tract were involved, then also, a general cultural clouding occurred.

*Cf. this interesting observation with Barber's Studies (Surgical Pathological Physiology, N. Y. University, 1915). "Dilatation of the Duodenum." (Ed. Note).

The association of discrete pneumonia with a disease-course of one to two days, was a picture, in clarity, never at all paralleled in the case of the disease of the gastrointestional tract. The theoretical attempts at an explanation for these facts will be found in the preceding chapter. There were cases of parallel protocols, differing in the main only in the lengths of their disease courses. In the case of the shorter reaction, hemorrhages occurred at certain points, while in the longer reaction, pus was present, at precisely the same points. (See Exp. Bact. Protocol Pigeon, page 213).

Cholecystitis, and cholelithiasis, are words scattered all through the pages of protocols. In not more than one or two instances, was there evidence of a localization in the wall of the gall-bladder, as indicated by acute inflammatory changes. But, in their rarity, these instances may claim interest. This mode of infection certainly did occur. Far more commonly, in an astonishingly short time, there appeared a yellow mucus—containing bile of excessive transparency, and even fine solid particles in the gall-bladder. In the case of this bacterial protoplasm, after animal inoculation so generally distributed and demonstrated initially to overload the liver, and secondarily to be supplied to this organ by the portal blood, we have an example of micro-organisms reaching the gall-bladder through the medium of the bile and from its springs of origin. This mode of infection of the gall-bladder and ducts, was experimentally demonstrated by Doerr. It has been especially, emphasized at the Mayo clinic.

Circulatory Disease:

Microscopically, it does not seem as if the glomeruli of the rabbit ever escaped inflammatory changes under conditions of experimental inoculation. Through the injections, they were overloaded, in the case of any preparation except in the case of the *supernatant fluids*. Following the employment of 1 c.c. of the fifth supernatant fluid of the *supernatant strain* (No. 165, page 122), a quite definite acute nephritis developed. On the

basis of separate, localized areas of involvement represented fundamentally by hemorrhages, not only were abscesses found in the kidneys, but also scattered areas of chronic inflammation. These were distinguished through parenchymatous tissue replacements by groups of round cells.

The right ventricle of the heart was the especially selected region of the circulatory system, and localizations here frequently accompanied the distant renal involvements. Hemorrhages, of that class belonging to the *typical reactions* were rather uncommon in the heart. When present, they usually lay near the septal line. The commonest distinct gross lesions of the heart were scattered, more or less fused abscesses containing pus, or a process of hyaline necrosis, both varieties involving the entire wall of the right ventricle. This latter lesion, to the naked eye, appeared as a small piece of whitish, crinkled paper. Endocarditis was observed but once. But, like mural cholecystitis, at least, it did occur, and in the face of any specific bacterial refinements.

CEREBRO-SPINAL SYSTEM:

The central nervous system provided, fairly frequently, considerable degrees of meningeal congestion. It is of interest in this connection, to recall the cases of human epidemic disease which so commonly were attended by terrific headaches. Dr. Baker of Forty-Fort, fortunately tapped the spinal sub-dural spaces of a number of human patients and found the fluid under high pressure. Under the clinical considerations, involvement of the corpora quadrigemina was mentioned. The best demonstration of the involvement of this system with complex reaction phases, was provided by the paralleled autopsies of rabbits No. 164 and No. 165. Special readjustments in the association between the units of the bacterial protoplasm and the factor controlling the affinity for animal tissues appeared to have been artificially effected. Apparently the power in or affinity for animal tissues was relative to the concentrations of material represented in the formed units. This differed from the original typical cultures, which were abstractly relative merely to the

intensity of the culture as a whole, and segregated especially in the non-centrifugable units. Therefore, we have in these protocols, two stages exhibited. Resulting from the inoculation of the smaller dose, there was a diffuse, inflammatory congestion of the brain and many hemorrhages in the stem. The larger dose induced in a shorter time also hemorrhages of the brain stem, and besides these and associated general inflammation, actually pus, beneath the arachnoids generally, and within the ventricles.

Miscellaneous Lesions:

Conjunctivitis, thymitis, orchitis, endometritis, adrenalitis, myositis, osteomyelitis, arthritis, all were recorded.

The finer histology of the splenic changes was not investigated beyond the recording of the tendencies toward lymph tissue hyperplasia, and hemorrhage. But grossly, a most remarkable series of splenic tumors were observed. In the presence of distinct disease, the spleen varied in response, from a condition in which there were none, to one, associated with elongation, in a rabbit, up to four inches. The human cases of enteric disease frequently differed from true cases of typhoid fever, only in the absence of a splenic tumor. Upon the other hand, the human spleen was often distinctly palpable. The observations recorded upon the blood included several cases of intense chronic anemia associated with focal infection.

Focal Disease:

Foci, in the present day understanding of what the term represents, would seem to have been experimentally established in the chronic cases of enteritis. The already described, mucus-filled sacs, formed by single loops of bowel, certainly acted as foci, upon the basis of which, months after a rabbit had been inoculated, acute processes of disease appeared to develop. These were both locally and generally emphasized, and almost invariably resulted in death.*

*Cf. Recent studies in intestinal obstruction. Also note that the author himself died from the absorption of toxins arising from an intestinal loop. (Ed. note.)

MICROSCOPIC PATHOLOGY IN GENERAL:

As indicated by the gross pathological findings, it is to be regretted that the more diseased parrots were not obtained. The investigation began after the acute stages of the epidemic, when most of the fatally ill parrots died in a few days.

The most marked lesions in any epidemic-parrot were found microscopically in the liver of No. 1, which showed various sized areas of small round-cell deposits with red blood cells and a necrosis, almost caseous in appearance. There seemed some tendency to deposit collections of round cells in the livers and kidneys of parrots. The organs of all were markedly congested. The glomeruli of the kidneys showed an increase in nuclei. The cells of the parenchymatous organs exhibited a variety of pictures, varying from cloudy swelling to complete destruction of the cytoplasm, with free nuclei, though even the nuclei in some cases were also destroyed.

In the study of the experimental rabbits, rapidly killed by the foul cultures of Gram negative rods, the two pictures of most interest were first the simple primitive hemorrhagic lesion, associated with large numbers of bacilli in clumps, and second, as in the organs of the birds, the presence of free nuclei in the parenchymatous tissues. Here the cytoplasms appeared more or less completely destroyed.

As will be seen in the protocols, a great variety of gross lesions associated with various time factors, and varying bacterial form and function were represented. The histologic lesions were either a simple hemorrhage, oedema, a collection of round cells, or leukocytes either with the formation of pus, or lying in a network of fibrin. The several cases of typical pneumonias showed fibrin and leukocytes very distinctly. The parenchymatous cells showed cloudy swelling, coarse granulation, a hyaline appearance, fat, or the destruction of the cytoplasm, or even of the nucleus.

Two general facts may safely be stated. First as might be presumed the less generalized more definite cases of local disease, such as pneumonia, presented the most clear cut pictures of cloudy swelling, but this process also did attend cases running

a rapid course, and revealing at autopsy, hemorrhagic lesions. The second fact was that in all cases variation in the degenerative pictures was the rule. A condition of spottiness tended to prevail, except following the use of the supernatant fluid preparation. Whether this was due to local faults in the nutritive supply, because of injury by contiguous lesions, or to variations in the very wide and general distribution of the bacterial units, has not been investigated. But by the use of smears, there seemed to be considerable evidence of the very general presence of bacterial protoplasm. There has been brought out abundant evidence of these variations in the intensity of activity between units of the bacterial protoplasm under discussion. These facts suggested that the spots (spottiness) were the homologues of the hemorrhages.

EXPERIMENTS IN THERAPY

TENTATIVE EXPERIMENTS IN THE PREVENTION AND CURE OF SUCH EXPERIMENTAL DISEASE REACTIONS AS MAY BE CAUSED BY THE TYPICAL CULTURE

The strong alkalization of the plain bouillon by cultures of this bacterial protoplasm suggested the following few attempts at the neutralization of its effects in an animal by the administration of a weak acid intravenously.

OUTLINE OF EXPERIMENTS IN THERAPY

The acid employed was hydrochloric, used in an amount equivalent to 3 c.c. of the N/10 solution.

PROTOCOLS:

RABBITS: Injected with plain bouillon cultures of *typical cultures*, as indicated.

No. 1669. 5/27/17. Two c.c. forty-eight hour sediment.

CLINICAL RECORDS:

Eighteen hours, very ill.

THERAPY:

Injected 7 c.c. N/20 HCL. Convulsions.

DEATH:
Immediately following the administration of the acid.

AUTOPSY:
Typical and generalized hemorrhagic reaction.

SMEARS AND CULTURE:
Typical.

No. 1654. 6/27/17. 2 c.c. 24 hour sediment.

CLINICAL RECORDS:

THERAPY:
At 24, 48, 72, 96 hours, injected intravenously with 7 c.c. N/20 HCL. In twenty-four hours there was nystagmus, and circular progression due to hemiataxia and paresis.

DEATH:
Thirty-five days.

AUTOPSY:
Scars in corpora quadrigemina. Congestion of the lungs. Dilatation of the heart. Parenchymatous degeneration.

SMEARS AND CULTURE:
Typical rods.

No. 1670. (Pregnant) 1 c.c. 24 hour sediment.

CLINICAL RECORDS:

THERAPY:
At 2, 15, and 24 hours, injected intravenously, 7 c.c. N/20 HCL.

DEATH:
Thirty-six hours.

AUTOPSY:
Beginning pneumonitis. Fine hemorrhages in appendix. Normal feces. Parenchyma degenerated. Splenic tumor.

SMEARS:
Appendix: Typical rod.

The usual effects from suspension of sediments or whole culture in .5 c.c. to 2 c.c. amounts, has been shown.

No. 1678. .5 c.c. 24 hour sediment.

CLINICAL RECORDS:

THERAPY:
At 1, 24, 32, 36 hours, 6 c.c. N/20 HCL.

DEATH:
Thirteen days.

AUTOPSY:
Gastrointestinal catarrh. Hyaline change, wall of right ventricle. Parenchymatous degeneration.

No. 1679. (Control) .5 c.c.

CLINICALLY:
Untreated.

DEATH:
Forty-eight hours.

No. 1680. .5 c.c.

CLINICAL RECORDS:

THERAPY:
At 1, 24, 32, 36 hours, 6 c.c. N/20 HCL.

DEATH:
Eighteen days.

AUTOPSY:
Healed abscess of the lung with old adhesions. Anemia, serious. Sclerosis. Degeneration of the heart muscle. Chronic splenic tumor.

SMEARS:
Lung: Gram negative-positive rods.

CULTURES:
Lung: Typical green.

For controls, the various protocols throughout the book may be referred to where the same dosage was employed of the same culture. The sediments were used here, but never has an animal been observed to live longer than forty-eight hours when untreated, and after inoculation with these preparations in doses above .5 c.c.

There was evidently in the use of the acid, the means of neutralizing the factor or factors causing rapid death and of preventing many of the lesions which would naturally occur.

In the pregnant rabbit No. 1670, although the administration of the acid was begun in the early stages of the vital interreaction and the occurrence of death was perhaps slightly delayed, no real benefit occurred. In rabbit No. 1669, the administration of acid precipitated death, associated with the common picture of a convulsion, within five minutes after its injection. In another unrecorded experiment with an alkali, NaOH, administered at a corresponding stage in the course of the disease reaction, as judged by the autopsy of the untreated control rabbits, appeared to prolong life, to exaggerate hemorrhage production, and to diminish the speed of putrefactive changes.

The well known susceptibility of pregnant rabbits to such attack by bacterial protoplasm, induced the author to have an analysis made of the bloods of a normal and pregnant rabbit, to be compared, in series, with those dying of the Typical reaction.

Through the courtesy of Dr. Victor Meyers, in charge of the chemical division of the Laboratories of the New York Post Graduate Hospital, the results are here recorded

No. 1. Healthy rabbit: Bled from carotid artery 11/7/17.

Report 11/16/17.
Non Prot. N. 43 mgms. per 100 c.c.
Creatinin. 2.7 mgms. per 100 c.c.
Sugar. 0.136%.
CO_2 Comb. Power, 28 c.c.—CO_2 per 100 c.c. Plasma.
H-ion. 7.2 per 100 c.c.

No. 2. Pregnant rabbit: Bled from carotid artery 11/7/17.

Report 11/16/17.
Non Prot. N. 54 mgms. per 100 c.c.
Creatinin. 2.5 mgms. per 100 c.c.
Sugar. 0.108%.
CO_2 Comb. Power, 41 c.c.—CO_2 per 100 c.c. Plasma.
H-ion. 7.5 per 100 c.c.

Unhappily, the analyses of the bloods of inoculated rabbits were never sought. Such investigation was, of course, merely a suggestion and further substantiation of well known facts. That fact of the general production of acidity in the tissues of animals, as a final result of bacterial septicemia, seems to hold in the case of the reactions herein investigated. The tendency of a hyperacidity, or H-ion concentration, in the blood of pregnant animals, unquestionably, may be correlated with these facts. Thus might be explained the cause of death in pregnant animals. But how explain the beneficial result observed even if demonstrated in only a few cases following the therapeutic employment of an acid. This phenomenon must bear no relationship to the final mortal tissue reactions, for then the acid merely precipitated death. It would seem to influence the living phenomena, expressed in the chemical reactions of the bacterial proto-

plasm, through the medium of the circulatory blood of the host. The effects of a mild acid upon the bacterial protoplasm in the culture tube previous to its inoculation into an animal, are shown elsewhere. If this were true, why then would not the inherent hyperacidity of the blood during pregnancy, be at least initially, of benefit to an animal host at that time? Of course, here are no such sudden changes in plasma reaction as result from the intravenous instillation of an acid. Very likely, this method of therapy may after all, have to be relegated to the realm of non-specific mechanisms. Whatever the explanation, the results are too suggestive to omit.

The above paragraphs illustrate the author's open habit of thought and demonstrate his appreciation of the protective mechanism of the body. (Ed. note)

SUMMARY PART I, II, III

A. Studies of Epidemic Material.

1. A group of parrots ill of a general disease were unwittingly exhibited to public view.

Human beings near the birds became ill of a general disease. In them, however, this was characterized by more evident local concentrations of activity in certain organs and systems; principally the respiratory and gastrointestinal tracts.

The disease was not transmitted by the human beings.

2. From individuals of both classes, bacterial protoplasm was obtained from the blood, sputum, feces and tissues.

The main characteristic of this low form of life was a generalized state of infinite variability in regard to the forms and functions it assumed.

Under the uniform influence of the artificial media employed (plain bouillon) for study, more or less metamorphosis and functional changes took place with regularity until a common state of relative fixation occurred.

Practically all cultures of this bacterial protoplasm obtained from the epidemic cases, exhibited some degree of pathogenicity.

The disease picture inducible in animals did not, however,

necessarily correspond to that observed in the host from which the specimen of bacterial protoplasm was obtained.

Yet, either as directly obtained or at different stages in the course of change, the inoculation with this protoplasm into animals resulted in disease pictures simulating all the different entities naturally arising during the epidemic.

3. The terminal state of adjustment, *the common state of relative fixation,* known as the *typical culture,* was in general so constituted that the unit forms were nearly all fine, short, motile, *Gram negative rods.* Two c.c. in an animal, effected death within twenty-four hours with hemorrhagic lesions indiscriminately located, and with more or less excessive putrefactive changes.

The bacterial protoplasm, in a particular culture (No. 1522), was proved to have latent in it these properties:

(a) Variability in form and function.

(b) Capacities not only for existence in, but an affinity for animal tissues, with resulting general disease, in courses, and localizations, simulating the pictures presented in the spontaneous cases at the time of the epidemic.

(c) In the supernatant fluids of the plain bouillon cultures obtained by centrifugation there were units in which the disease producing power was so enormous, as compared to its related mass, the animal tissue affinity so great, that an amount of material, easily imagined to be transferable through the air, was found sufficient to induce a general disease in the animals inoculated.

B. Studies of Material from the Intestines of Healthy Parrots.

In the intestines of healthy parrots similar bacterial protoplasm was found. The latent capacity for growth in animal tissues was aroused by artificial meat substance media, and accelerated in the tissues of mice and rabbits. The succeeding stages in the development of this adjustment to living tissues were shown to be correlated to an increasing ability on the part of the bacterial protoplasm to subsist in tissues further and further removed from the direct neighborhood of the gut tube. Finally, it ac-

quired a capacity to attack any portion of an animal tissue. The stages of this experimental adjustment were marked out by disease entities which were similar both to those naturally arising in the epidemic, and to those experimentally induced by means of the bacterial protoplasm obtained from epidemic sources.

Metamorphoses and functional changes were observed in subcultures from the animals thus diseased and the same *terminal, common state of relative fixation resulted;* the *typical culture.* This was found to be a relatively fixed state, but in character comparable with those in general obtained from epidemic sources, and with No. 1522 in particular.

The ordinary amount of this typical culture—usually two to four c.c.—in a test tube, must be considered as a whole, in which units rose and fell according to the various temporary demands of the culture. All viable portions of the culture transmitted a similar generalized life, which might withstand the effects of dessication, the ravages of time, and great heat, (up to 80 degrees Centigrade). With lightning-like rapidity adjustments were effected in the face of all food problems. Even if in a condition of powerlessness when meeting the tissues of an animal following artificial inoculation, all the barriers were passed until the lumen of the animals gut tube provided a haven. Just as in that *typical culture* provided by the epidemic, so centrifugation revealed in the *supernatant fluid* units of protoplasm representing a very small amount of material by which an affinity for animal tissues was exhibited to a very marked degree. Most clearly defined, therefore, was the division of labor evidenced by the segregation of those activities, developed by contact with animal tissues, in certain units of matter which were thus endowed with a distinctive, digestive and replacing power, by which the life of the bacterial protoplasm might be substituted for that of the tissues and body of an animal.

The fact, that although viable protoplasm might pass the pores of a porcelain filter, while filtered supernatant fluids were inocuous when injected into animals, substantiated the observation that the special power represented by an affinity for animal tissues was temporarily segregated in protoplasm which existed

as formed, non-filterable units. These were rather large Gram positive, and distinctly non-vegetative and single. Therefore, the protoplasm composing them represented the lightest of the cultures.

That the typical cultures were but temporary states of adjustment, was indicated by the fact that the protoplasm which passed through the porcelain filter carried latent capacities for the characteristic adjustments, and these all recurred following incubation. Just as in the case of fluids cleared by the centrifuge, in the absence of any fresh culture material, repopulation in the filtrate was rapid and complete. So universal and perpetual were the digestive functions of this bacterial protoplasm that, relatively speaking, the excretory products of today were the food of tomorrow. Yet, after about a week, the life activities in a culture were markedly decreased, and a "hibernation" set in, in which the protoplasm was observed to be capable of retaining its viability for at least a year.

C. Correlation.

In order to understand how such bacterial protoplasm passed from the intestines of parrots to human tissues and how disease was there produced, a scheme was developed, based largely upon the results of the studies during the epidemic and those made later upon healthy parrot intestinal flora. Under experimental conditions, the activation, by plain bouillon, of that chemical reaction in bacterial protoplasm providing an affinity for animal tissues, was employed to hypothesize a similar action by living tissue extracts under natural conditions. The subsequent refinement or intensification of this power by a sojourn of the bacterial protoplasm in animal tissues has been repeatedly demonstrated. The segregation of the power in *typical cultures,* in special concentration, in light single formed units, suggested how air transmission was possible. Bacterial protoplasm-animal tissue contact was then studied; the initial intensification of the already activated power in the former, and the beginning of disease in the latter.

Under experimental conditions, the following working theory

was developed to explain the processes of contact, and those following contact between activated bacterial protoplasm and animal organisms:

C-1. For any abstract location in the animal, the succeeding possibilities exist:

C-1a. A fixed point must be selected to start from; therefore, let it be a given degree of that power capable of being exhibited by the bacterial protoplasm in animal tissues. Variations will occur thus:

1. Primarily executed through units of mass, the degree of power demonstrated will be proportionate to the number of units present.

2. In the tissues saturated with dead organized matter the degree of power demonstrated will be greatest.

3. It will also be greatest when in the presence of materials such as have served already in an activating or catalytic role, to develop just the intensity of activity in existence at the time in a given portion of bacterial protoplasm.

C-1b. The direct contact is effected with the tissues by two means:

1. Bacterial protoplasm of great power retains its unit body form in the tissues.

2. While, on the contrary, units of bacterial protoplasm of relatively less power, assume an amorphous state effecting a much more intimate contact with the tissues.

The researches of Dr. Victor Vaughan may explain the exact results of interaction reaction, on the basis of a liberation of the protein poison by the body ferments from bacterial matter dying in the contact, and presumably vice versa.

C-1c. The immediate local effects upon the tissues (living tissues) according to the intensity of the interaction will be:

1. Cloudy swelling, a diffused process.

2. Cytolysis, a spotty process.

3. Nuclearlysis, as well as cytolysis,

merge imperceptically into:

C-1d. Lesion production. The community tissue effects (animal organism) will be:

1. Hemorrhage, developed by reflex vascular dilatation stimulated by the new local chemical activities, with blood vessel ruptured, due to injuries of the walls, diseased by the local processes just described.

2. The deposit of serum, leukocytes, fibrin, pus, round cells, fibrosis.

Assuming a given degree of general, defensive reaction-power on the part of the host, the inflammatory process will depend upon two factors:

A. The degree of concentration of a given amount of bacterial power both as to total animal body area, and locally. The more concentrated it was, the less the initial local tissue destruction, the less the animal organism would be generally embarrassed. Other things being equal, the more intense would be the chemotactic effects and the finer the reaction picture that would be drawn by the animal. If the factor controlling the intensity of such a concentrated power of affinity could be fixed for the bacterial protoplasm, the animal would soon be overrun, but generalized protoplasm is too flexible; its power quickly diffuses, and therefore examples of acute septicema were not observed. The finest evidences of chemataxis seen, the pneumonia of No. 145, destroyed the rabbit because of the local activities.

B. The second factor was that of time. The degree of lesion production would depend, other things being equal, upon the duration of time allowed for the process to develop.

Thus far only the abstract lesion has been considered.

C-2. As special experiments showed, the generalized bacterial protoplasm was completely and diffusely distributed throughout the animal organism, following the experimental introduction of a culture into the venous circulation. Therefore, all these possibilities considered for an abstract area might occur anywhere in the animal's body.

Lesion location for a given degree of power of the bacterial protoplasm, which had already been considered in relation to the kind of lesion produced, had been shown to depend upon three factors. Given a tissue composed of essential cells, plus a certain quantity of dead material or tissue pabulum, the location of lesion would depend upon:

1. The degree of intensity of activity underlying the affinity existing in the bacterial protoplasm for animal tissues. The less the degree of this intensity the nearer would the lesion develop to the gut tube.
2. The closeness in relationship of the tissues concerned with that type of tissue which activated the chemical reaction involved in providing the tissue affinity. This power, naturally, must have been sustained in bacterial protoplasm by continuous activation, and a slightly different environment might temporarily reduce the effective power.

Thus lines might be roughly drawn between the gut contents, dead tissues, the gastrointestinal system, and the more purely essential tissues, (perhaps the lungs are thus distinct from the heart and kidneys) on the basis of the first factor.

The second factor might explain the more delicate shadings of the disease pictures, if high degrees of selective election could be established. This would not be expected of such generalized matter, with variable characters.

C-3. Accidents of first contact especially occurring in the capillaries of the lungs, which thus receive primarily the full dose injected. Animal organism considered as a whole.

Theoretically, at least some degree of interaction reaction takes place throughout the entire animal organism. The results

for any area, and for those areas where the results will be most marked, have been indicated.

The total amount of microscopic lesion production, the death of the animal, and the subsequent intensity of putrefactive changes were shown to depend upon the total amount of power by which generalized bacterial protoplasm in a culture demonstrates an affinity for animal tissues, and the distribution of the power throughout the protoplasmic material of a culture. The distribution of this power in *typical cultures* has been shown to be very unequal, and especially segregated in the supernatant borne units following centrifugation.

C-3a. Macroscopic Lesion Production:

Other things being equal, this will be greatest:

1. The greater the total amount of power in the culture of sufficient intensity, that is, above a certain minimum, as already indicated.

2. The more complete the segregation of the total power in a part of the bacterial protoplasm injected.

C-3b. Death of the animal:

This will be most rapid:

1. The greater the total amount of power in the culture above a certain minimum, which is for any one unit below that required for visible lesion production.

2. The more complete the diffusion of the total power throughout the bacterial protoplasm of a culture.

C-3c. Putrefaction intensity:

This will be most marked:

1. The greater the extent to which this power is represented by reaction activities of the bacterial protoplasm adjusted to the chemistry represented by dying or dead tissues.

The summary of the disease pictures included the clinical aspects, the macro- and microscopic pathology, and some suggestive experiments in therapy.

PARTS I–III

CONCLUSIONS:[1]

In the presence of cold, the chilling of the parrots resulted, most likely, in an intestinal disturbance, with irritation, as evidenced by a diarrhoea. The altered secretions, containing inflammatory elements, exuded from the walls of the gut tube. These constituted extracts of living tissues. The stimulating effect of plain meat substance bouillon upon unspecialized bacterial protoplasm has been many times experimentally demonstrated in the preceding pages. The effects of genuine, fresh tissue extracts must be more marked.

The influence of meat substance bouillon plus that provided by extremely short sojourns in animal tissues, have also been demonstrated. First, there appeared to be a general increase in the power of all the units representing the bacterial protoplasm; then the power or intensity of reaction underlying the affinity for animal (including bird) tissues was refined and segregated, as demonstrated by the study of the supernatant fluids of centrifuged *typical cultures.*

The effects of contact between the personal saprophytic bacterial protoplasm from the intestinal tracts of different birds undergoing this stimulation at the same time, has not been studied. Nor has there been investigated the effects upon the bacterial protoplasm of contact with parrot tissues. There must have frequently taken place such transfers of bacterial protoplasm from the intestinal tract, not only to the tissues belonging to the body of the same bird, but to the tissues of others, and from these been transmitted on through an unknown number of individuals. How much more intense the power became under such natural influences than it did under those which were artificial can only be imagined.

Admitting these untested possibilities the picture may be thus simply drawn. The whole group of parrots, on the train en route from the point where they were first chilled and in the

[1] Pages 192 to 195 are similar to the conclusions to Part I, pages 73 to 76, the use of which was questioned by the author.—(Editor's note.)

store, were one great culture. In the intestines of many birds the bacterial protoplasm received the stimulus provided by an excess of exudates from the gut walls, practically tissues in solution and natural preparations imitated by artificially prepared plain bouillon. The "culture," comprised by the group of parrots, was then inoculated by this bacterial protoplasm, aroused to a state of activity already capable of demonstrating an actual affinity for living tissues. Acceleration of this activity of the bacterial protoplasm occurred in the tissues of the parrots, which formed, naturally, the principal component of the culture. This attack was made easy by the undermining of the birds' resistance, due to the chilling of their bodies. The effect of cold has the well known influence of reducing the resistance of higher organisms against bacterial infection. All about the parrots, dead animal material was deposited. Thus, there existed a state of affairs to which that entity known as "*the typical culture of generalized bacterial protoplasm in plain bouillon,*" may roughly be compared.

The parallel between the two becomes more evident if one considers that the factor provided by the bird bodies in the parrot group culture is represented in the typical culture by the bodies of the rabbits through which the bacterial protoplasm was passed, previous to the development of the typical culture. In the latter, it has been shown that the power of the bacterial protoplasm providing the affinity for animal tissues, was segregated in the "bodies" of certain units which were proved to be the lightest of all the units in the culture by the fact that they remained in the supernatant fluid after the culture was centrifuged.

In the culture composed of the group of parrots, the action of the centrifuge was represented by the force of gravity. The more saprophytic portions of the protoplasm clumped, and therefore, being heavy, remained upon the ground, while the units of disease-bearing power floated above, about the parrots in the air, represented by the supernatant fluid of the *typical culture.*

Thus, all bacterial protoplasm, as finally distributed to human beings, was in a high state of activity. Energy had flamed up and become disproportionate to its associated material mass, or

we may say, every particle of material mass involved in the reactions when the epidemic was active among the parrots was aflame with energy. Such material was diffused in the zone about the parrots and alighted upon human tissue.

In human beings, there occurred first a period during which the enormous energy carried in the tiny particles of matter was producing protoplasm, which was being diffused throughout the body. This is the period for which there has been no true experimental analogue provided. Vaughan has explained it, especially in relation to the disease producing activities of members of the typhoid-colon group, as time consumed by reproduction on the part of the bacteria, and specific ferment mobilization on the part of the animal organism. He considers its termination, as taking place at the moment when the body ferments begin to feed upon the bacterial substance. In the case of the generalized bacterial protoplasm under consideration, the only modification to this conception suggested by the investigation is that the ferment attack and the bacterial reaction comprised a process which began immediately and which very slowly and gradually developed. Specific ferments would seem undevelopable against such generalized bacterial matter.

Whatever the process of incubation, the disease here was general, with special exaggerations of signs and symptoms referring to the respiratory or gastrointestinal tracts, but variation in the clinical pictures was the rule.

A natural conclusion would be that the general lack of fixation in any one so-called character of the protoplasm, so frequently observed, must hold true also for the relationship between the protoplasm and this character of great activity in animal tissues. And indeed there has been shown experimentally, in the studies upon the supernatant fluids of *typical culture* No. 24, a dissolution of the relationship between the bacterial protoplasm and the degree of tissue affinity it may exhibit in a rabbit's tissues, as far as the supernatant fluid units were concerned; i. e., a rapid dissipation of their energy. At any rate, in the first line of human bodies, as clinically reported, the epidemic faded and great masses of bacterial protoplasm of a generalized

character then lay about the human community, with hardly more than saprophytic power.

The epidemic was over.

It was this protoplasm that the author first procured; protoplasm capable of various degrees of activity in and affinity for animal tissues. Being generalized under the influences of a common environment, metamorphosing and adjusting its functions until a common ground was reached, the *typical culture,* developing in plain bouillon, provided such far reaching results.

PART IV

(AN APPENDIX)

INTRODUCTION

The epidemic among parrots, transmitted to neighboring human beings, has been shown to have been due to generalized bacterial protoplasm, such as was found to constitute the saprophytic flora in the feces of the healthy parrots studied.

All through the experimental work there was observed on the part of the bacterial protoplasm, here and there, tendencies to persistency in a character, usually a very temporary condition. This was most often the case with regard to a given unit form, which when transferred to a new environment, tended to retain the shape assumed in the original environment.

In the cases of sporadic disease of parrots observed in the laboratory, this persistency in the exhibition of a certain character in the face of a changed environment, in other words, this fixation, was beautifully demonstrated by the bacterial protoplasm from sporadically diseased parrot No. 3. The character concerned was that involved in the production of disease in animals. The bacterial protoplasm from a pigeon, canaries and a rabbit, was compared to that involved in the epidemic, for the original purpose of controlling various experiments performed upon the latter. Examples of fixation were accidentally discovered.

Therefore, these studies are grouped in Part IV to constitute a separate division of the work, by which the results of the investigation of the local epidemic may be linked up to those reported for previous epidemics; the clinical picture described by Dieulafoy, and the bacterial cause reported by Nocard.

Chapter XI

THE BACTERIAL PROTOPLASM INVOLVED IN THE EPIDEMIC AND FOUND TO EXIST IN THE CONTENTS OF THE INTESTINES OF HEATHY PARROTS, CONSIDERED IN ITS RELATIONSHIP TO SPORADIC DISEASE IN PARROTS

With the presence of four new parrots in the laboratory, it was a foregone conclusion that, especially in this climate, individual or sporadic disease should occur.

Three of them were obtained in the spring. They were kept scrupulously clean, but not apart. They died within a period of 3 months, but their deaths were separated by some weeks, and no evidences were observed to link them up with a common cause.

As controlled by observations upon other birds, as well as by care at the time, the fact was safely established that naturally arising factors of disease did not act at the time when they could have influenced the experimental studies.

The symptoms of the sporadic disease were in the main, diarrhoea. In these four birds, fortunately, there were represented in two, disease with a short course, one was a case showing a prolongation of the same disease, both types being associated with gastrointestinal irritation. Opposed to these was a case constituting an exception.

PARROTS NOS. I AND II

Parrots No. I and No. II both suffered from acute disease with the clinical symptom of diarrhoea.

At autopsy, there was found in each a gastroenteritis.

Associated bacterial cultures showed a functional power closely comparable to that exhibited in the *typical cultures* of

No. 1522 and No. 24, intensively studied in the preceding chapters.

We have in these parrots probably the most simple form of disease arising naturally. They appeared to be isolated cases in which the normal bacterial protoplasm, represented by that in the intestines, became disturbed in its adjustments.

Probably the latent capacity for growth in animal (bird) tissues, considered in Chapter VI, was aroused by the tissue extracts exuded from the intestinal walls under the influence of whatever began the intestinal irritation. This could of course be due to diet, cold, etc. Such individual cases multiplied and exchanging bacterial protoplasm so stimulated to activity, may be imagined as a basis of an epidemic.

PARROT NO. I

CLINICAL HISTORY:

This parrot was ill and suffered diarrhoea quite acutely. But its most marked characteristic was its sudden death.

AUTOPSY:

Mild gastroenteritis.

BACTERIAL FINDINGS:

CULTURE:

Nose: Foul, and contained *Gram negative rods.*

RABBIT:

No. 1779. Two c.c. plain bouillon culture.

DEATH:

12 hours.

AUTOPSY:

Hemorrhages lacking. Parenchymatous organs and spleen profoundly dark.

SMEARS:

Heart blood: Gram positive rods.
Spleen: Same.

CULTURES:

Heart blood: Gram negative rods.
Bile: Gram negative-positive rods, and cocci.

RABBIT:

No. 1783. Two c.c. 24 hour culture of heart of No. 1779.

DEATH:

24 hours.

AUTOPSY:

Large and small hemorrhages of the lungs. Intestine congested, contained gas and mucus. Parenchyma degenerated. Splenic tumor.

URINE:

Albumin negative.

BACTERIAL FINDINGS:

Same; somewhat variable forms tending to gram negative rods.

The first rabbit (No. 1779) presented a rare example of an extreme case of 12 hour death without lesions; practically putrefaction in life. The subculture showed the lung hemorrhages, considered as associated with a high disease producing power. The extremes, shown in such a short time, demonstrated the great flexibility so often observed in cultures of the type of bacterial protoplasm.

RABBIT:

No. 1. Two c.c. plain bouillon culture of feces.

DEATH:

1½ hours.

This result was frequently seen from all varieties of cultures of this bacterial protoplasm. The study was carried no further.

PARROT NO. II

CLINICAL HISTORY:

Ill for 3 weeks. Isolated. Had diarrhoea and was molting.

AUTOPSY:

Mild enteritis with mucus. Lungs showed congestion. Parenchyma degenerated.

BACTERIAL INVESTIGATION:

RABBIT:

No. 125. Two c.c. subculture of a 5 day old plain bouillon culture of rectal contents.

DEATH:

8 to 12 hours.

AUTOPSY:

Putrefaction rapid. Hemorrhages and congestion of tonsils, pharynx, trachea, and stomach. Hemorrhages of thymus and kidneys. Intestine obscured. Kidneys pale. Liver very dark. Splenic tumor.

CULTURE:

Heart blood: Plain bouillon. Foul. *Contained Gram negative rods.*

PARROT NO. III

In November a parrot was bought and put in a closed, small and well lighted room. It was intended that a lot of work should have been done upon this bird.

At this time healthy fecal germs were being studied. A culture was taken of the feces and set aside for a day or so. In the interim the parrot had diarrhoea, unknown to the author. The culture was injected, but the disease in the rabbit did not follow the usual course. The animal became slowly ill and died within 24 hours. There was found a high grade gastrointestinal inflammation, but in the most early hemorrhagic stages, with mesenteric adenitis, parenchymatous degeneration and splenic tumor. Two c.c. of healthy fecal culture either kills suddenly in an hour or slowly in a week or so; rarely it causes slow emaciation with final death.

The condition of the parrot was investigated and it was found to be suffering from diarrhoea. From this time on there were periods of alternating health and diarrhoea, so that the parrot could only be studied as a case of sporadic disease.

The supernatant of the above mentioned culture showed the highest, or a 24 hour rabbit-killing power, although without distinct pathology. Three weeks later a fresh culture killed a rabbit in 72 hours, with a picture remarkable in its resemblance to typhoid fever, with lymph plaque involvement, bronchitis, and a progressive clinical course. The supernatant fluid required 216 hours to kill and caused general abscesses. Comparing this to the usual results following the inoculation of supernatant fluids of the *typical cultures,* and to the effects of the supernatant fluids of the supernatant strain, it may be concluded that there is here represented a diffusion of supernatant unit power.

According to this reasoning, we have here a more common distribution of power throughout the whole culture, contrary to that state of affairs observed in the case of epidemic cultures, and in rapidly transferred and reacting protoplasm such as the artificially induced strain, No. 24.

On February 26, 1918, the parrot died after a period of diarrhoea.

The post mortem examination revealed a very active inflammation of the whole intestinal tract, which also contained food, not usually associated with severe types of diarrhoea. There was parenchymatous degeneration and splenic tumor.

Cultures were made from the chyme, heart blood, liver, kidneys, and nose. The feces were cultured directly, also portions were emulsified and centrifuged until the suspension of bacterial units observed in the smears equalled that of the other cultures.

The nose culture was typically saprophytic, had a powerful odor, and killed a rabbit in an hour; thus the respiratory tract might be excluded as a factor in the disease.

The fecal emulsion, three separate cultures of the contents and three of the feces, at six to nine day intervals, to allow for retrogression, and a culture of the kidney were injected in equal amounts into rabbits; nine animals in all. The results were remarkably uniform and the autopsies compared closely to that of the animal inoculated in December. The results then were: Death in 36 to 96 hours, with a high grade of gastroenteritis, mesenteric adenitis, and in 6 also cholecystitis; in two, a tendency to pneumonia; in one, liver abscesses; in all, parenchymatous degeneration and splenic tumor. The exceptions were indiscriminately distributed between the fecal and organ cultures.

The supernatant culture of the feces killed in 120 hours, a feeble parallel to the others.

The bacterial forms were variable both in parrot smears and in all cultures. On the special media, gelatine was not digested, just as it was not in the case of the supernatant fluid of No. 24. Milk coagulation varied, but no digestion was observed. On Russel's double sugar agar (differentiating typhoid, paratyphoid, and colon bacillus) each one differed; one appeared as bacillus typhosus, one as the paratyphosus; another decolorized, and some changed after 48 hours. A putrefactive odor was feeble or absent and alkalinity was slight; cloudiness was rather slight and the pellicles were absent. Later, the saprophytic characters were slowly resumed.

Thus there existed a persistent gastrointestinal disease. Associated with it was a bacterial flora which exhibited variability in form, and in the coarser artificial media, digestive functions. In the initial stages of the course it demonstrated practically the reaction of a *typical culture*.

The principal peculiarity lay in the fact that as time passed, with persistent clinical evidences of intestinal disease, changes occurred in the chemical adjustments of the protoplasm in cultures, as realized in the response to animal inoculations.

1. By analysis, with the passage of time the supernatant borne units known to be the most refined pathogenetically, killed more slowly than typically.

2. Cultures of the feces in plain bouillon when inoculated into animal tissues acted like the suspension in salt solution of the feces; i. e., in plain bouillon there was not observed the unequal redistribution of the power exhibited in animal tissues, with the resulting partition into the refined supernatant units and others more saprophytic.

These factors suggested that there was a tendency towards the development of units of definite individual values such as may be supposed to constitute the population of the cultures of definite so-called bacterial species.

3. And more, repeated efforts failed to bring out a change in function in the cultures taken after the death of the bird, a change similar to that observed during the epidemic and experimental studies and resulting in the *typical culture*.

These facts in time would seem to indicate a fixation, as between the bacterial protoplasm and the factor regulating the powers of disease production under consideration. Presumably this took place in the course of time in a fixed environment. There was no such flashing as observed during the epidemic. This is all suggestive of the underlying phenomenon which may provide the possibilities of the apparently opposed reported findings as to the aeteological factors of a common clinical entity, when the studies are made in two different localities, one associated with epidemic, one with purely endemic disease.

PARROT NO. III

CLINICAL HISTORY:

This was a healthy parrot when bought in November.

I. ANIMALS OF 11/15/17.

RABBIT:

No. 138. Two c.c. whole culture of feces in plain bouillon.

DEATH:

24 hours.

AUTOPSY:

Putrefaction rapid. Hemorrhages of the intestine. Congestion of the stomach and lymph patches of the intestine. Splenic tumor.

SMEARS:

Bile: Rather large Gram positive rods.
Nose: Same.

CULTURES:

Heart: Gram negative rods varying in length.
Bile: Same.

RABBIT:

No. 148. Two c.c. doubly kaolinized and twice centrifuged (15 minutes) supernatant.

DEATH:

24 hours.

AUTOPSY:

Curiously little pathology except a yellow liver.

CULTURES:

Bile, nose, urine, heart blood, contents, all positive and moderately rich.

NOTE: An interesting observation was made upon the fecal changes attendant upon the beneficial effects of coffee.

Preliminary to its use the forms seen in the fecal smears were numerous. Following its use the number of forms decreased greatly.*

4TH NEW PARROT CULTURES IN ARTIFICIAL MEDIA

Feces of 1/26/18 when most nearly healthy. *Never injected.*
Large Gram positive rods.

Fecal Emulsion.
Gram positive rods.

Plate:
Homogeneous, grey green.
Large Gram negative rods.

Slimy with white points.

* During the War between the States, coffee was used on the Confederate side almost universally to dress suppurating wounds. (Ed. note.)

NOTE: White points appear as more or less formless units or variable ones as if germination occurs here. Transfers from points or grey homogeneous portions result in the development of the same two kinds of colony.

Milk:

Complete digestion. — *Slow coagulation. Pink top in 72 hours.*

Gelatine:

Digested. ++++ — *Digested.* ++

Russel's Double Sugar Media:

Pellicle. +		—
Foul odor.		++++
Indol. —		—
Alkaline. +		+
Gas: Only a little.		++
Stab: Colony.		*Pink.*

Contents (Chyme)	*Heart:*	*Liver:*
Mixed rods and Gram negative cocci.	*Short Gram negative rods and cocci.*	*Short Gram positive rods.*

Plate:

Homogeneous and slimy.	*No white points.*	
Large Gram negative rods.	*Short Gram positive and negative rods.*	*Short Gram positive and negative rods.*

Milk:

Rapid coagulation. Pink top in 24 hours.	*Very slow coagulation. Pink top in 72 hours.*	*Negative. Alkaline in 72 hours.*

Gelatine:

Digested: +	*Digested:* ++	*Digested:* —

Russel's Double Sugar Media.

Pellicle: —	—	—
Foul odor: ?	+	—
Indol: —	—	—
Alkaline: +	+	+
Gas: +++	+	++
Stab: Pink, whole.	+	+

Kidney: — *Nose:*

Short Gram positive rods and cocci. — *Mixed short rods and cocci, some Gram negative.*

Plate:

Grey green, many white points.....

Short Gram positive and negative rods. — *Gram negative cocci.*

Milk:	
Very slow coagulation.	*Moderate coagulation.*
Pink top in 72 hours.	*Pink top in 24 hours.*
Gelatine:	
Negative.	*Negative.*
Russel's Double Sugar Media:	
Pellicle: —	—
Foul odor: —	+
Indol: —	—
Alkaline: +	+
Gas: +	++
Stab: +	+

II. Animals of 12/8/1917.

Rabbit:

No. 162. Two c.c. 24 hour plain bouillon culture of feces.

Clinical History:

Progressively weaker.

Death:

72 hours.

Autopsy:

Hemorrhagic inflammation of duodenum and all lymphatic areas in the intestinal walls. Hemorrhagic adenitis. Bronchitis. Intense degeneration of the liver and kidneys and spleen.

Urine:

Diazo and urochromogen negative. (The same result obtained in human typhoid-like cases.)

Rabbit:

No. 172. Two c.c. supernatant treated as was that inoculated into No. 148.

Death:

216 hours.

Autopsy:

Abscesses of liver, kidneys, and appendix. *Cholecystitis* with pus. Gastrointestinal catarrh.

Urine:

Albumin positive.

Sugar positive.

Culture:

Light growth. Did not digest milk or gelatine.

III. 2/28/1917. Parrot Died.

For studies upon the associated bacterial protoplasm in artificial media, see special sheet. In rabbits, cultures from the nose, kidneys, intestinal chyme, feces and a salt solution emulsion of the feces were injected. As will be seen, the inoculation of the feces and chyme were repeated, the former twice, the latter three times.

ANIMALS:

A. NOSE CULTURE.

RABBIT:

No. 250. Two c.c.

DEATH:

1½ hours.

B. KIDNEY CULTURE.

RABBIT:

No. 249. Two c.c.

CLINICAL HISTORY:

Became immediately ill.

8 hours: Leukocytes 2400. Temperature 103°.

24 hours: Leukocytes 7000. Temperature 103°.

DEATH:

36 hours.

AUTOPSY:

Heart showed acute myocarditis of the right ventricle.

Lungs showed pneumonia, fibrin and consolidation. Severe *enteritis*. Mesenteric *adenitis*. Feces soft. Kidneys dark and congested. Liver and gall-bladder hardly affected.

SMEARS:

Nose, bile, spleen, lung, pericardium, and mesenteric node; Gram positive rods and coccal form.

CULTURES:

Heart: Irregular forms.

Lung: Small Gram negative rods.

Bile: Same.

C. Emulsion of feces in salt solution, centrifuged to remove large particles, and then the cloud of bacteria was standardized against the bouillon cultures.

RABBIT:

No. 247. Two c.c.

CLINICAL HISTORY:

24 hours: Leukocytes 25000. Temp. 102.2°. Sick.

48 hours: Leukocytes 56000. Temp. 104°. Ate. No diarrhoea.

72 hours: Leukocytes 52000. Temp. 103°. Weaker.

DEATH:

72 hours.

AUTOPSY:

Moderate *enteritis*. Much gas and mucus. *Cholecystitis*. Hemorrhagic *adenitis*. Acute parenchymatous degeneration. Huge splenic tumor.

URINE:
Negative.

SMEARS:
Nose: Gram positive cocci and rods.
Bile: Same.
Spleen: Same.
Appendix: Irregular Gram negative forms.
Mesenteric node: Same.

D. Feces Culture in Plain Bouillon.

RABBIT:
No. 245. Two c.c. 48 hour old culture.

CLINICAL HISTORY:
18 hours: Quiet. Leukocytes 8000. Temp. 101°.
24 hours: Better. Leukocytes 8000. Temp. 104.3°
Feces O.K. Ate well.
72 hours: Leukocytes 9800. Temp. 104°.

DEATH:
96 hours.

AUTOPSY:
Heart: Showed acute *myocarditis.*
Lungs: Atelectatic, *inflamed,* almost pneumonic.
Catarrh of intestine: Mesenteric nodes slightly hemorrhagic.
Appendicitis. Acute *cholecystitis* and inflammation of the ducts. Mild parenchymatous degeneration. Huge splenic tumor.

URINE:
Albumin positive.

SMEARS:
Rich in cocci and short rods of various length and stain.

CULTURES:
Bile and heart as irregular as the smears.

RABBIT:
No. 251. Two c.c. fresh bouillon culture sediment used in No. 245, six days later.

DEATH:
60 hours.

AUTOPSY:
Practically the same.

RABBIT:
No. 255. Three c.c. 7 days later, same kind of subculture.

DEATH:
72 hours.

AUTOPSY:
Almost duplicate findings.

RABBIT:

No. 246. Two c.c. supernatant fluid.

DEATH:

120 hours.

AUTOPSY:

Practically the same, except for an immense spleen, about 4 inches long.

E. Chyme Culture.

RABBIT:

No. 248. 1.75 c.c. 96 hour culture in plain bouillon.

CLINICAL HISTORY:

Ill in 45 minutes.

24 hours: Leukocytes 5000. Temp. 105°.

DEATH:

48 hours.

AUTOPSY:

Heart *inflamed* in right ventricle, with transparency of muscle. Intense hemorrhagic *gastritis*. *Duodenitis* and *appendicitis* with hemorrhages. Cæcum *inflamed*, with hemorrhages. Hemorrhagic *mesenteric adenitis*. *Cholecystitis*. Huge splenic tumor.

RABBIT:

No. 254. Two c.c. 48 hour culture of sediment of tube used 7 days before for rabbit No. 248.

DEATH:

96 hours. Convulsions.

AUTOPSY:

Except that the acute inflammation involved the kidneys, and not the heart and with the addition of abscesses of the liver and pus in the gall-bladder, this rabbit resembled No. 248.

URINE:

Albumin positive.
Indican positive.
Hyaline casts.

RABBIT:

No. 263. Two c.c. 5 day subculture of the same bouillon then about 3 weeks old.

CLINICAL HISTORY:

Leukocytes:	2 hours:	4000.
Leukocytes:	24 hours:	34000.
Leukocytes:	48 hours:	51000.
Leukocytes:	72 hours:	126000.
Leukocytes:	120 hours:	49600.

Temperature, high at first, fell slowly.

DEATH:
288 hours.

AUTOPSY:
Practically the same as that of No. 254, but this also showed abscesses of the appendix.

RABBIT:
No. 268. Subculture of a subculture of the heart culture of rabbit No. 248, nearly 2 months old.

DEATH:
48 hours.

AUTOPSY:
Almost a duplicate of No. 248 plus acute nephritis as in No. 254, with albumin, casts, cells, etc.

A Typical reaction never occurred.

SUMMARY PARROT NO. 3

Persistent low grade intestinal disease, after the initial arousal of the factor for disease production in the generalized bacterial protoplasm, gradually influenced the development of this activity to a certain degree, which in time became quite fixed. Associated with this state of affairs was a loss of the property by which gelatine is digested, but other characters were typically variable. That the factor of disease production in the bacterial protoplasm was inherently active, diffusely present and not aroused by plain bouillon, was indicated by the production of practically similar disease pictures with a fecal emulsion in salt solution as well as with a culture in plain bouillon. Thus there was distinctly absent that flexibility and arousability of the factor demonstrating an activity in animal tissues, effected among the saprophytes in the healthy droppings. We have here an example of a persistently infectious bacterial strain, as opposed to that, freshly aroused, profoundly active, but rapidly weakening, provided in the course of the epidemic.

PARROT NO. IV

CLINICAL HISTORY:

Sick for 2 weeks. Was sleepy, and had paroxysms of sneezing and choking. It did not seem to grow worse, but sat in the bottom of its cage. The feces did not show softening or an increase in amount.

AUTOPSY:

Brain, lungs, heart, liver, and kidneys were negative. One loop of the small bowel was somewhat congested. Rectum was dark. Practically no gross changes.

BACTERIAL FINDINGS:

SMEARS:

Throat: Mixed.

Lung: Mixed.

Feces: (Normal areas). Negative. (Dark areas). Diptheroids and slim Gram positive rods.

CULTURES:

Heart blood, lung, dark intestine, pale intestine, splanchnic blood from both contracted and dilated vessels, marrow, throat, feces, all positive.

Heart blood: Cloudy, pellicled, odorless and acid. Five days later, still acid. Showed at first Gram negative rods in reculture, also cocci.

Lung: Same, plus sediment.

Throat: Same.

Marrow: Mostly cocci.

Intestine: Mixed and irregular forms.

Time showed no change in the cocci, while the rods did change and became diphtheroidal, and bizarre.

ANIMALS:

RABBIT:

No. 42. Two c.c. 48 hour plain bouillon culture of throat.

DEATH:

7 months.

AUTOPSY:

Suppurative appendicitis with adenitis. Atrophy of the heart. Gastric catarrh. Acute enteritis. Nephritis. Splenic tumor.

No. 47. Two c.c. 96 hour plain bouillon culture of lung.

DEATH:

6 months.

AUTOPSY:

Chronic emaciation. Acute enteritis. Adenitis. Soft feces.

No. 41. Two c.c. 48 hour plain bouillon culture of feces.

DEATH:

5 months.

AUTOPSY:

Acute enteritis. Chronic cholecystitis. Atrophy of the heart.

SMEARS:

Bile: Formless.

CULTURE:
Bile: Gram negative rod.
This suggests a metamorphosis, but from a fecal culture.

No. 46. Two c.c. 96 hour plain bouillon culture of contents of dark intestine.

DEATH:
3½ hours.

AUTOPSY:
No pathology developed. Putrefaction very rapid.

PARROT NO. IV

In the case of this parrot there is no pretense at making a positive diagnosis. The investigation is presented in the role of a control compared to the three other parrots. In this case, there was a definite absence of the clinical evidences of intestinal irritation. Smears and cultures from all localities examined after death were positive. The forms seen, in themselves were not different from those seen in the other three parrots and those involved in the epidemic. It was mainly in the peculiar functional responses, in association with animal tissues, that very distinct differences seemed to lie. True, rabbit No. 46 which was injected with material from the intestinal contents of an area of apparent congestion, did show a *typical reaction.* But no further conclusion can be drawn from this than that the normal flora had become adjusted, by unknown means, so that the environment of living tissue had become more suitable. In the absence of other varieties or states of bacterial protoplasm, this, if alone present, could be considered as an actual factor in the disease picture.

In the rabbits injected with cultures of bacteria from other areas, the disease courses lasted over a period of months and there were exhibited, in the autopsies, at least in part, acute changes. These have been considered secondary reactions in the study of the inoculated rabbits. They should take place with greater ease in rabbits which had been injected. Of course, as observed from studies in the preceding pages, such secondary reactions might involve the use of the protoplasm which had been

previously injected as a factor in the new or acute disease equations. In No. 41, this apparently occurred.

Further, the bird was sick long enough so that a fixation in the bacteria would be likely, as in Parrot No. 3; and no metamorphosis was observed in the unit forms to link together those originally so unlike, except in the case of No. 41. Here the culture showed Gram negative forms, but the rabbit had been inoculated with a culture of the feces. Nevertheless, if form were fixed, so may have been the factors for disease production in animals. On the other hand there were no evidences whatever of inherent disease production observed in the bacterial units except as stated in the case of rabbit No. 46.

Of course in time a gradual functional adjustment, as well as a metamorphosis, might have been observed by rabbit passage, but this would only have proved the latent generalizability; the lack of a profound fixativeness in the bacterial protoplasm obtained. It must remain a puzzle.

CONCLUSIONS

1. Disease, based upon changes in the normal activity of the bacterial protoplasm existing in the gut tube, arises sporadically, in individual parrots.

The bacterial protoplasm involved in the adjustment of the disease equation, closely paralleled in characters bacterial protoplasm involved during the epidemic among parrots and human beings, and that from healthy parrots, artificially induced to produce similar disease pictures.

2. This adjustment between the protoplasm and the factor for disease production, may be in time quite fixed and stable, and apparently the only factor so involved, as in parrot No. 3. Presumably, that was the condition of affairs most directly opposed to the findings at the time of the epidemic. It is likely that in those regions where the disease was endemic, such fixation occurred, accounting to some extent, for the differences between the findings in the local epidemic and those previously reported.

Chapter XII

THE STUDY OF THE BACTERIAL PROTOPLASM, SAPROPHYTIC IN THE INTESTINAL TRACTS OF THE PIGEON, CANARY, AND RABBIT. THE COMPARISON OF THESE STRAINS WITH ONE ANOTHER AND WITH THAT OF THE PARROT ALREADY INVESTIGATED

Thus far, there has been studied a common type of bacterial protoplasm, found in the tissues of men and parrots ill through the medium of an epidemic. It was obtained also in the droppings of healthy parrots and acquired from cases of sporadic disease of parrots.

It is difficult to keep parrots continuously well, and after losing one, it often requires a long time to procure another. Therefore, other birds were selected, two of which were presumed to be herbivorous, the canary and the pigeon. They were employed as controls in the interpretation of bacterial life in parrot feces and in the interests of comparison, but especially for the purpose of repeating certain of the most important preceding experiments.

The forms and functions in dead and living matter, of the intestinal saprophytic life of the canary and the pigeon will be described.

For the purpose of comparison, there were repeated the two most vital experiments in the study of bacterial function, in the case of the fecal flora of parrots. These of course were the experiments of the induction of disease producing power whereby the previous effects upon the bacterial protoplasm of growth in ox bile and glucose bouillon, and plain bouillon, respectively, were compared.

As a further control, the intestinal saprophytic life of rabbits

was studied, and these same experiments were performed with this bacterial protoplasm.

The specimens were acquired from animals and birds under healthy conditions, away from the laboratory. The results of the use of diseased birds and animals, shown in the citation of controls, will be exhibited as proof that the relationships studied, existed under normal conditions.

PIGEONS (from the country).

Fresh, dark green, and white fecal material was selected, and a portion, an eighth of an inch in diameter, emulsified in plain bouillon, and divided.

I. SMEAR OF EMULSION:

(a) Large and small, and irregularly rounded Gram positive masses.
(b) Gram negative rods, varying in size.
(c) Diphtheroid forms.

II. ON BLOOD PLATE:

(a) Tiny, transparent colonies of rather large Gram negative rods.
(b) Larger, grey, slimy, colonies of smaller rods.

Later on, changes occurred in the amount, character, and color of freshly developing masses,

1. Light, yellow brown deposits.
2. Less of very dark brown material.
3. White staphyloid matter.

Subcultures from the primary grey plate colonies, into:

(a) Bile and glucose bouillon:
Gram positive cocci and short rods.
(b) Plain bouillon, three tubes:
1. Gram positive, large masses, Gram negative rods.
2. Gram positive, large masses, Gram negative rods, diphtheroids, and irregular forms.
3. Smaller, and all irregular.

Subculture of these, on blood plate, showed all forms practically Gram negative rods, and alike.

III. IN PLAIN BOUILLON:

(a) Tube inoculated with one loop of emulsion, showed Gram negative rods, varying greatly in length.
(b) Tube inoculated with ten loops of emulsion, showed Gram negative rods, varying very little.

IV. IN GLUCOSE BOUILLON:

Gram negative rods.

V. SPECIAL MEDIA:

(a) From plain bouillon:
1. Litmus milk, digested.
2. Nutrient gelatin, digested.

SECOND SPECIMEN FECES:

I. INOCULATED PLAIN BOUILLON:

Formed pellicle for only seventy-two hours, then gelatinous streamers descended. 120 hours, heavy, granular cloud and sediment.

FORM:

Various gram negative rods and irregular masses.

A. Subculture of blood plate colony, confluent, white, dry, hummocky masses, with tongue-like spreaders, looking like drops of clear water.

A-a. INOCULATION INTO RABBITS:

No. 280. 5/16/18. Two c.c. whole culture (48 hr.).

CLINICAL HISTORY:

One and one-half hours, vibratory breathing, slowly recovered. Continued ill. In seven days, weaker, in nine days, worse.

DEATH:

Nine days.

AUTOPSY:

Gastrointestinal catarrh. Mesenteric adenitis. Kidneys swollen, hemorrhagic, intense *glomerulitis,* round cell infiltration, cytoplasmic destruction. Heart, congested, fair striation. Nuclei slightly obscured. Liver, fatty degeneration and congestion. Spleen, small, little changed.

BACTERIAL FINDINGS:

SMEARS:

Showed very little.

CULTURES:

Heart: Showed mostly Gram negative rods, varying greatly in length.

Bile: Same.

Urine: Same.

Kidneys: Same.

No. 285. 5/27/18. Two c.c. supernatant, after three hours centrifugation, fluid was slightly cloudy. Forms, rather irregularly outlined Gram negative rods.

CLINICAL HISTORY:

No changes. 14 hrs. later, temperature 103 degrees. Leukocytes 14,000.

DEATH:

Accidental. Ten days.

AUTOPSY:

Emaciated. Intestine showed loops distended, with mucus, rich in cells. *Mesenteric adenitis. Remainder,* negative.

BACTERIAL FINDINGS:

CULTURE:

Mesenteric node: Gram negative rods.

Study of effects of artificial media upon the functions of the bacterial protoplasm as demonstrated in animals.

METHOD:

14 c.c. of forty-eight hour cultures of plain, and bile and glucose bouillon centrifuged to clarity. Bouillon replaced by salt solution, and the bile and glucose sediment, usually the lighter, covered with 14 c.c. salt solution, and plain sediment covered with enough fluid to cause an equal degree of density upon distribution of sediment.

I. PLAIN BOUILLON:

Two c.c. of 48 hr. subculture of seven day subculture of culture used for rabbit No. 280, every two hours for seven doses.

DEATH:

Within twenty hours.

AUTOPSY:

Rhinitis, bronchitis, hemorrhages of heart, right ventricle, with yellow degeneration; of the kidneys, with pink congestion; of the whole of small gut, especially caput duodeni, and appendix; also, of mesenteric nodes; of skeletal muscles. Stomach, diffuse inflammation. Feces, normal. Liver, yellow. Bile, thick. Splenic tumor. Urine, whitish, albumin positive.

BACTERIAL FINDINGS:

SMEARS:

Stomach: Rather rare, Gram negative rods.
Chyme of ileum: Same.
Bile: Same.
Chyme of duodenum: Gram positive rods.
Mesenteric nodes: Gram positive rods.
Heart blood: Cocci.
Spleen: Cocci.

CULTURES:

All rich and foul smelling.

II. IN BILE AND GLUCOSE:

RABBIT:

No. 286. 2 c.c. of 48 hr. subculture of original bile and glucose tube, directly inoculated from original plate colony (grey).

CLINICAL HISTORY:

Unfortunately, the rabbit was pregnant, but the results have an interest outside of the experiment. In twenty-four hours, the rabbit miscarried, but did not appear ill.

DEATH:
Four days.

AUTOPSY:
Peritonitis, metritis, right cornu of uterus filled with dead embryos, beginning to be gangrenous. *Pleuritis, pericarditis.* Gastro-intestinal tract showed only gas. Acute parenchymatous degeneration. Acute splenic tumor.

NOTE: Thus, we have very definite pathology, far beyond mere hemorrhagic lesions.

BACTERIAL FINDINGS:

SMEARS:
Peritoneal fluid: Gram negative rods.
Bile: Gram negative rods.
Chyme: Gram negative rods.

CULTURES:
All rich, alkaline, all foul, and green, but heart blood.
Peritoneal fluid: Gram negative cocci and rods of varying lengths.
Bile: Same.
Fœtus: Same.
Uterus: Same.
Heart: Showed only rods.

RABBIT:
No. 292. Two c.c. of uterus culture.

DEATH:
20 hours.

AUTOPSY:
Hemorrhages of lungs, small intestine, appendix, mesenteric nodes. Parenchymatous degeneration. Splenic tumor.

CULTURES:
Foul.

This experiment demonstrates, clearly, the development of disease as an equation; a point of low resistance; and the bacterial protoplasm at hand.

CANARIES: (2 birds)
Material emusified as before.

SMEARS:
Both specimens, formless.
After twelve hours:
No. 1. Gram negative cocci rods.
No. 2. Gram positive rods.

CULTURES:
(When inoculated with fresh material, growth was feeble, or absent. Therefore, inoculation was made with 12 hour old, foul, material.)

I. On Blood Plates:

Both showed rather coarse, grey, green, slimy, rapidly growing colonies. Later, everywhere overgrown with brown material and spreaders of dry delicate tendrils.

Subcultures:

Gelatin: Digested.
Milk: Digested.
Russel's double sugar: Colon type.
Bile and glucose: Gram negative cocci, and rare rods.

II. Plain Bouillon:

Cloud, foul odor, long and short Gram negative rods.

III. In Glucose Bouillon:

Cloud was less, no odor, more Gram positive.

IV. Bile and Glucose:

At first, Gram negative rods.
48 hrs., nearly all Gram positive cocci.

Subcultures of all, on blood plates, bore close resemblances to one another. Some streaks showed the whitish points where units were formless or at least, poorly outlined and stained.

Rabbit: (Small).

No. 281. 1½ c.c. of 48 hr., plain bouillon culture.

Clinical History:

One and one-half hours vibratory breathing, very weak. In twenty-four hours, improved. Anorexia. Eight days, weaker, nine days, worse.

Death:

Eleven days.

Autopsy:

Abscesses, large, and in great numbers, about every rib, along the spine, at tip of spleen, causing pelvic peritonitis, pleuritis, etc. *Gastrointestinal catarrh.* Urine, albumin positive.

Bacterial Findings:

Smears:

Pus: Negative.
Gall bladder: Negative.
Mesenteric node: Negative.
Chyme: Negative.

Cultures:

Heart blood: Rich, heavy sediment, no odor.
Pus: Rich, heavy sediment, no odor.
Chyme: Rich, heavy sediment, no odor.

FORMS:

As injected and in cultures: Gram negative cocci and quite long rods.

Study of effects of artificial media upon the functions of the bacterial protoplasm, as demonstrated in animals.

METHOD:

Same as in pigeon.

I. PLAIN BOUILLON:

Two c.c. every two hours for seven doses.

CLINICAL HISTORY:

Ill at fourth dose, weak, droopy. In twenty-four hours, leukocytes were, 7,000 at 10:30 A. M., 5,000 at 12:30 P. M., 5,000 at 2:30 P. M. In forty-eight hours leukocytes were 20,000. Sick, anorexia.

DEATH:

Etherized in 48 hours.

AUTOPSY:

Stomach, half full. Duodenum, hemorrhagic, especially about caput. Small gut, congested with gas and mucus, some hemorrhages. Appendix showed exudate and one area of hemorrhage. Heart degenerated. Kidneys, degenerated. Liver, degenerated and abscessed. Bile, thick, yellow, with mucus. Large and acute splenic tumor. Tested twenty-four hours on ice, putrefaction was moderate.

BACTERIAL FINDINGS:

SMEARS:

Heart blood: Rare, regular Gram negative rod.
Bile: Rare, regular Gram negative rod.
Chyme: Rare regular Gram negative rod.
Appendix (hemorrhage): Rare regular Gram negative rod.
Liver: Irregular forms.
Spleen: Irregular Gram positive masses.
Nose: Negative.
Duodenum: Negative.

CULTURES:

Rich, odorless, without pellicles. Forms, Gram negative rods. On blood plates, same type colonies as before injected.

II. BILE AND GLUCOSE:

This culture contained chained cocci and rods, largely Gram positive.

CLINICAL HISTORY:

Showed nothing.

Leukocytes, 24 hours, 17,000.
Leukocytes, 26 hours, 17,000.
Leukocytes, 28 hours, 30,000.
Leukocytes, 48 hours, 34,000.

Anorexia.

DEATH:

Etherized in forty-eight hours.

AUTOPSY:

Stomach, half full. Duodenum congested. Small gut, slightly catarrhal. Mesenteric nodes hyperplastic. *Appendix,* lesion exactly same location as in previous rabbit, but here contained *pus. Heart,* dilated, left auricle *inflamed.* Microscopically, the heart was spotted, congested, showed poor striations and loss of cytoplasm here and there. Muscle was yellow, right ventricle glassy, transparent, hyaline change. *Kidneys,* pale, swollen, hemorrhagic. Microscopically, the kidneys showed hemorrhages, with *glomerulitis.*

Urine: Albumin, positive. Casts, granular, and red blood cell.
Liver: Negative.
Bile: Normal.
Spleen: Slight tumor.
Tested 24 hours on ice, putrefaction, negative.

BACTERIAL FINDINGS:

SMEARS:

Chyme: Gram negative rods.
Appendix: Gram negative rods.
Kidneys: Gram positive cocci.
Spleen: Gram positive cocci.
Heart: Negative.
Nose: Negative.
Duodenum: Negative.

CULTURES:

Heart: Light.
Chyme: Moderate.
Urine: Heavy and of a fearful odor.
All irregular Gram negative forms.

Although from these canaries the bacterial protoplasm was not as flexible as from the pigeon, perhaps due to human associations, with the prevalence of more specialized forms the media previously grown in had considerable determining effects upon function. Two c.c. in bouillon killed in eleven days. Fourteen c.c. of the sediment of the plain bouillon culture was not as deadly as in the case of the pigeon, but more generally effective than the sediment from bile and glucose. Of especial interest, was the typically coccal form of heart and kidney disease following the inoculation with the bile and glucose sediment, and caused by Gram positive coccal chains, formed from Gram negative rod material. Although these rabbits were etherized, and

thereby facts revealed such as would not be possible in any other way, it seems hardly likely that the course following the injection of the bile and glucose sediment would ever have compared with that observed in case No. 1809. It is as an exception, that the result of this experiment can lay claim to a great suggestiveness.

RABBITS: (From the country).
One piece fresh feces shaken in 1 c.c. salt solution.

SMEAR:
Forms, round and long, largely Gram positive.

I. IN PLAIN BOUILLON:
Rich, foul odor, light pellicle. Digested gelatin and milk.

RABBIT:
No. 287. Two c.c. of above culture injected.

CLINICAL HISTORY:
Ill, respirations deep and rapid, at times. Anorexia.
In 24 hours, leukocytes 13,000.
In 48 hours, stronger, leukocytes 14,000.
In 72 hours, eating better, leukocytes 17,000.
In 120 hours, leukocytes 25,000.
In 12 days, normal condition, leukocytes 15,000.

DEATH:
Eighteen days, following cold spell which killed several others.

AUTOPSY:
Emaciated. Brain, oedematous and congested. Duodenal mucosa, at caput, pigmented. Mesentery nodes, pigmented. Kidneys, scarred. Urine: *albumin positive;* casts, negative; red blood cells present.

BACTERIAL FINDINGS:

SMEARS:
Practically negative.

CULTURES:
Heart blood: Very rich.
Bile: Very rich.
Chyme: Very rich.
Kidneys: Slightly putrid.
Forms: Cocci and Gram negative rods, different lengths.
Urine: Cocci, Gram negative rods, different lengths, few primary Gram positive forms.

NOTE:
Bouillon culture of healthy human feces has usually no effects whatever in a rabbit.

METHOD:

As used with specimens of pigeon and canaries to compare the effects of artificial media.

I. IN PLAIN BOUILLON:

ANIMALS:

RABBITS:

No. 271. Two c.c. of sediment of culture containing irregular and regular Gram positive and negative cocci and rods, rich and foul as mentioned, every two hours for seven doses.

CLINICAL HISTORY:

24 hours, quiet.
48 hours, leukocytes 25,000.
96 hours, quiet.
12 days, well.
30 days, well.

DEATH:

Etherized, 37 days.

AUTOPSY:

Well nourished. *Rhinitis.* Heart, scar of an abscess tip of left ventricle, some effects resulting from dilatation. Aorta, a bit thick. Stomach, contents rather moist, normal in amount. Rectum contained gas, but normal feces. Kidneys, rather yellow, right kidney showed scar of an abscess. Urine: *Albumin,* trace, pus, and urates. Spleen was large and firm. Bile, contained some mucus. Liver and lungs were negative. Gastro-intestinal system, strikingly free.

BACTERIAL FINDINGS:

CULTURES:

Chyme: Sterile.
Bile: Sterile.
Heart blood: Few Gram positive cocci.
Urine: Rich, pale green on plate, haemolizing, rather dry.
Cæcum: Much the same.

RABBIT:

No. 293. 1½ c.c. plain bouillon cæcum culture.

DEATH:

12 days.

AUTOPSY:

Abscesses right ventricle of heart. Adrenals congested. *Gastrointestinal catarrh.*

No. 293 was thus such a reaction as was obtainable from the cultures of the normal feces of birds.

II. In Bile and Glucose Bouillon:

Animals:

Rabbits:

No. 272. Two c.c. of culture containing long Gram negative rods, every two hours for seven doses.

Clinical History:

Same course as No. 271.

Death:

Etherized in 37 days.

Autopsy:

Emaciated. Intestine, one loop distended, filled with gas and yellow mucus. Hyperplasia of mesenteric nodes. Heart, atrophied. Otherwise, negative.

Bacterial Findings:

Chyme: Rich in cells and bacteria. Culture, Gram negative rods, foul odor.

Rabbit:

No. 294. 1½ c.c. of above culture.

Result:

Negative.

EXPERIMENTS WHICH INDICATE STATUS OF NORMALCY IN THOSE PRECEDING

Canary Feces:

Bacterial protoplasm, very variable in forms, resembled canary fecal life reported, except that it colored its culture green, thus far, a phenomenon never observed except associated with active, disease-producing power.

Animals:

Rabbits:

No. 279. Two c.c. culture containing Gram negative rods.

Clinical History:

Continuously weak.

Death:

In 60 hours.

Autopsy:

Meningitis, encephalitis, pericarditis, myocarditis, dilatation and pulmonary congestion. Nephritis, (round cell deposits). Urine: Albumin, a trace; indican, a trace. Stomach, aphthous ulcers. Appendicitis (local at tip). Lymph hyperplasia. Splenic tumor.

Bacterial Findings:

Smears:

Spleen: Gram positive, rather small, rods.

Brain: Gram negative, rather small rods.
Bile: Gram negative, rather small, rods.

CULTURES:
Brain: Gram positive rods.
Bile: Cocci.
Heart blood: Regular Gram negative rods.

It was recognized that this was not a normal fecal strain. The activity demonstrated could easily have been due to intestinal disease of the canary upon the basis of its own flora. The canary was perfectly well. It was found that the canary belonged to a patient convalescing from pneumonia. Fortunately, the sputum from this case had been examined at this laboratory, and showed a very variable form of bacterial protoplasm of the same type as that recovered from the canary feces. This pneumonia closely resembled that seen in the parrot epidemic, as did the generalized bacterial flora associated with it. These will be described in the next publication.

RABBIT FECES:

Through a misunderstanding, pieces of feces from the rabbit pen were assumed to be normal and so investigated.

Smears, and plain bouillon cultures showed Gram negative, fairly regular, cocci and rods, varying only in length. This bouillon was rich and foul.

ANIMALS:

RABBITS:

No. 261. 1.9 c.c. of 60 hour plain bouillon culture.

CLINICAL HISTORY:
Immediately ill.
12 hours, improved, leukocytes, 24,000.
21 hours, weak, temperature, 94 degrees.
Diarrhoea continuously.

DEATH:
21½ hours.

AUTOPSY:
Intense gastroenteritis.

At this time, an uninoculated rabbit was found dead in the yard and showed this identical form of gastroenteritis. The feces of this rabbit, sick with this spontaneous disease, evidently comprised the specimen above alluded to and thought to be normal.

SUMMARY AND CONCLUSIONS

The analysis of these experiments upon the fecal saprophytic life in the pigeon, canary, and rabbit, while substantiating the facts observed in the case of the intestinal flora of parrots, pro-

vides also interesting data upon another very important subject. This is, the capacity for and presence of, a fixation between certain characters of fundamentally generalized bacterial protoplasm. The flora from the pigeon, in its susceptibility to the effects of plain bouillon and to the animal tissue reactions, was practically indistinguishable from that derived from the parrot intestines.

The flora from the normal canaries exhibited no such differences in its sensitiveness to the two media, bile-and-glucose bouillon, and plain bouillon. Mild results followed animal inoculation with bacterial protoplasm, which was developed preliminarily in both. In character, that developed in bile and glucose induced disease results similar to those usually due to coccal bacteria, as opposed to the more bacillary type of pathology, following the preparatory growth of the bacterial protoplasm in plain bouillon.

From the intestine of a rabbit, bacterial protoplasm was obtained, which, after development in both bile and glucose bouillon and plain bouillon, had almost no effects in animal tissues.

Although the experiments which can be compared upon this basis are few, nevertheless, the failure of plain bouillon to provide a preliminary, stimulating effect upon the bacterial protoplasm of rabbit intestines, as observable through its reaction in animal tissues, was striking.

The question is likely to be asked, if plain bouillon does not stimulate rabbit intestinal flora to attack rabbit tissues, why expect it to stimulate parrot intestinal flora to attack parrot tissues? This point certainly has not been experimentally demonstrated in this investigation. Either there is some substance in plain bouillon to which the bacterial protoplasm from bird intestines is peculiarly sensitive, or the bacterial protoplasm in rabbit intestines, and therefore in all birds and animals, is always in a state of temporary, special adjustment to the class comprising its natural host. If not as suggested above, it may be that fecal bacterial protoplasm differs fundamentally in its flexibility, that of rabbits being permanently less generalized and therefore less sensitive to new influences.

It is not unlikely that, although the bacterial protoplasm from the intestines of both classes is certainly generalized, there are greater degrees of specialization, differentiating the flora of birds and certain mammals. The first two possibilities mentioned, cannot be excluded.

In the experimental induction of disease-producing power on the part of the bacterial protoplasm of parrots, the tissues of the latter were not employed as they must have been in the natural processes of the epidemic, but adjustments were forced in mammal tissues. Although this may be considered a factor of weakness in the foundations laid for the final argument, if it is true that the flora of parrot intestines is characterized primarily, by absolute flexibility, as opposed to a relative fixation observed as characterizing that of rabbit intestines, then the deductions from our experimental data, as to what took place naturally in the parrots during the epidemic, may not only be interesting, but sound.

CONCLUSIONS

Bacterial protoplasm provides stigmata for differentiation of its tendencies towards a fixation in the mechanism-determining character. This may occur at different points and stabilize various degrees in the activity of a given mechanism. One or more characters may be so fixed. Generalized bacterial protoplasm may be best studied and designated therefore, in terms of the particular one or more characters fixed, the degree of activity at which fixation occurred, and the permanency.

A lack of fixation, a flexibility, sensitiveness, arousability in the mechanism of character demonstration, is of supreme importance in the case of that involved in the activities in and affinities for animal tissues. Such an epidemic, as has been described, depended for its occurrence upon the flexibility in healthy fecal bacterial protoplasm of parrots under experimental conditions, and presumably at the time of the epidemic, flexibility was the rule. In the rabbit, flexibility was not exhibited, and fixation, or stability, was at the high point of activity. In the sporadically diseased parrot No. 3, fixation occurred at a point of high ac-

tivity in the mechanism of disease production, and associated with this, was the fact of the failure to digest nutrient gelatin.

CONCLUSIONS PARTS I–IV

An epidemic of disease among parrots occurred in Wilkes-Barre, Pennsylvania, March 2, 1917. This was followed by an epidemic among human beings. The cause of this epidemic was, originally, the chilling of the parrots, which, by changing the intestinal secretions, affected the generalized bacterial protoplasm in their intestines, so that it assumed powers of disease production in living tissues. Energy became extraordinarily heightened; was concentrated, and associated matter, excessively light, was passed upon the surrounding air to human beings.

The human disease belonged to the class typified by Psittacosis and which has been described by Dieulafoy as resulting from an epidemic in Paris in 1892. The cause of the French epidemic was proved by Nocard in 1892, to be a fixed bacillus.

The name psittacosis has been applied to the local epidemic because it is so fitting in its etymological sense, viz., from parrots, even though its use has been hitherto restricted to such happenings as occurred in France. There, it was evidently a general disease of the human organism, beginning like typhoid fever, but pneumonic involvement was regularly observed. The mortality was four to six times that observed locally. Here, it was evidently a general disease of the human organism, but the mode of inception, the course, the regions of the body involved, all varied. It has been developed in this investigation, that the type of the local epidemic was due to the general flexibility in the mechanism of character-demonstration of the generalized bacterial protoplasm, saprophytic in the parrot intestines. The origin of the epidemic depended upon the arousal to an intense activity of that mechanism capable of creating an affinity for animal tissue. This was accomplished by the intestinal secretions, which were abnormally rich in tissue extracts, as a result of the intestinal irritation which followed a chilling of the birds' bodies.

In both epidemics, the disease came to human beings from parrots. In both, also, the medium was bacterial protoplasm. In France, the bacterial protoplasm as examined by Nocard, exhibited great fixation in many characters, and fixation at a point of a high degree of activity in the character providing an affinity for animal tissues. Here, in Wilkes-Barre, there was practically no fixation of any characters exhibited by the bacterial protoplasm.

Between these two extremes, there are an infinite number of possibilities. Probably, not all parrots will show a saprophytic intestinal flora generalized as herein described. Should those parrots supporting a less generalized flora become involved in an epidemic, there would be developed characteristics peculiar, not only to the epidemic and the transmitted bacterial protoplasm, but even to the clinical disease pictures. And epidemics among parrots supporting a generalized saprophytic intestinal flora, may well provide, in the associated diseased organisms, bacterial protoplasm exhibiting various complexes of character fixation. Such a fixed organism as that described by Nocard, undoubtedly arose upon a basis of some such state of affairs.

Indeed, the infinite possibilities, resulting from the various degrees and combinations in the fixation of characters by generalized bacterial protoplasm, open a great field for study. Psittacosis probably has guises, in number equaling that of the localities in which it has occurred, or where it may again appear.

GLOSSARY

A description of the terms to which a special meaning has been attached in this investigation. Also an explanation of the terms in common parlance, for the use of any lay person who may find time to read this book.

AFFINITY FOR ANIMAL TISSUES: This is a phrase arbitrarily employed to designate not merely the capacity of bacteria to live in animal tissues, but the avidity of bacteria for these tissues. It refers to active attack on the part of the micro-organisms, and carries a sense of the destruction, and replacement of the living tissues of an animal.

AGGLUTINATION: This term refers to a protective property in the serum or liquid portion of the blood of animals. By means of this property the bodies of the units of bacterial protoplasm are caused to adhere to one another, to stick together in clumps. Under such circumstances they are not necessarily dead, but are incapable of performing those functions leading to the production of disease.

AMORPHOUS: Refers to matter which lacks in definiteness of form.

BACILLUS: The term applied to certain forms of bacteria which are rod shaped.

BACILLUS PARA TYPHOSUS: Rod shaped bacterium closely resembling Bacillus typhosus and the specific causes of intestinal diseases simulating typhoid fever.

BACILLUS TYPHOSUS: A rod shaped bacterium closely resembling Bacillus coli communis, and the specific cause of typhoid fever.

BACTERIA: The term applied in general to germs, or the miscroscopic units of that low form of life often involved in the production of infectious diseases.

BACTERIAL PROTOPLASM: The term used in this book to refer in an abstract sense, to that low form of living matter represented by the life processes of bacteria and conveying no expression of specific form or function.

CAPACITY TO LIVE IN ANIMAL TISSUES: Is a phrase frequently employed in this book to replace the term "pathogenic," in order, under certain circumstances, to convey the idea of a state in which bacterial life in animal tissues is restricted and disease production is at a minimum.

CARRIERS: This name is in common use to denote individuals, men, animals, etc., who carry about in their tissues, pathogenic, or disease-producing and virulent bacteria, which are not active in producing disease in the carrier. Either disease had been induced, and the carrier recovered although the bacteria remained virulent, or, because of some special immunity in the carrier, the virulent bacteria, lodging in his tissues, were incapable of producing disease in him but retained their

virulence. In this book, the term is used rather loosely, in reference to experimentally inoculated animals, which following an injection, for a long time exhibited few or no symptoms of disease, until suddenly an acute process arose and proved to be associated with bacterial protoplasm related to that previously injected. But, it has been discovered that these so-called "chronic carriers" do suffer from a mild form of enteritis, during the period when they exhibit no symptoms.

CATALYST: (Catalyzer). Is the term applied to any agent employed which by contact may accelerate or speed up a chemical reaction which is already prone to occur. In this book it is used in rather a restricted sense and theoretically, to designate the property of animal matter, both living, dead, and artificially prepared, of arousing to more intense activity, and accelerating latent chemical activity of the life processes of bacterial protoplasm. The chemical reactions thus intensified are presumed to be supplied with fuel by such material and to have been previously latent, in the presence of "food" materials of another nature. The kinetic energy, accumulated in this process of arousal and intensification, is conceived of as providing that degree of destruction, digestion, and possibly replacement of living animal tissues, by bacterial life. It is this degree of bacterial activity which is intended to be expressed by the use of the phrase "Affinity for animal tissues." This, as has been explained, is employed in contrast to the phrase "Capacity for growth in animal tissues," which refers to an activity only sufficient to provide an adjustment to the environment.

CENTRIFUGABLE: This is used to refer to any solid material suspended in a liquid, and capable of being thrown out of the liquid by centrifugation, thus forming the "sediment."

CENTRIFUGATION: This is the process of spinning, at high speed, in a horizontal position, long narrow containers of liquid in which particles of a solid are suspended. This causes the solid particles, of greater specific gravity than the liquid, to move towards, and become collected at, the closed bottoms of the containers. These solid particles comprise the "Sediment." The liquid from which the particles have been removed, is termed the "Supernatant Fluid."

CHEMOTAXIS: This refers to that influence exerted by chemical substances, living or dead, at any given point, whereby mobile cells such as leukocytes are attracted to or repelled from that point. In this book the term is applied to the influences exerted by the units of bacterial protoplasm in the tissues of an animal, whereby it attracts leukocytes and other inflammatory elements by which the body builds up its defense against the invader. For the sake of argument this may be considered as the vis-a-vis of that reaction activated in the bacterial protoplasm by the catalysts composed of the body tissues.

COCCI: Refers to all bacteria which are round in form.

COLONIES: Are groups or clumps or masses of bacteria formed by the development of great numbers of units upon the surfaces of solid media and visible to the naked eye.

DIPHTHEROIDS: Is the name given to rod shaped bacterial forms which are characterized by the concentration of apparently most of the material composing their bodies, at one or more points. The effect is that of a transparent rod, containing after the manner of a shell, one or more dense cocci. The group has derived its name from the bacterium of this type which is the specific cause of diphtheria.

DIPLOCOCCI: Refers to cocci appearing in pairs. The units are usually oval, practically the shortest imaginable rod forms. They frequently appear fused.

GENERALIZED: Is a term used, principally, in conjunction with the phrase "bacterial protoplasm," explained above. It is intended to embrace in its meaning that the life processes of bacterial protoplasm, so qualified, do not exhibit any necessary relationships, or fixed associations between any of the characters of form or function by which it is recognized. That is to say, as living material is represented to-day by certain forms and functions; to-morrow, under different environmental influences, entirely different characters may be exhibited. And further, although certain characters may usually appear in a given environment, at any time they may not do so because of some hidden factor.

GRAM: This is the name applied to a method of staining. Gram was the man who discovered that by the use of certain dyes and chemicals, bacteria could be divided into two great groups, accordingly as they did or did not take up, and become colored by, a certain stain. In general, staphylococci, streptococci and pneumococci become stained, and are therefore Gram positive, while the members of the typhoid-colon group, do not become stained, and are therefore Gram negative. In this book, this method of staining is principally employed to differentiate the initial stages from those that are terminal, in the metamorphosis already explained. The failure to take the stain is characteristic of the typical rod in the typical culture; it is therefore termed Gram negative.

HAEMOLYSIS: Refers to the property by which the red blood corpuscles are fractured and the contained red coloring matter is liberated.

MEDIA: Is the term given to artificially prepared substances suited to the growth of bacteria. They may be liquid, such as ordinary soup or bouillon, to which may be added various sugars, milk, etc.; or they may be solid, such as ordinary gelatine, or agar. The latter is prepared by simply adding agar-agar to plain bouillon in order to stiffen it.

METAMORPHOSIS: The term used in this book, according to its usual meaning, to designate a definite change in form undergone by the units of bacterial protoplasm. Stages in the metamorphosis, refers to a period of time, during which the unit forms temporarily show a marked tendency to desist from changing and to resemble one another more closely.

MOTILITY: Is the capacity for active motion. Motion is effected by bacteria by means of flagella, or delicate hair-like appendages, manipu-

lated in a whipping motion, thereby driving the body of the bacterium through the reaches of a liquid.

PARASITE: This is the term applied to those bacteria which are in that state of existence, during which their substance depends upon living material. Broadly speaking they often cause disease in order to live.

PATHOGENESIS: The term in common use, applied to the capacity of bacteria to cause disease.

PNEUMOCOCCI: This is the name of a specific group of micro-organisms, the members of which under certain circumstances, are capable of causing croupous or lobar pneumonia. In shape, these are oval diplococci.

POWER TO PRODUCE DISEASE: This phrase has a more general meaning. It may embrace the meaning of those preceding.

PRIMARY ROD: Refers to the first bacterial form observed in the blood cultures of human beings, ill of the epidemic disease. The word "primary" is intended, principally, as an indication of the initial stage in a metamorphosis.

PUTREFACTION: This is the name of the process involved in the digestion, and ultimate dissolution, of dead animal matter through the action of ferments, principally from bacteria, which are for the most part in a state of saprophytic existence. The process is attended by the evolution of characteristic and foul odors.

ROD: The term frequently employed in this book, and used in a more general sense than "bacillus."

SAPROPHYTE: The term applied to those bacteria which are in that state of existence during which their subsistence depends upon dead material. They are unable under ordinary circumstances to cause disease.

STAPHYLOCOCCI: A term used in a more restricted sense to designate cocci which are grouped in bunches and which on the surface of a solid medium, grow in masses or colonies, smooth, white, and coarse in appearance.

STREPTOCOCCI: The name applied to cocci which form chains and grow on the surface of a solid medium in masses or colonies; which are mostly transparent, and very small and fine.

TYPHOID COLON GROUP: The name given to that group of rod shaped bacteria which includes those just mentioned; rods causing the various types of dysentery, and others of less importance.

TYPICAL ADJUSTMENT: This refers to the living chemical reactions, which are presumed to underlie the more or less transient but inherent property of the bacterial protoplasm for growth in any given substance. These chemical reactions, and therefore the functions by which they are demonstrated, are considered to be typically adjusted in a culture when that culture is capable of producing a typical reaction in animals.

TYPICAL CULTURE: Refers to the suspension of typical, Gram negative rod units, developing and living in ordinary meat-substance bouillon.

TYPICAL GRAM NEGATIVE ROD: The special term used to designate the form assumed by the units of the generalized bacterial protoplasm, involved in the epidemic investigated in this book, when under the influence of a common environment, namely that provided by artificial media composed of meat substances. This form resembled the members of the typhoid-colon group.

TYPICAL REACTION: This refers to the reaction in a rabbit which follows the intravenous injection of about a half-teaspoonful of a typical culture, completely developed, of typical Gram negative rods. The reaction is characterized by death, within, at most, 24 hours, the autopsy revealing hemorrhages indiscriminately located.

VIRULENCE: This is the term used to designate the degree to which bacteria are able to cause disease.

WIDAL: The name given to that method of testing a serum, to discover if the property of agglutinating a certain type of bacterium be present. Widal is the name of the man who invented this method. In the first instance he employed this means of detecting the presence of the typhoid bacillus in man, through *its* tendency to stimulate in the serum the development of such a property, specifically directed against itself.

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APPENDIX

Andrew Todd McClintock, as related in the biographical sketch by his friend, Dorrance Reynolds, was long the victim of chronic intestinal invalidism. The symptoms were obscure. Finally, his own diagnosis of intermittent obstruction in the large bowel was corroborated by X-ray study and the operation, which he himself had some time before considered necessary, was done.

As a matter of record, the surgical notes together with copies of the radiogram and a model of the intraabdominal lesion are appended.

Finally there is appended an abstract of the Deed of gift relating to the Andrew Todd McClintock Memorial.

ABSTRACT OF HISTORY OF OPERATION UPON ANDREW TODD McCLINTOCK, FOR RELIEF OF COLONIC OBSTRUCTION.

IDYLEASE INN, NEWFOUNDLAND, NEW JERSEY
JULY 15, 1922

Left mesiad incision. The hand could not be introduced except into the left lower quadrant, the adhesions being distributed over the remaining three quadrants. When these adhesions, which were mostly between the parietal peritoneum and omentum, were separated, another and denser set, evidently long antedating the above, were shown to exist in the right quadrants. They extended from the liver, first and second portions of the duodenum and the gall-bladder to the colon. They appeared to be fasciculi from the omentum, probably congenital. In addition there was a dense pericolic membrane, frankly of inflammatory origin and heavily contractured, which obscured and completely buried the cecum and the first portion of the ascending colon. This was separated with the utmost difficulty, and three-quarters of an hour was consumed in liberating the colon so that a study could be made. On the left side, omental bands passed down across the splenic flexure, causing, in part, the peculiar condition shown on the enema X-ray. When the omental bands were divided, an extraordinary condition was revealed at the splenic flexure. Just before reaching the spleen, to which it was tightly attached, the transverse colon made a complete circle about eight or ten centimeters in diameter. It was densely festooned with a pericolic membrane which presented very little evidence of circulation, probably due to contracture. Every effort was made to preserve this specimen intact, but of necessity many of the bands were torn or cut in making the necessary and difficult dissection to remove it. There was serious bleeding when it was being separated from the spleen, but this fortunately ceased under a compression suture. The descending colon was narrow and thickened and there appeared to be no sigmoid. It looked as though all of the slack both from the sigmoid and from the transverse colon had been gradually taken up into this coiled mass at the splenic flexure. The terminal ileum contained the usual amount of dark fluid material so apt to be present in cases of partial colonic obstruction, but it was free and its mesentery contained no glands. There were many large glands on the right side beneath the adhesions. Usual total colectomy followed by heterostaltic lateral anastomosis.

On attempting to wash out this specimen with 10% formalin immediately after the operation, it was found that the entire colon, together with about ten centimeters of ileum, could be filled with fluid and held vertically

without any fluid running through the coiled area, and it was only on manipulating this that a small stream could be induced to flow past it. This is conclusive proof of the existence of an obstruction which undoubtedly became complete under conditions of torsion or inflammation and which fully accounted for the patient's clinical symptoms.*

SECOND OPERATION, FOR RELIEF OF DUODENO-JEJUNAL OBSTRUCTION.

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Abdomen opened on right side. The small bowel at about its center was attached so densely to the site of the previous operation wound that it was cut away with great difficulty. Here the bowel was greatly dilated and obstruction had been nearly complete. At another point a fistula was found, a spontaneous opening having occurred between the small gut and the sigmoid. At a point in the ileum there was another well defined obstruction. These bands were all freed and the anastomosis was found to be patent. It readily admitted three fingers. It was therefore necessary only to relieve the adhesions and close the abdomen. This was done. The patient survived eighteen hours.

JOHN WILLIAM DRAPER.

*This entire specimen has been preserved.—EDITOR.

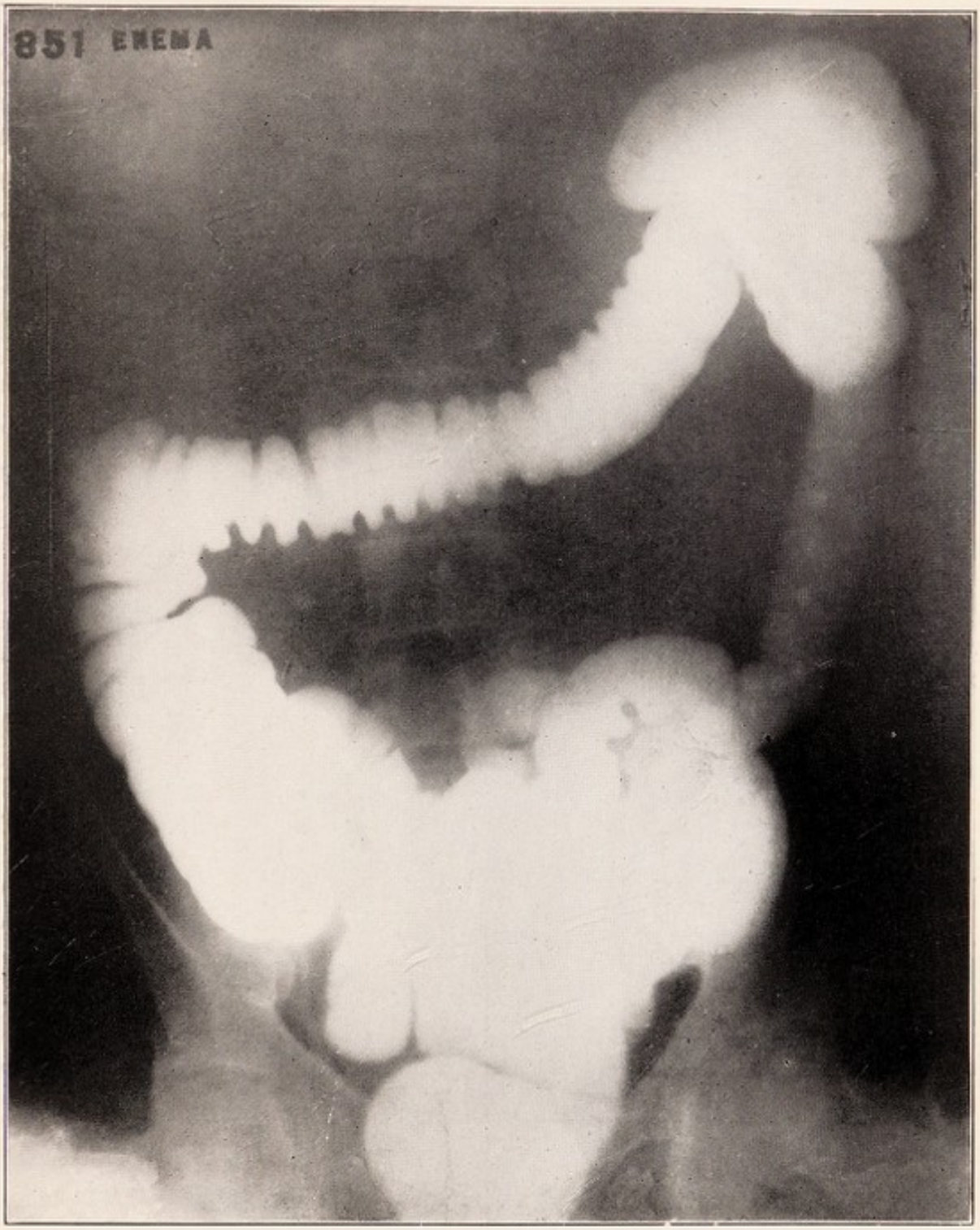

FIGURE 1. X-ray study of the colon of Andrew Todd McClintock made prior to operation for colectomy. The obstructive lesion is seen in the upper right hand corner at the splenic flexure.

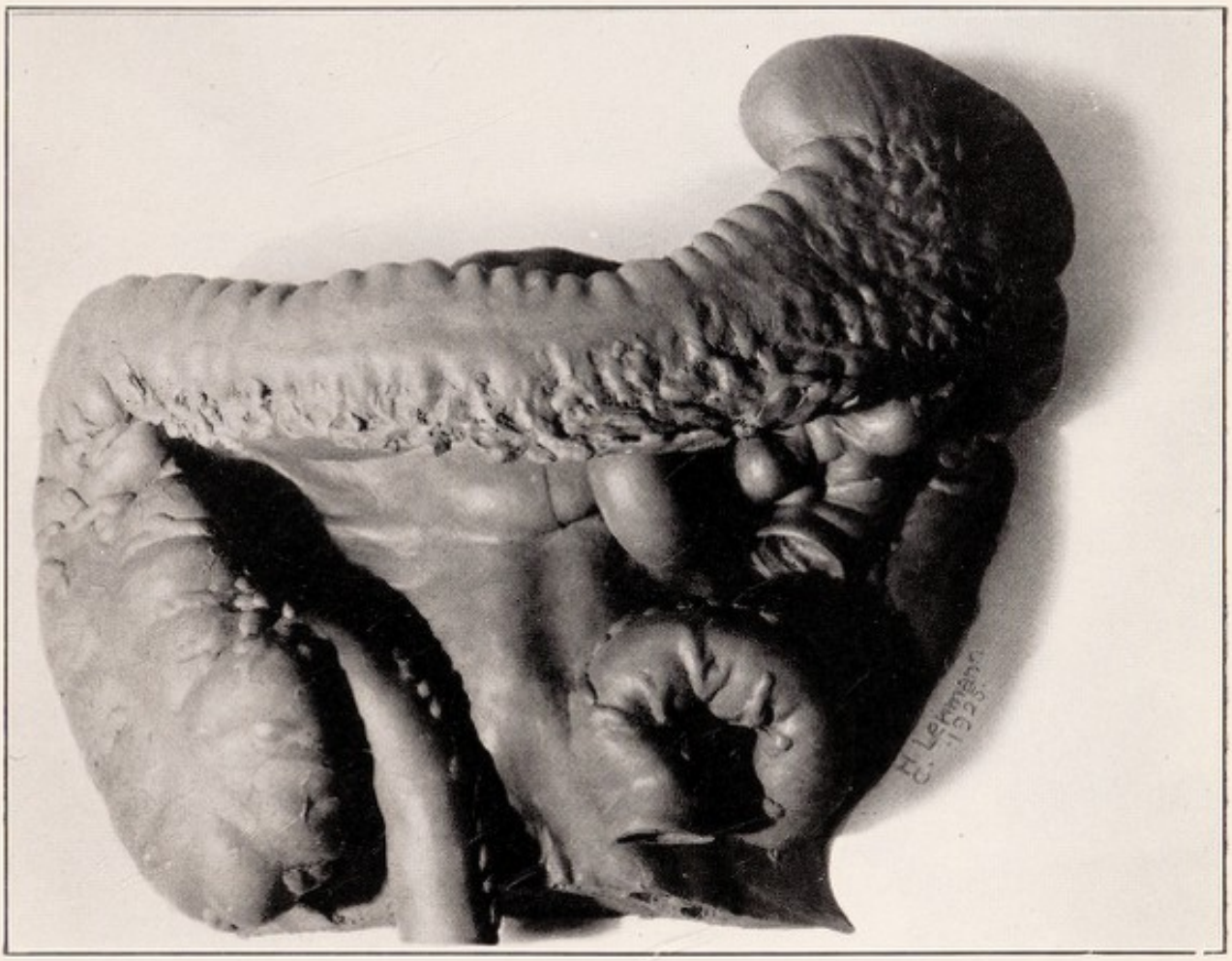

FIGURE 2. Photograph of wax model showing conditions found in the abdomen of Andrew Todd McClintock. The terminal ileum, the right and left portions of the colon and the obstruction at the splenic flexure are seen. On the right side, between the transverse colon and the sigmoid, are the adherent and obstructed coils of the jejunum.

THE ANDREW TODD McCLINTOCK MEMORIAL

FOR THE STUDY OF

DISEASES OF THE ALIMENTARY CANAL

The terms and conditions of the gift by which Isabel Cairns McClintock established this Foundation in memory of her late husband are in abstract* as follows:

Whereas, the late Andrew Todd McClintock, a physician of Wilkes-Barre, Pennsylvania, during a period of several years prior to his death made exhaustive research into the causes of diseases pertaining to the gastrointestinal tract, and it was his intention to devote the remainder of his life, and his fortune, if necessary, to the scientific study of the afore-mentioned diseases, and

Whereas, said Dr. McClintock became a victim and finally died as a result of one of these diseases, and

Whereas, the Grantor is desirous of carrying on the work in which her husband was so deeply interested by establishing in memory of him and of his devotion to his work and in appreciation of his fellowship, a trust fund to be known as "THE ANDREW TODD McCLINTOCK MEMORIAL," the income from which is to be used at first in part and eventually in its entirety to encourage, promote, conduct and maintain bacteriological and other researches into the nature and causes and treatment of primarily gastrointestinal diseases and to make available to physicians, investigators and others the work and findings of these investigations, either by publications issued as the The Andrew Todd McClintock publications, or by such other means as may from time to time seem most efficient and practical.

Now, therefore, in consideration of mutual covenants and agreements, the Grantor hereby conveys unto the Trustee, (The Wyoming National Bank of Wilkes-Barre), all right and title in the fortune bequeathed to her by her late husband, Andrew Todd McClintock, directing the said Trustee to establish a Memorial, devising income and finally the entire principal to the establishment of laboratory and other agencies for the continuation and perpetuation of the researches in the diseases of the alimentary canal, as begun by her late husband, Andrew Todd McClintock.

*Abstracted from the Deed of Gift on file in the County Court House, Wilkes-Barre, Pennsylvania.

It seems entirely fitting, therefore, that Doctor McClintock's manuscript, "Pleomorphism in Bacterial Protoplasm, a study in Psittascosis," comprising this book, should constitute the first Volume of the Archives of this Foundation.

Endowments or funds of this general character have been given by several public spirited citizens for the furtherance of both general and specialized research in medicine, but so far as is known to the Director, this Foundation is the first for the study of diseases of the alimentary canal. Since it is now widely recognized that the majority of general diseases, formerly supposed to be separate entities, are in reality often merely terminal manifestations of primary gastrointestinal lesions and that these in turn are often curable in their incipiency, the far-reaching importance of this specialized gift becomes evident.

JOHN WILLIAM DRAPER, *Director.*

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