Thesis

on

"Some Recent Advances, in Pathological Research."

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The history of medicine, viewed in its twofold aspect as a science and an art, presents itself to the retrospect of those who occupy the vantage-ground of the present, not so much in the mode of a continuous evolution, uniformly progressive, like the symmetrical unfolding of a leaf bud, but as a grouping, rather, of a series of terraced plateaux, apparently disconnected with one another, because the winding upward pathway can be often traced with difficulty, but commanding, with each successive ascent, an ever-widening area, in the landscape, of scientific research. Just as in the wider, and more general, history of mankind, so, in the more restricted history of medical discovery, there have been outstanding events, often trivial enough, in their origin, but epoch-making in their character, and consequences, and those too, generally, associated, inseparably, with the names of observers, equally famous, that serve as landmarks, in the line of progress, that emerges
emerges from the mists, records of obscure empiricism, in the past, and is projected into the somewhat more scientifically accurate sphere of the present. There has thus been a rising, so to speak, upon stepping-stones of dead theories to higher facts. Subjected in the rigid crucible of the scientific method, to the fierce heat of protracted criticism, the hypothetical became in time the probable, and that, in turn, the actual, until what there was of untruth and error, in theory and practice, was either relegated in limbo patrum, or gradually eliminated, as though exposed to a process of destructive distillation. Certain ideas have dominated the field of scientific interest at certain periods of time, and round these, as a sort of regnum protisticum, or battle-ground, has raged the intellectual warfare of conflicting opinions, until, from the smoke and din of controversy, have been borne at length, in triumph, the trophies of established truth, while round the brows of the victors are now entwined, as the meed of their genius, symbolic laurel wreaths by a discriminating and appreciative posterity.
But however fascinating might be the study, in detail, of any one of those past epochs of medical discovery that formed a new departure in the science of the time, yet such a study might prove somewhat too discursive, and, hence, it is my present purpose, in order, as far as may be, to avoid difficulties, and lack of definiteness, to limit my range of subject, and seek to deal with one or two more strictly modern expansions in the field of medical research. I shall associate these with the names and, where desirable or necessary, with a few facts in the life-history of those who conceived, and instituted them, and with allusion to analogous facts, and methods, in what may be termed comparative research, so far as that may serve to cast light upon the platform of our present state of knowledge.

Somewhere about the year 1838, (to go no further back) the microscope was so far perfected as to prove a reliable instrument in the observation of histological facts. By its use Schleiden founded what may be termed the cell-theory, restricting it, however, to the vegetable kingdom, and defining a cell, as a
pelicle with semi-fluid contents. So there was added a third element by Schwann, namely, the nucleus, which he considered to be formed by the semi-fluid substance, within the cell. His words are these: "The cell, when once formed, continues to grow by its own individual powers, but at the same time, directed by the influence of the entire organism, in such a manner as the whole requires in its design. This is the fundamental phenomenon of all animal and vegetable life."

In the year 1841, Haeckel followed up these observations, by pointing out the method in which cells multiply, by fission, and quotation, and, in the same year, Martin Berry showed the reproduction of cells by division of the parent nucleus. His proposition was confirmed by Goodier, who stated that the secretion, within a primitive cell, is always situated between the nucleus and the cell-wall, and would appear to be a product of the nucleus. The comparative unimportance of the cell-wall was demonstrated by Nægeli in 1845, and certain non-nucleated cells were described by Max Schultze, in 1854, so that doubts were thrown on the universality of the nucleus, while, in 1857, Leydig proved definitely that the cell-wall is non-essential. But probably the
the greatest stimulus was given to the systematic study of histology, when, in 1856, Lord S. G. Osborne discovered the process of carmine-dying of vegetable and animal tissues, which was so successfully adopted and modified, by Lewin Reale, and others, that the theory of biplasm, or protoplasm, superseded the previous cellular theory of Schleiden and Schwann. Näckel says, "The protoplasm or sarcode theory,—that is, that this albuminous material is the original, active, substratum of all vital phenomena,—may, perhaps, be considered one of the greatest achievements of modern biology, and one of the richest in results."

The morphological unit, then, by whose aggregation and co-ordination, the animal organism is constructed, was discovered to be a simple mass of undifferentiated protoplasm, apparently structureless, according to our present methods of observation and experiment. Thus protoplasm, in its arrangement and modifications, in normal, healthy, animal tissues and organs, became the subject of study, giving an impulse to the department of Physiology, and where these were abnormally modified, by the various forms and conditions of disease, to that of Pathology. While, in the former case, there remains comparatively
comparatively little to be done, the ground having been so carefully, and systematically, surveyed by competent observers; in the latter case, from the preconceived notions that pathological processes assume, much work, in the direction of observation, classification, and induction, still awaits the explorer of the hidden secrets of Nature. A parallel is thus presented, to a region of country that is first seen over, by a trigonometrical Survey, whose duty it is to map out the contour of each district; following upon their footsteps, next come the Geological Survey, who, adopting, as their base of operations, the maps, and charts, of the former, proceed to fill in the prevailing formations, and mineralogical characteristics, of each successive district. But perhaps, it might be held that the above illustration might be applied, more aptly, to the relation of Anatomy to Physiology, than, of the latter to Pathology, whose votaries might rather be considered to resemble a body of engineers, engaged in the study of the etiology of earthquakes, landslips, water-spouts, and other meteorological abnormalities.

Such is a brief outline, necessarily in perfect, of the trend of comparatively recent biological
biological research, and such a sketch, dealing with the elementary, and ultimate, structure of the human organism, seemed to be logically required, since the relation which it perhaps most frequently bears to the contagium, or materia morbi, of many (if not most) pathological affections, is that of a field to the seed, whether by accident, or intention, sown therein. Such a relation might be illustrated by instancing the case of any one of the so-called exanthematosus disorders, as, for example, that of scarlatina, in which the infective element, in the form of spores, or seeds, finds an entrance into the evil of the organism, where, a period of incubation having elapsed, such seeds, or germs, undergo a process of germination, and pass through the stages, successively, of growth, fertilization, and efflorescence, precisely analogous to the corresponding growth, and reproduction, of wheat, in a wheatsfield. Thus, from an exhaustive analysis of the quality, and disposition, of the molecular elements that constitute the evil, namely, the human organism, men's minds passed naturally, to consider the various forms of pathogenic seed, liable to be deposited within it, with the view of determining their causal
causal, and their merely concurrent, relationship to certain, morbid, clinical, manifestations, previously observed, and described.

As a concrete illustration of these general remarks, I shall briefly indicate the history of one of the most successful, as well as important, of recent pathological investigations. I refer to the researches of Koch into the etiology of tubercular disease. Koch first made himself known by the penetration, skill, and thoroughness, of his researches, on the contagium of spleenic fever. By a process of infective inoculation, he traced the virus of the above grave disease, through all its stages of development, and through its various modes of action. This masterly investigation caused the young physician to be transferred from a quiet country practice, in the vicinity of Brandan, to occupy the position of Government Adviser, in the "Imperial Health Department of Berlin." From this department has lately issued a most important series of investigations, on the etiology of infective disorders. Koch undertook the study of one, which, as regards mortality, heads the list. If the seriousness of a malady be measured by the number of its victims, then, the most dreaded scourges
Scourges that have hitherto ravaged the world, plague and cholera included, must stand far behind tuberculosis. Koch makes the startling statement that one seventh of the deaths of the human race, are due to tubercular disease, while fully one third of those who die in active middle age, succumb to the same cause. Prior to the time of Koch, it had been placed beyond doubt, that the disease was communicable, but the aim of the sterile physician, has been to determine the precise character of the contagium, which previous experiments on inoculation, and inhalation, had proved to be capable of indefinite transfer, and reproduction. He subjected the affected organs of a number of men, and animals, to microscopic examination, and found, in all cases, the tubercles infected with a minute red-shaped parasite, which, by means of a special dye, he was able to differentiate from the surrounding tissue. It was in the highest degree impressive, to observe, in the centre of the tubercle-cell the minute organism that had created it. Transferring, directly, by inoculation, the tuberculous matter, from diseased animals, healthy ones, Koch, in most instances, reproduced the disease. To meet the objection that it was not the parasite itself, but some virus in which it was imbedded, in the
discussed organ, that was the real contagium, he
cultivated his bacilli, artificially, for considerable
periods of time, and through many successive gener-
ations, of bacillus life. With a specie of matter, for
example, from a tuberculous, human, lung, he infused
a substance, prepared after much trial by himself,
with the view of affording nutriment to the parasite.
Were he permitted it to grow, and multiply. From this
new generation, he took a minute sample, and infused
therewith, fresh nutritive matter, thus producing
another brood. Generation after generation of bacilli
were developed, in this way, without the intervention
of disease. At the end of the process, which some-
times embraced successive cultivations, extending over
half a year, the purified, or modified, bacilli were
introduced into the circulation of healthy animals
of various kinds. In every case, inoculation was
followed by the growth, and multiplication, of the
micro-organism, and the production of a measure
of the original disease.

The following is a brief resume of
a series of his experiments. Of six guinea-pigs,
all in good health, four were inoculated with
bacilli, derived, originally, from a human lung that
had produced, in sixty-four days, five successive generations,
Two of the six animals were not infected. In every one of the infected cases, the guinea-pig thickened, and lost flesh. After thirty-two days one of them died, and after thirty-five days, the remaining five were killed, and examined. In the guinea-pig that died, and in the three remaining infected ones, strongly pronounced tubercular disease had set in. Spleen, liver, and lungs were found, filled with tubercles; while, in the two uninfected animals, no trace of the disease was observed. In a second experiment, six out of eight, guinea-pigs, were inoculated with cultivated bacilli, derived, originally, from the tuberculous lung of a monkey, bred, and reared, for ninety-five days, until eight generations had been produced. Every one of these animals was attacked, while the two uninfected guinea-pigs remained perfectly healthy. Similar experiments were made with cats, rabbits, rats, mice, and other animals, and without exception, it was found, that the injection of the contagium, into the animal system, was followed by decided, and, in most cases, virulent, tubercular disease.

In the cases thus far mentioned, inoculation had been effected in the abdomen. The site of injection, was afterwards changed to the aqueous humour of the eye. Three rabbits received, each, a speck of bacillio-culture, taken from a human lung.
affected with pneumonia, eighty nine days having been devoted to the culture of the organism. The injected rabbits, rapidly lost flesh, and, after twenty-five days, were killed, and examined. The lungs of every one of them were found charged with tubercles. Of three other rabbits, one received an injection of pure blood-serum, in the aqueous humour of the eye, while the other two were infected in a similar way, with the same serum, containing bacilli derived, originally, from a diseased lung, and subjected to ninety-one days' cultivation. After twenty-eight days, the rabbits were killed. The one that had received an injection of pure serum was found perfectly healthy, while the lungs of the two others were found overspread with tubercles.

Other experiments were performed, from which the most useful, and practical, conclusions were drawn. Koch determined the limits of temperature between which the tubercle bacillus can develop, and multiply. The minimum temperature he found to be 86° Fahrenheit, and the maximum 104°. He observed that, unlike the bacillus anthracis, of splenic fever, which can flourish, freely, outside the animal body, in the temperate zone, animal warmth is necessary for the propagation of tubercle bacillus.
In a vast number of cases Koch has examined the matter expectorated from the lungs of persons affected with phthisis, and has found in it, swarms of bacilli, while in matter expectorated from the lungs of those not thus affected, he has seldom or never found the organism. The sputum, in the former cases, was actively contagious, not did dryly destroy its virulence. Guinea-pigs infected with expectorated matter, that had been kept dry for two, four, and eight weeks, respectively, were inflected with tubercular disease, quite as virulent, as that produced by fresh expectoration. Koch was thus able to point out the grave danger of inhaling air, in which particles of the dried spume of consumptive patients, mingled with dust of other kinds.

Having thus far described, in outline, the experiments of Koch, I shall now add some details, descriptive of the tubercle bacillus, and of the method by which Koch obtained his results.

Tuberculosis, including all the varieties of consumption, in man, the "Porteuch" of cattle, and the corresponding disease in the ape, and other animals, is a parasite disease of the internal organs: the parasite is a bacillus, which may be distinguished from all other bacilli, by its remarkable behaviour towards the coloured
coloured reagent mixture. It is not the actively moving tubercle-organism of Klebs, for it is stationary, nor is it the spherical micro-coccus of Schüller, nor of Troussainting, for it is red like. It is not the rod-shaped organism, that Aufricht found (along with two sorts of micro-cocci), for that was only twice as long, as it was broad, whereas, the length of Koch's bacillus exceeds its breadth five times. It is a slender, rod-shaped, motileless body, in length, one-quarter, one-half, or even the whole length, of the diameter of a red blood-corpuscle, with, or without, spores, clinging to it. It approximates, in morphological features, most closely to the bacillus of leprosy, although the latter is a little more slender, as well as more acuminate at the poles.

So much in description of the microbe itself, and now a few details may be given of Koch's method of isolating it. The tuberculous substance was either spread out, upon a cover-glass, dried, and exposed to heat, or a piece of a tuberculous organ was placed in alcohol, and afterwards cut into fine sections. A particular solution of methylene-blue was next made, a weak solution of potash being added. The cover-glass, coated with tuberculous matter (or a section of the organ), was then placed in the solution for
Forty, to twenty-four, hours but half an hour suf-

ficient, if the solution were warmed, in a water-bath, up to 40° C. The cover-glass, which comes out a deep blue, is then treated with a concentrated, aqueous, solution of resorcin, for two or three minutes, and is afterwards washed with distilled water. The blue of the methylene has then, visibly, changed to brown, and, under the microscope, all the amorphous detritus, and fragments of tissue, spread out on the glass, are brown; but the tubercle-bacilli remain blue.

The bacillus of leprosy is the only organism that shares, with the bacillus of tubercle, the remarkable faculty of resisting the brown staining of resorcin, and even the former will take on a certain colouring (derived by Heigert) which the latter resists, so that Koch has, therefore, a means of isolating the tubercle-bacillus, that bears his name, from the most puzzling surroundings. Curiously enough it was in the interior of giant-cells, destined evidently to sustain all their former interest, that the bacilli were oftenest found, and in slowly-progressing cases of tuberculosis, they occurred nowhere, but in the sub-

stance of these, multinuclear, elements. With a 450-

miller immersion lens, the bacilli occupy the field of microscopic vision, as small, blue rods, sometimes one, sometimes
sometimes several, up to twenty, or more, in the midst of a prevailing environment of brown. Not every group of giant-cells, and not every giant-cell in a group, show the bacilli; many were quite free from them, but there were, usually, effete cells, that had previously contained bacilli also, but had somehow eliminated them. The bacilli are probably enclosed by the giant cells, as foreign bodies, just as those, multinuclear, masses are found to enclose vegetable fibres, and the ova of Strongylus. When the tuberculous eruption has passed its maximum intensity, the bacilli become fewer, and may even disappear altogether. They are extremely numerous, in cavities, in the lung; the well-known small, caseous, friable masses, in the interior of cavities, consist almost entirely of bacilli, which are often covered with spores. In the larger kind of cavities, they are mingled with other kinds of bacteria, but under the above-described staining process, the pathogenic bacilli alone, are blue, while all the others are brown. It is right to state, however, that the bacillus of tubercle may, on some occasions, appear independently of the staining procedure.

Klebs, Schüller, Joussaint, and Buyschel, have each cultivated a bacterium from tuberculous matter...
matter, and have indicated tuberculoceiosis by injecting it, but they cultivated the organism in an albuminous fluid, that became turbid, in about three days, after the piece of tuberculous tissue had been deposited in it. Koch, however, found that his bacillus was troublesome to cultivate in fluid, and hence, he did not persevere with that method. He took, instead, some blood plasma of an ox, or sheep, in a test tube, and, after having carefully sterilized it, by repeated exposure to the action of heat, he reduced it, by boiling, to a coagulum, at the same time, inclining the test tube, so that the coagulum might cover a considerable surface. The clot, thus obtained, was a firm, transparent, yellow-coloured, jelly, and upon this, as a nutrient soil, he proposed to cultivate the bacillus, without the interposition of moisture. He now took a piece of tuberculous tissue, from the lung of man, or of an ape (taking all due precaution, in rendering the instruments, and reagents thoroughly disinfected) and washed it several times, in a solution of corrosive sublimate. Then the outer layers were removed, and from the interior was taken a fragment, free from septic bacteria. The plug of cotton-wool was removed, for a few moments, from the test-tube containing the layer of coagulated plasma. The piece of tubercle was rapidly introduced, broken up, and
and scattered over the surface. The plug was then re-placed, and the test-tube deposited in an oven, kept at a uniform temperature of 37°-38°. Usually, after the tenth day, there became visible, to the naked eye, on the dry surface of the evagulum, a number of very minute points, or dry-looking scales, which surrounded the pieces of tubercle, that had been deposited. These were considered to be colonies of the bacillus. After a few weeks' exposure to dry heat, the scales, or zoogloea, ceased to enlarge. The plug was then withdrawn, and they were transferred on glowing platinum wire, to another test-tube similarly prepared. After a like interval, zoogloea again appeared, became confluent and covered more, or less, of the evagulum. And so, from test-tube to test-tube, the culture continued to be transferred, for about twelve times, and through a period of about one-hundred-and-fifty, days. With the evagulating bacillus-cultures, in the form of dry scales-grown, several animals were inoculated, as formerly described, either subcutaneously, into the anterior chamber of the eye, or into a vein. Without a single exception, all the inoculated animals, chiefly rabbits, and guinea-pigs, developed tuberculosis of the lungs, liver, spleen, brain, intestines, and other organs, and the resulting tubercles possessed the structure of the original tubercle.
including giant-cells, which contained specimens of tubercle bacilli. Subsequently, potato, gelatine, and agar-agar, were employed, as nutrient media of cultivation."

Such, in mere set outline, is a study of a materially important contribution to our pathological knowledge. And now let us pass from Germany to France: from "Unter den Linden" to the "Champs Elysées": from the "Imperial Health Department of Berlin", to the "École Normale Supérieure of Paris": from Koch to Pasteur,—a name inferior to none, upon the list of investigators, in the long roll of fame.

There is a little house in the Rue des Tanneurs, at Dôle, in France, upon whose façade may be seen a plate, bearing, in gilt letters, the words "Here was born Louis Pasteur December 27, 1822." It was placed there, in presence of the distinguished scientist, on the occasion of an ovation, that he received, in the town of his birth, and early days. Born of parents in a humble sphere of life, his father having taken to the trade of a tanner, after a term of honourable service in the French army, Pasteur received the priceless benefit of a liberal education. At Besançon he took the degree of "bachelier des lettres", and subsequently passed
fourteenth, and in the following year, fourth, in the scientific examination, at the Ecole Normale, in Paris, after which, he seems to have devoted himself to the special study of chemistry. While engaged in investigating the distinction between crystals, derived from organic, and inorganic, sources respectively, he was induced to direct his attention to the subject of fermentation. He was at that time Dean of the Faculty of Science at Lille. The true brewer's, or yeast-plant, had just been discovered, and a German manufacturer of chemicals had observed the fact, that ordinary calcium-tartrate underwent a process of fermentation on being dissolved, and subjected to a moderate temperature. The original clear solution became turbid, from the presence, and multiplication, of a microscopic organism. Pasteur recognized in this organism, a living ferment, and pointed out that substances, formerly regarded as ferments, were in reality the fabulums, or food, of ferments. He further instituted a series of experiments, by means of which he was able, conclusively, to negative the theory of abiogenesis, or spontaneous generation, and, moreover, traced the deterioration of wine, and beets to the presence of a specific organism, which acted as a ferment, and could be destroyed, at a temperature of
122. Fahrenheit, without injury to the liquor itself.

The chief forms of fermentation, now known to us, are (1) the alcoholic, (2) the lactic, (3) the vinous, (4) the acetic, (5) the ammoniacal, (6) the butyric, and (7) the putrid fermentation. The agents are always microbes, destitute of chlorophyll, and are, generally, anaerobic, obtaining the sustenance they require for growth, and activity, from the oxidised compound in the liquid, in which they grow. The micrococcus of area, and, of the phosphorescence of putrid meat and fish, are, however, aerobic. The ammoniacal, and the putrid fermentations are those of most pathological interest. The former is exemplified, in the action of the micrococcus ureae (and the bacillus ureae), in converting urea into ammonium-carbonate. The micrococcus ureae appears, when cultivated, in the form of minute, spherical, cells, either separate, or united in diploceles, or in tetrads, or chains. They do not fluidify gelatine.

The development of the microbe is not interfered with by the alkalinity of the fluid, and it can continue, when the proportion of ammonium-carbonate is as much as 13 per cent. The most favourable temperature for its development is from 30° to 33°C. The bacillus ureae is short, with rounded poles, about twice as long, as broad. Growth takes place, only on the surface, and,
like the micrococcus, its action, as is now well known, is to decompose urea, into ammonia-carbonate, with absorption of water, thus:*

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\text{CO} \left( \frac{\text{NH}_3}{\text{NH}_2} \right) + 2 \text{H}_2\text{O} = (\text{NH}_4)_2\text{CO}_3.
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Pasteur's attention was next directed to the investigation of pébrine, the muscardine disease among silk-worms, that broke out in 1849, and spread so rapidly that it threatened to destroy the whole silk-industry in France. Induced, by the representation of his former instructor, Dumas, Pasteur started for Alsace, with the view of discovering some means of combating the epidemic (or ophelministic) and had not been there many hours, when he was able to demonstrate, to some members of the Agricultural Commission, certain minute bodies, the hydropsia bassiana, of our present nomenclature, in the ovum, the larva, and the imago, or moth; in the latter, the development being so distinct as to render recognition, a matter of no difficulty. From healthy moths, healthy eggs were sure to spring from healthy eggs, healthy silk-worms, and from these fine cocoons, so that the problem, of the restoration, to France, of its silk-industry, reduced itself to the separation of the healthy, from the unhealthy moths, the destruction of the latter, and the exclusive employment of the eggs, of the former. The simple method
method, by which Pasteur insured the cultivator against a recurrence of the disease, is now universally adopted. As soon as the ova are deposited, the moth is crushed in a mortar, and mixed with a little water; the mixture is examined, under the microscope, and should a germ of the botrytis bassiana be found, the ova are immediately destroyed, with everything belonging to them. Laboratories are met with, everywhere, at the time of the cultivation of silk-worms, in which operators are steadily employed, under strict supervision, in pounding and examining the moths, setting aside healthy ova, and destroying the rest.

Brief reference need, here, only be made, to Pasteur's recent, successful investigation into the subject of "charbon," or soot choler, an outbreak of disease, that decimated the folks of Paris, and which Pasteur proved to be due to a specific microbe, that he was able to isolate, and attenuate, through a series of cultivations, in chicken-broth.

Resuming his researches, into living forms, Pasteur drew nearer to a knowledge of the causes of contagious diseases. The publishing of certain of these researches, on fermentation, had the effect of stimulating, yet another worker, in the scientific field.
means of suggesting to the, no less philosophic, mind of Lister, a fruitful process of inductive reasoning, and experiment, that culminated in the elaboration of what is now known, as Lister's method, of anti-septic surgery—a method that has produced a revolution in the department of modern operative surgery.

The ancient theory of parasites and living contagia, was revived, and placed on a scientific basis, largely owing to Pasteur's researches regarding fermentation. Taking up the investigation of Lavoisier, Kaper, Koch, Greenfield, and others, Pasteur approached the subject of anthrax, or splenic fever, which had long eluded research. Since most diseases are, in their nature, non-recurrent why should there not, he argued, be found, for each of them, a preventive disease, which, being similar, but less virulent, should act, as a safe-guard, against the more virulent type. Pasteur found that lengthened contact with atmospheric air, weakened the contagium, or microbes: they are living organisms, demanding certain elements of life, for their growth, and activity, and they may be assimilated the elements, essential to their growth within the organism, as to prevent the cultivation of
of a second crop. An attenuated cultivation may serve to exhaust the soil, so that a more concentrated virus, may be introduced into the system, with negative results. This is the rationale of Jenner's famous discovery, but he applied it, empirically only, in the case of variola, leaving the field to be explored by Pasteur, who, with growing scientific instinct, grasped the principle of the discovery, and employed it, in other pathological processes, with the most fruitful, and suggestive, practical results.

It was in 1881, that Pasteur communicated to the Académie des Sciences his discovery that, by repeated cultivation of pathogenic microbes, much of their virulence could be modified, destroyed, that, in fact, they might be rendered benign; and, though much applause followed his exposition, some of his colleagues seemed inclined to suggest that there was romance in the theory. The President of the Society of Agriculture at Melun, invited Pasteur, however, to make a public experiment, of epidemic fever, protective inoculation. The invitation was accepted, and, on May 5th, 1881, a large concourse of interested spectators, assembled to witness the result. A flock of sheep was divided into
into two groups; those, in one, were inoculated with the prophylactic; those, in the other, were left alone. A number of cows were similarly treated. After fourteen days, all the animals were inoculated, with a virulent type of bacillus anthracis, and two days subsequently, twenty-one sheep, that had not been protected, were dead, and the remaining ones of the same group, were dying. Those previously protected, on the other hand, appeared, hardly, to have suffered. Great enthusiasm followed these results, and although every new discovery is destined to meet with hostile criticism, the significant fact remains, that Pasteur is overwhelmed with applicants for supplies of protective fluid.*

Passing from the consideration of anthrax, I shall next sketch, in outline, the most recent, and in many respects, the most important, of Pasteur's researches, upon a subject, that still engages his attention, namely, that of rabies, or, as it is popularly termed, from one of its most prominent symptoms, hydrophobia. In the preventive treatment of this disease, Pasteur has devoted the past six years of his life. His attention was first directed to it, in the year 1880, when a child, who had been bitten, some time previously, by a rabid dog, and in whom, the usual symptoms of the
the malady were, rapidly, developing themselves, admitted into the Bourgeois Hospital, under the care of M. Laennec's. Pasteur obtained saliva from this child, and injected it under the skin of a rabbit. The rabbit died in forty-eight hours, and its blood and saliva, served to inoculate another rabbit, with a similar result, and so, with a whole series. There seemed, however, to be some doubt whether the disease communicated, in this manner, was true rabies. The incubation period was too short, and the symptoms seemed rather those of septicemia. M. Gaëtien had, previously, stated that the disease was incommunicable to rabbits, but his results were proved to be erroneous, by Pasteur, who collected the cerebro-spinal fluid, as being purer, and less exposed to septic contamination, than the buccal saliva.

In a communication addressed to the Académie des Sciences, on March 30th, 1881, Pasteur announced that he was able to transmit the disease readily, by cerebro-spinal fluid, taken from any part of the central nervous system. The method adopted consisted in making a small opening in the skull of a rabbit, and in injecting a few drops of rabies poison, under the membranes of the brain. In the rabbit, the operation, performed under chloroform, was trivial.
one, and the wound even heals under aseptic treatment. Pasteur discovered, moreover, that, not only is the liquor expressed from the brain, and spinal cord, capable of reproducing the disease, but that nerve tissue has the same effect. He was able to convey rabies to a dog or rabbit, by subcutaneous injection of portions taken from the pneumogastric nerve, at its origin from the brain, at its point of exit from the skull, and in its peripheral distribution, respectively. He also established the fact that, when the intra-cranial method of communicating rabies is adopted, the salivary glands, and saliva, are as virulent, as those directly affected by the toxic bite, of a rabid animal.

The next step, in the history of the subject, was the attempt, made by Pasteur, to modify the activity of rabies-poison, in order to eke out utilisation, when diluted, as a protective vaccine. As a result of his experiments, he discovered that the poison has a uniform standard, or constant index, of virulence, among animals of the same species. Thus the rabies of the street-dog is of the same intensity, when inoculated into the dog. It is fatal after the same incubation-period, and runs a similar course. But if the virus be transferred from one genus to another, it is altered in intensity, becoming attenuated.
attenuated, on the one hand, or concentrated, on the other. For, if the disease be transmitted from the dog to the rabbit, its virulence becomes exaggerated, while, conversely, if it be transferred from the dog to the ape, thus ascending, instead of descending, in the scale of animal being, the virus becomes attenuated, and, if communicated successively through a series of monkeys, it becomes more, and more, and more, until, at length, it may lose all pathogenic energy, whatever, whether it be injected subcutaneously or intracranially. Further, if the virus, modified to a certain extent, be re-introduced into the dog, rabbit, or guinea-pig, it retains, for a time, its attenuated properties, though these, by successive circulations, are again lost. The virulence, on the other hand, becomes enhanced when the poison is passed, from rabbit to rabbit, and, when at its maximum intensity, in this animal, it can be re-transmitted to the dog, possessing a virulence more intense than that peculiar to canine rabies.

As the outcome of experiment, the following process has been adopted by Pasteur. The spinal-cord of a rabbit, dead of rabies, is suspended, in a sterilized glass-flask, about one-quarter filled with potash, to render the air as dry as possible.
The flask is plugged with sterilized cotton, and the temperature kept constant. Gradually, the activity of the poison, contained in the spinal-cord diminishes, until, about the twelfth to the fifteenth day of exposure, its power to reproduce the disease has entirely vanished. The decapsulation has destroyed all the activity of the poison, and it is henceforth inert. This procedure, though not rigidly accurate, in its results, is still sufficiently so, to furnish the poison, for practical purposes, in any required state of attenuation.

The method of preparing the prophylactic is as follows. A rabbit is first rendered insensible by chloroform. A small circular piece of bone is removed from the skull, and, with a Pavaz-syringe, a few drops of the cerebro-spinal fluid of a rabbit, previously dead from the disease, are injected beneath the membranes, covering the brain. When the animal recovers from the anaesthesia, it does not appear to be the worse for the operation. It eats, and moves about, as though nothing had happened, until the seventh day, when the paralytic symptoms, characteristic of the disease, in the rabbit, usually manifest themselves. It gradually becomes comatose, and dies in about four days afterwards. Immediately after the animal has...
has died, a portion of the spinal-cord is rapidly excised, placed in the dissecting-flask, and left there, for the necessary length of time. When an animal is about to be protected, a portion of the spinal-cord of such a rabbit, which has been dried for fifteen days, is beat up, with sterilized bouillon, and a few drops of the liquid are injected hypodermically. On the following day, some fluid, from a cord, exposed for fourteen days, is injected, and this is continued, until a cord is reached, that has been exposed, for only one day, after excision.

By this time, the animal has become refractory to the strongest poison, so that, of fifty dogs, thus treated, not one was found to be susceptible of rabies, either by hypodermic, or intra-cranial injection. In contrast to this, fifty unprotected dogs, infected, in various ways, with the same virus, became rabid, in direct ratio with the method of injection.

Recently, M. Pasteur has made a communication to the Académie des Sciences, in which he states, that the prophylactic treatment can be rendered more effective, if the injections are repeated, at shorter intervals, than before, and with virus, obtained from spinal-cords exposed for less time. He formerly commenced the treatment with
with a spinal-cord, that had been exposed, for fifteen
days, and repeated the injection, daily, as above
described, until a cord was made use of, that had
been exposed for only twenty-four hours. He now
finds, from experiments upon dogs, that better
results are obtained, by beginning with a cord of
ten days' exposure, and by administering three in-
jections daily—in the morning, at two o'clock, in
the afternoon, and at nine o'clock, in the evening.
The spinal-cords used, at the first three injections,
had been desiccated for ten, nine, and eight days,
respectively. On the second day of the treatment,
he employs cords of the seventh, sixth, and fifth
days' exposure, and so on, until the series is com-
pleted. On the morning of the fourth day, a
single injection, taken from a cord exposed for
twenty-four hours. This cord may be considered
as, practically, fresh, since the potassium has had the
effect, simply, of drying the surface, while the
centre remains unaltered.

When the first course of treatment is
finished, Pasteur waits for a day, or two, and then
begins a second, in which a single injection, daily,
is deemed sufficient, but one, initiated with a spinal
cord, exposed for only seven days, in the desiccating flask.
At first, Pasteur had not ventured to apply his mode of treatment to the human subject, but, on the 6th of July 1885, two persons presented themselves at the door of his laboratory. One of them was Theodore Vincenot, a merchant of Meissenrode, near Schelstadt, who had been bitten, two days previously, on the arm, by his own dog, which had become rabid. The other was Joseph Meister, a boy of nine years, who had, also, been bitten, by the same dog, but much more severely. In the case of the man, there had been no abrasion, but merely bruising, and slight discoloration of the skin. He was, accordingly, deemed as free from danger. The boy, however, had been attacked by the pangs of the animal, in no less than fourteen different places, and the wounds had been cauterized with phenol, twelve hours after infliction. That the dog was indeed rabid, was assumed from its excited state, and, from the fact that its stomach contained hay, straw, fragments of wood, and other objects, that had come in its way. After consulting with various phlebotomists, and Dr. Lepou, who were of opinion, that the boy must, inevitably, succumb to the disease, Pasteur resolved to put it to trial, the treatment he had found so successful, among the lower animals. The subcutaneous injection of the liquid, obtained
from dedicated spinal-cords was commenced; the first, taken from one that had been exposed for fourteen days. A few drops of all the liquids employed were, at the same time, injected into rabbits, so as to have a series of control experiments, in order to test the potency of the virus. These showed that the first five injections, were incapable of initiating the disease, that is to say, the poison had become inert, while the remainder increased in virulence, according to the relative brevities, in the time of exposure. The injections were continued so far, that the last one was made with liquid, from a spinal-cord, that had been exposed to dry air, for only one day, that is to say, with virus, that caused rabies in the rabbit, after seven days, and in the dog, after from seven to ten days' incubation.

The further history of the case was, that Joseph Meister made a complete recovery, and appeared to be none the worse for his experience, either of the rabid dog, or of the "homoeopathic" treatment. Hence, Pasteur's laboratory, in the Rue Normale, in Paris, was soon besieged, by dog-bitten patients, from all parts of the world, and, instead of being, as heretofore, an establishment for experimenting upon dogs, rabbits, apes, guinea-pigs, other animals, if
it became converted into a hospital, for the reception and treatment of human beings. Pasteur has lately had from one hundred and fifty to two hundred patients, under his care, at a time. The injections are made every morning, hypodermically, on the anterior aspect of the body, by means of a Pasteur syringe. Each time it is used, the syringe is dipped into boiling water by an assistant, who, next, half fills it with the prophylactic solution, and hands it to the operator. A vascular fold is made in the skin; the point of the syringe is carefully introduced, and the solution rapidly injected. Very rarely do any evil effects follow the injection, but this fact, on the other hand, has been urged by the opponents of the method, as an argument against its efficacy. But statistics go far to establish a strong prima-facie case in favour of the process. In a lecture, delivered at the Hygienic Exhibition, in July 1886, M. Puecher gave the statistics of 1335 cases, treated up till the 10th June of the same year. He classified them as follows:

1. Those bitten by a dog proved experimentally, to have suffered from rabies.
2. Those in which the rabies of the dog had been established, by veterinary surgeons, from the symptoms only.
3. Those bitten by a wolf.
the bite of which is even more dangerous, than that of the dog. Of the first group, 10 in 1,000, of the second, 5 in 1,000, and of the third, 14 in 100—had succumbed to the disease, notwithstanding the treatment. But while these statistics, undoubted, are striking, as contrasted with those of hydrophobia, formally, yet, it is by no means conclusively settled, that Pasteur's prophylactic method has all the infallibility, which it claimed for it by its advocates.

Meanwhile, the report of the English Commission, appointed to inquire into the whole question, will be awaited with interest, as such a report will, presumably, have the advantage of being based upon the crucial experiments of experts, in whose hands we must, at present, be content to leave the subject; which, however, must, I think, be regarded by most minds, as, still, sub judice.

And, now, I shall collect yet one more series of investigations, and it is but fitting, that, in this case, the venue should be this country, and the observers, British. Here, there is a difficulty in selecting, where there is so much to choose from. It might prove interesting to give a résumé, for example, of the recent researches of Klein, into the epidemic of scarlatina, at Hendon, as a result of which...
he has been able to obtain, from the blood, in cases of ordinary human scarlatina, a micro-organism identical in its morphological characters with that inhabiting the ulcers of the London cow-disease.

But, if the importance of the subject be considered to dominate the choice, then some reference to the recent instructive researches of Roy. Graham-Brown, and Sherrington on the etiology, and pathology, of Asiatic cholera, may, perhaps, most suitably, close this paper. These researches appeared in the form of a preliminary report, in a recent number of the "Proceedings of the Royal Society of London," on the pathology of cholera Asiatica, as observed in Spain, in 1887.

To quote the full text of the report would exceed the limits of the present paper; hence I must forsake extenuation itself with giving some of the more important and suggestive extracts from the communication.

The following critical reference is made, in passing, to Ferran's method of protective inoculation. An opportunity was afforded the above investigators, of examining, under the microscope, the prophylactic cultures sent, at the request of the Spanish Government, from Ferran's laboratory, to be employed in inoculating troops. These cultures were in possession of
of agents, duly authorized by Ferran. They were found to contain several forms of bacilli, and micrococci, but neither comma bacilli, nor any of the peronosphora-like bodies, described by Ferran, as present in her protective fluid. The administration of such fluids, as were seen, in the hands of Ferran's agents, must expose the recipient victim, to serious peril of septicaemic infection. The theory, moreover, upon which Ferran's system is based, namely, that one attack of cholera, protects against a second, was entirely opposed to the facts observed by the above-mentioned investigators.

The differences of opinion, among pathologists, as to the relation of certain micro-organisms— and, more especially, of a curved bacillus, described by Koch,— to Asiatic cholera, led to these observers being deputed, by the "Association for the Promotion of Research in Medicine," acting in conjunction with the "Royal Society of London," and the "University of Cambridge," to proceed to Spain, in order to make further investigations, on the subject.

In Madrid, autopsies were made upon twenty-five typical cases of cholera. The post-mortem examinations, were made, in the case of many of them, either immediately after death, or else within a
a very few hours of it. The comma bacillus of Koch, was not discovered in the intestinal contents of all the cases. Thin, dried flake of the mucous film, and fluid, contained in the intestine, were examined, stained by methods, that would have shown the bacillus had it been present. Such films, from one or two fatal cholera cases, showed, that the intestinal contents contained larger numbers of comma bacilli than of other parasites. In some of the cases, the comma bacillus, though certainly present, was not the most marked feature, in the preparation, and in certain of these, such bacilli were found, only after prolonged, and careful examination. In many cases of undoubted cholera asiatica, where death occurred, before the reaction stage had set in, comma bacilli were unable to be detected, in any of the films or cultures, prepared from the intestinal contents, taken from different regions, of the alimentary canal. A number of plate-cultivations were made, in gelatine, and agar-agar, the cultivating media, employed, being prepared, in the manner recommended by Koch. The results, however, both of the examinations of films, and of the plate-cultures, from the contents, of the intestines, were found to be opposed to the facts obtained by Koch, though it is possible that
the comma bacillus may prove to be the direct cause of the premonitory diarrhoea, that precedes but is not merely, a milder form of true cholera. Sections of the intestinal wall were next prepared and examined. The tissues, intended for histological examination, were placed in absolute alcohol, while the autopsy was being made, and the method of staining employed, was that with methylgreen-blue, which is well fitted to show the comma bacillus, when it is present, in the tissues. In only a few cases, was the bacillus found, situated in the substance of the mucous membrane, of the intestines and in these cases, it was present, either in, or close to, the tubular glands of Lieberkühn, or else, close to the free surface of the intestinal mucous membrane. The situation of the bacilli was such, as to suggest the belief, that they might have penetrated the epithelium, either after death, or in a few hours preceding it. In the great majority of the cases, careful search was insufficient to discover comma bacilli, in the intestinal mucous membrane, or, indeed, in any of the tissues, or organs examined. It was, therefore, considered unnecessary to make special investigation, into the pathogenic "potency" of the comma bacillus, when administered to the lower animals.
The observers were further unable to convince themselves that the pathogenic effects, described by Koch, Van Ettenmogn, and others, when comma bacilli are given to certain animals, are identical with the phenomena that characterize cholera Asiatica, in man.

With regard to the macro and microscopic, morbid appearances to be found in fatal cholera cases, the results were, completely, in accord with the classical descriptions given by Braun, Rocard, Roux, and Thudier and, as to the comma bacilli, the observations of Braun, supported by those of Klein, in which he was unable to satisfy himself of the causal relation between the comma bacilli of Koch, and Asiatic cholera, were fully in accordance with the experience of the observers. Though specially commissioned to investigate the latter relationship, attention was directed by them to the pathology, as well as to the etiology of cholera.

That the hypersecretion, from the mucous membrane of the stomach, and intestines, in cases of cholera, is not due merely to catarrh was clearly shown, both by the chemical characters of the choleraic defecum, and by the absence, from the mucous membrane, when examined, after death, of the usual pathological changes, occurring in catarrh.
That it is not due to any irritant, present in the contents of the intestine, may be concluded from the absence of necrosis (or ulceration), as well as of any well-marked inflammatory change, in the mucosa. The experiments of Moreau, who found well-marked hypersecretion of the mucous membrane, of the small intestine, resulting from section of its nerves, would appear to have an important bearing upon the pathology of cholera. The chemical characters of the fluid, so abundantly secreted, during the first few hours after section, of the nerves, of the isolated loop, of intestine, are shown, by Kühlne, to be, practically, identical with those of the alvine dejecta, of cholera cases. There can be little doubt, that hypersecretion from the intestine, with the more or less complete arrest of absorptive power, by the mucosa, is the cause of the tarry thickening of the blood, and the dryness of the tissues in cholera. Its pathology, therefore, is centred in the question as to what is the true cause, of this intestinal secretion, and diminished, and devitalized, power of absorption. By a series of experiments, the results of Moreau, were corroborated, namely, that the characters of the paralytic secretion, following intestinal nerve-section, are, practically, identical with.
three of the intestinal contents, in fatal cases of cholera. As to whether the epithelium, covering the free surface of the mucosa, is, simply, less firmly attached, than in the normal intestine, or, becomes actually detached, before death, observation seemed to point towards the former alternative.

The authors are of opinion that the pathology of cholera, can be best explained, by the hypothesis of some cause, acting on the gland, or nerves, of the intestine, and producing effects, similar to those, that result from paralysis, of the intestinal nerves. This conclusion makes it probable, that the cause of the disease, if discoverable, by the microscope, may be found, in the mucosa, itself, in the nerves, distributed in the mesentery, or in the ganglia, from which those nerves proceed.

In the course of observation, that followed their return, from Spain, the above writers were fortunate in discovering, in sections of the intestinal wall, certain minute granules, varying in size, but, for the most part, smaller than the nuclei of the surrounding cells. These granules had invariably one, or more, processes, attached to them. The granules could be stained with fuchsin, methylene blue, and other aniline dyes, but the processes resisted staining.
staining. In a specimen from a kidney, several of these granules were seen, lying in the lymph space, outside the membrana propria, of a convoluted tubule, and proceeding from the granules, was distinctly visible, the arrangement of filaments, seen in other preparations. The inference was very strongly suggested, that these were a form of vegetable parasite. The specimen was submitted to specialists, in that department, who stated that such forms were characteristic of the Natural order, Chytridiaceae, an order, that includes many rapidly growing, and virulent, parasites, of vegetables. There was some difficulty, in staining the parasite satisfactorily, but finally, Löffler's method, with methylene-blue was adopted—a method, that had not hitherto, been employed, in fatal cholera cases, hence the failure, of previous, competent, observers, to discover the micro-organism. During the autopsy, pieces of the intestinal wall, and of the other organs, were placed in absolute alcohol, and all the fluids, through which the tissues, and sections, required to be passed, for the purposes of cutting, staining, mounting, etc., were carefully sterilised, by boiling, and protected from the invasion of vegetable organisms, by the addition of thymol. The micro-organism, found in fatal cholera cases,
cases is described, as follows. It is composed of terminal, or nodal, swellings, connected by filaments, varying greatly in thickness, and showing no differentiation, into component cells. Stained with mosty bone-blue, the granules, and filaments, take on the colouring matter equally, although, when treated with fuchsine, followed by dilute nitric acid, the filaments become decolorized, before the granules. Certain minute bodies, in the mycelium, and granules, suggest the presence of spores, but the development of zoosporangia, from the terminal granules, such as have been described, in some forms of the Chytridiaceae, were not to be detected.

In every one, of the twenty-five fatal cases, of Asiatic cholera, an organism having the above characters, was found, lying at various depths in the mucous membrane of the intestine. Its distance, from the free surface, the absence of other microbes, and the precautions taken, to prevent post-mortem contamination, all tend to make it evident, that it must have been there, during life. The vigour of its growth, would appear to proceed, pari passu, with the extent of the histological changes, in the structure of the mucosa. In some cases, it was found in
or between the epithelial cells, of the tubular glands, of the intestine. As to other organs, and tissues, it was found, in the kidney, in the liver, and in the blood vessels; and the supposition was entertained, that the filaments, and bodies, described by Strauss, as growing from the blood-corpuscles, may have been the micro-organism above described.

It would further appear to no means necessary, that the micro-organism should grow more readily, in an alkaline cultivating medium, such as that best suited, for the nutrition of the comma bacillus of Koch. Possibly, acid media may be found most conducive, to its growth, and a correlative fact is, the almost constant association, of epidemic outbreaks of cholera, in tropical, and subtropical, countries, with a superabundance of over-ripe fruits, and putrescent states, of the surface soil.

As to whether the micro-organism is, frequently, or constantly, present, in the contents of the intestine, the above cultivations did not determine, nor did the microscopic inspection of stained films, decide for, or against, the presence of the microbe. In the intestinal contents, or the dejecta
dejecta of cholera-cases, appearances were seen that resembled, in many points, the characters of the microbe, in question. None of the films, however, showed structures, that were, unmistakably, identical with those found, within the substance of the mucous membrane.

Not having, hitherto, cultivated the organism, in question, artificially, the observers would not seek to claim the right of stating absolutely that it is the true cause, of Asiatic cholera. They point out, however, that it was present, in certain instances, of all the fatal, cholera cases, they had an opportunity of examining. Further investigation, in the two-fold form, of observation, and experiment, must decide the question, whether, and this micro-organism can be proved to be the direct cause, or merely a concomitant, of the disease.

Finis.

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