LECTURES ON THE OCCURRENCE OF ORGANIC FORMS IN CONNECTION WITH CONTAGIOUS AND INFECTIVE DISEASES.

Delivered at Owens College, Manchester.

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LECTURE II.

CHANNELS BY WHICH BACTERIA MAY ENTER THE ANIMAL ORGANISM.

IN the bodies of men and of the higher animals, there are innumerable doorways by which particles as small as septic bacteria must constantly enter, yet no putrefactive changes occur. Bacteria, as we have seen, are, if I may so express myself, potentially present in all articles of food; for every kind of ordinary liquid has the faculty, when added, even in the smallest proportion, to a "cultivation liquid" (*i. e.*, a liquid fitted to serve as soil), of determining in it at once the development of bacteria. The fact that, even when we employ the highest powers of the microscope, we can see absolutely nothing in these liquids, only teaches us that our modes of observation are insufficient; for we have the proof, not merely that they act zymotically, but that we can deprive them at will of this power, either by heat or by agents such as carbolic acid, which are destructive to bacterial life. Thus, in ordinary drinking water, in cold meat, in fresh vegetables, in cheese above all, and in all ordinary specimens of milk, we are constantly taking in efficient sources of saprogenous life.

When such aliments come into relation with the absorbing apparatus of the intestine, whatever of course is chemically soluble enters the blood-current by the veins, while the insoluble parts enter the lymphatic system by the villi. The mechanism of absorption by the villi is such that even inorganic solids, in a state of fine division, are very readily taken in, and thus find their way from the intestinal cavity into the lacteals, through the thoracic duct, into the blood-stream. Anatomically, this was formerly very difficult to understand; for, so long as it was supposed that the epithelial covering of the villi was set on a structureless hyaline basement-membrane, it was difficult to see how particles could pass through such a membrane. Now we know that this membrane is not structureless, but that here, as in other examples of supposed structurelessness, the substance is penetrated by channels lined with protoplasm, which in the one direction are ready for the taking in nutritive particles, especially oleaginous ones, and in the other communicate with the commencing lacteal.

But, before these anatomical conditions were known and understood, it was proved experimentally that solid bodies in a state of fine division readily entered the lacteals. It is a great many years since Oesterlen showed that, if charcoal in extremely fine powder were mixed with the food of animals, the particles could afterwards be found in the circulating blood. *A fortiori*, therefore, if bodies relatively so enormous as charcoal-particles can be taken into the blood-stream by the villi, bacteria must certainly get into the vascular system whenever they are present in the intestinal liquids. Yet, notwithstanding the ubiquitousness of bacterial germs, it is manifest that the chance of their entrance into the organism by the intestine must depend in a considerable degree on the quality of the food, and particularly on its liability to septic change, and on the readiness with which its constituents are absorbed. Of these two conditions, the latter is likely to be the more influential.

Those alimentary substances which are soluble, as has been already said, enter the circulation by diffusion—a process which would exclude even particles so small as bacteria. But the fatty constituents can only obtain admission by interstitial channels, such as those which form the beginnings of the lacteal absorbents in the intestinal villi. Hence we are led to anticipate that a liquid like milk, of which the oleaginous particles are of extreme minuteness and in enormous numbers, all of which enter the absorbent vessels without undergoing any chemical change, would have a better chance of serving as a vehicle for saprogenic germs than any other sort of food. Whether this is so, is still a matter of speculation. It is supported by what was ascertained

in the inquiry relating to the remarkable epidemic of typhoid fever in London last year. With reference to that epidemic, it was, I think, clearly shown that the spread of typhoid was due to a widely spread contamination of a certain supply of milk; but it was not shown what was the nature of that contamination.

Notwithstanding the trouble that was expended in the research, it cannot be admitted that a case was made out in evidence of its specific origin—*i. e.*, its origin from previously existing cases of typhoid fever. This question must, I think, be allowed to remain open. For my purpose, it is sufficient that it was contaminated, and that the contamination had a very wide distribution. For if this be admitted, a practical lesson may be learnt from it—*i. e.*, that, if it happened once, it may and will happen again; and that greater precautions against contamination than are at present used are necessary. In England, we use our milk raw. In Germany, and in all countries in which acid fermentation products are consumed by the population, the milk is all boiled at the dairy before anything else is done to it; for, if it were not so, it would not be possible to produce sour milk, without constantly running the risk of septic fermentation. It would pass through the stage of lactic acid fermentation into that of putrefaction. I have myself ascertained experimentally that it is not possible, as regards London milk, to keep it until it is acid without its becoming at the same time putrid. Sour milk, as used in Germany, is a refreshing and wholesome beverage, but a little leaven of septic bacteria is sufficient to deprive it of both these qualities, as any one knows who has attempted to prepare it from London milk.

The other doorway for the introduction of bacteria is the mucous membrane of the lungs. Experiment has proved that, in animals exposed to a smoky atmosphere, the black particles are absorbed by the mucous membrane, and can be traced step by step along the course of the absorbent channels. It was indeed in this way that the first step was made towards the acquisition of that knowledge of the lymphatic system of the lungs which has lately been brought to comparative completeness by the anatomical researches of my colleague at the Brown Institution, Dr. Klein; for it was by Knauff's experiments* on the effects of a smoky atmosphere on dogs that the manner in which smoke particles find their way through the pulmonary epithelium, and thence into the network of superficial lymphatics, that the course and distribution of these channels first began to be understood. In the pulmonary mucous membrane, we have, then, a second unguarded portal by which morbid particles can enter the animal organism.

GERMLESSNESS OF HEALTHY LIVING TISSUE.

These considerations lead us to another question, which lies nearer to the object we have in view in these lectures—the elucidation of the relation of bacteria to disease. From experiments made in 1871, I came to the conclusion, that the blood and tissues of healthy animals were barren; that is, that, when subjected to conditions of temperature favourable to the development of microphytes, no such organisms were developed in them, provided that they were protected from external contamination. The general truthfulness of that conclusion has been confirmed by better and more exact experiments than mine, so that "barrenness" may now be accepted, as regards muscle and blood, as a criterion of health. But, from recent experiments which have been made at Heidelberg under the direction of Professor Kühne,† and which I have lately repeated a good many times, following his method exactly, it appears that the negative property of germlessness is not one which belongs to all organs, and that, in particular, those parts of the animal body which are in closest proximity to absorbing mucous membranes are most liable to be found pregnant with microphytic life when tested by suitable methods. Kühne's mode of experiment is as follows. An animal having been killed by opening a large artery, the organ of which the germlessness is to be tested is rapidly exposed, and a small bit cut out with a sharp scalpel, to which a previously boiled silk cord is attached. The preparation is then, without a moment's loss of time, dipped in paraffin at 120 deg. to 130 deg. cent. (about 250 deg. to 260 deg. Fahr.), and immediately afterwards withdrawn, allowed to cool, and again dipped. This process having been repeated several times, it is finally plunged into paraffin at a lower temperature (52 deg. cent. = 125.6 Fahr.), and allowed to remain until the whole mass (which is contained in a paper box like that used by histologists for "embedding") is cool. The thread having been burnt away, the whole is covered by an additional layer of paraffin, and the cake is placed in the "warm chamber" at a temperature of 30 deg. cent. (86 deg. Fahr.) In this process, the surface-layer of the embedded bit of tissue is of course

* See my paper on Artificial Tuberculosis, in the *Report of the Medical Officer of the Privy Council for 1868*.

† Tielgel, Ueber Coccobacteria septica im gesunden Wirbelthierkörper. (*Virchow's Archiv*, vol. lx, p. 453.)

cooked, *i.e.*, its albumen is coagulated, but the interior remains unaltered. If the tissue be germless, it remains free from organisms, and is found to be so when the preparation is examined microscopically after remaining four or five days in the chamber. I place before you several such cakes of paraffin, some containing muscle, others liver, kidney, or spleen. I cannot assert that any of them are free from organisms, but it is quite possible that that of muscle may be. As regards the others, I will answer for it that all are full of bacteria—bacteria, be it understood, of which the germs unquestionably did not enter after death—germs which existed and retained their latent vitality in the living tissue. Muscle and blood, if healthy, are germless; liver and spleen never are.*

The experiment I have been describing is by no means the only one by which it can be shown that blood and muscle are germless. As regards blood, the earliest is that of Professor von Recklinghausen, to which, as may be remembered, Professor Tyndall referred in his famous lecture on Dust and Disease. This consists simply in allowing blood to flow from an artery into a platinum capsule, which has been heated to redness and allowed to cool. It is then placed in a moist chamber, *i.e.*, a chamber of which the atmosphere is saturated, and kept at the temperature of the body, or rather below it (35 deg. cent. = 95 deg. Fahr.) The blood so kept remains without putrefaction for months, if protected from contamination. The coagulum first formed disintegrates; the colouring matter of the corpuscles leaves them, becomes dissolved in the liquor sanguinis, and crystallises, if crystallisable; but, after this, no further change occurs. This method has been improved and modified by Klebs; for in the form originally devised it often fails.

Instead of using a capsule, he employs a glass tube of the same nature as the one exhibited at last lecture. When it is desired to collect a specimen of blood, the drawn out end of the tube is introduced into a vein and secured in its place by a ligature. This done, the point (which is of course within the vein) is seized by the thumb and finger and broken off. The tube being half vacuous, the blood fills a great part of it. The tube is then withdrawn, and immediately closed hermetically. The specimens of blood thus collected may be kept for any time without undergoing putrefactive change. If the blood of animals whose colouring matter is readily crystallisable be used, and the temperature be suitable, the liquid in the tube contains crystals of hæmoglobin. Most of it, however, undergoes a peculiar change, which results in the production of an amorphous coloured mass, differing from hæmatin in its insolubility in alkalis.

The other method—that of cultivation—was employed by Dr. Ferrier and myself in our original experiments in 1871. It was by it that the fact of the absence of the septic ferment in the blood was first established. Having found that all exposed liquids possessed what I then called the zymotic property—that is, the power of starting the development of *Bacteria Termo* in Pasteur's liquid—we extended our inquiries, not only to other animal liquids, but also to healthy tissue, and concluded that all the liquids and tissues of the body are germless.†

RELATION BETWEEN SEPTIC PROCESSES AND THE PRODUCTION OF FEVER.

We must now apply ourselves to the consideration how far the organisms with which we have been occupying ourselves have to do with the processes of disease. As I said, the inquiry must be approached from the pathological side. As naturalists, we may be interested in all the lessons to be learnt from bacteria: in studying the remarkable influence of the conditions under which they originate and grow, in modifying their form, or the question how they come into existence, as it were, out of nothing. But all this is beside the mark; at all events, beside our mark as pathologists. To us, who are concerned about disease, and have its prevention and cure as our ultimate

object, the subject loses its interest the moment it becomes unconnected with bodily disorder. Life is short, and the work to be done long and difficult. We must, therefore, keep close to our own subjects, if we wish to make progress.

There are three sorts of diseased processes in which bacteria are supposed to be concerned: the process of fever, certain processes dependent on inflammation, and the various processes of specific infection. The relation of the development of bacteria to the febrile process can be investigated experimentally; for liquids are within our reach that have this property, *viz.*, when introduced in a very small dose into the circulating blood of a living animal, they produce fever—a fever which derives its claim to be so called not merely from its being attended with rise of temperature, but from its consisting of the successive stages which go to make up a febrile accession or attack; *viz.*, a period of latency, a rigor with rapid rise of temperature, followed by loss of muscular power, and a period of decline, the whole process being of short duration—not more than five or six hours, and not being accompanied by any local disease whatever, but being throughout a blood-process.

The liquids in question are exclusively derivable from one of two sources; *viz.* (a) products of inflammation, (b) products of putrefactive decomposition of blood, muscle, or other animal tissues. I shall speak of them as pyrogenic liquids. Among inflammation products, those of serous and catarrhal inflammations are the most frequent sources. As regards the former, *i.e.*, liquids of serous inflammations, those are most active which are of infective origin, *i.e.*, those of which the producing processes are secondary. Of catarrhal products, there is one which has been used by Senator, and which I have also used myself as a source of pyrogenic material. I refer to the expectoration of the purulent stage of phthisis. From this liquid (which is of course always to be had in any quantity), an extract can be obtained by treating it with two per cent. solution of common salt, which admits of filtration. This extract is always pyrogenous.

It is to be noted first that all of these liquids are apt to contain bacteria; they are not, however, putrid in the ordinary sense; the last mentioned, for example, retains the peculiar odour of purulent expectoration; and, secondly, that all of them are more active when freshly prepared than at any subsequent period. If, *e.g.*, they are subjected to the temperature suitable for putrefaction, they very rapidly lose their activity, and the rate at which this occurs varies with that of the putrefactive process. This must not be understood to mean that they were not at first in a state of decomposition, but simply that the *pyrogen* (as we may, for shortness, call it, whatever its chemical nature) is a product which is produced at an early stage in the septic process.

But there is, as I have said, another source from which pyrogenous material can be derived, *viz.*, from any animal liquid or tissue in the early stage of septic decomposition. The favourite tissue for the purpose is muscle. If a quantity of muscle be chopped up and placed in water at a temperature of from 60 deg. to 70 deg. Fahr. for a couple of weeks or so, an infusion is obtained which is pyrogenous. The exact period at which it acquires this property depends on various conditions, more than all on temperature. If this period be allowed to go by, the pyrogenous activity declines, while the obvious signs of putrefaction become more apparent. If the extract be used in the crude state, the result of introducing it into the circulation is often rapidly fatal. The reason of this is that embolism occurs. Little plugs are formed which find their way into the pulmonary artery and produce sudden death. Even if the liquid be strained, the production of fever (the effect which at this moment interests us) is not the only one which presents itself. Local effects are produced of the class to which I have applied the term secondary inflammation, *viz.*, infective nodules in the internal organs—serous and mucous inflammation. Those animals which are prone to tuberculosis become, in consequence of such an injection, eventually tuberculous, although at the time they may apparently recover from the immediate effects. These effects—all of which must be regarded as in so far embolic that they are dependent on the circumstance that the liquid contains particles of irritating material which are carried by the blood-stream to various parts of the body, and there prove foci or centres of infective inflammation—have evidently no necessary connection with fever in the sense in which we have agreed to use the word, *viz.*, as consisting in rapid augment of temperature, attended with the characteristic muscular phenomena of rigor, followed by adynamia, and succeeded by a much more gradual decline.

It may, however, be possible by suitable methods to obtain this purely pyrogenic effect without the others. In other words, there may be in muscle-extract, at an early stage in the process of putrefaction, a substance which possesses the pyrogenic activity without that of producing secondary inflammation.

Before proceeding to state what others have done, and what I have

* It is desirable to note that in the experiments I have myself made with reference to the germlessness of muscular tissue, that of the dog was used. In substituting muscle of the rabbit, I have failed. I believe this stands in relation with the observed proneness of the latter animal to traumatic infection as compared with the immunity of the former.

† Under conditions not as yet sufficiently known, organic forms, probably allied to bacteria, are met with in the blood of healthy persons. About three years ago, a discovery was made simultaneously by Dr. Ferrier, and by Dr. Löffler of Vienna, that in certain individuals bodies exist in the blood which in form resemble sarcina. They are to be found in blood which has been received into calcined tubes with all the necessary precautions. If such tubes are kept at the temperature of the body for a week or two, they are found to multiply rapidly. This observation has been confirmed by Cohn (*Beiträge, loc. cit.*, p. 223). A still more remarkable observation was made by Dr. Osler in my laboratory two years ago. He found that in the blood of certain apparently healthy persons, peculiar aggregations of pale particles of extreme minuteness, but definite form, existed in considerable numbers. These were studied under the microscope, on a stage of which the temperature was maintained at 38 deg. (about 100 deg. Fahr.), and exhibited a series of changes, all of which are carefully figured in his paper (*Proceedings of the Royal Society*, No. 153). In short, each aggregation resolved itself in the course of half an hour or an hour into individuals, each of which possessed the power of active locomotion.

done myself, in the effort to find such a substance, let me draw your attention to the remarkable fact that no therapeutical agent, no synthetic product of the laboratory, no poison, no drug is known which possesses the property of producing fever. The only liquids which have this endowment are liquids which either contain bacteria or have a marked proneness to their production.

Further, let me remind you that, whatever effort we may make to discover a disease-producing agent, we must take, both as our starting point and as our guide post, the one fact that we know about it, viz., its pathological action. For our problem is this. In a mixed liquid, we have reason to believe that a body exists which has the property of producing fever. Let us suppose that, by the use of appropriate chemical methods, we succeed in separating from it a crystalline principle which forms compounds of definite characters and constitution. To us this is of no value, unless it can be shown that the body so obtained contains the fever-producing property. Or let us suppose that a certain organic form is present, and that, by appropriate methods, we can determine its botanical characteristics, it is of no interest unless it can be proved that the form encloses the property. It was from forgetting this principle that Hallier, whose indefatigable labours as a botanical investigator of the forms of vegetation which he believed to associate themselves with diseased products were referred to in the last lecture, lost his way. He forgot that, in the investigation of morbid agents, pathological experiment and observation are the only safe guides. His results are valueless, not so much because the botanists say they are wrong botanically, but because he went on developing form out of form, without ascertaining as he went along, by repeated experiments, that the clue he was following was the right one.

Let me now return to my own experiments. I will not weary you by detailing the steps by which I was led to adopt my present method, but will content myself with reminding you that the purpose is (assuming that, in all probability, the pyrogenous is separable from what has been called the "phlogogenic" action of the material in question, viz., the septic extract of muscle) to effect this separation by obtaining the "pyrogen" in a convenient form.

The plan is as follows. The liquid is first precipitated by absolute alcohol. The precipitate is separated by decantation, again extracted with boiling alcohol, and then filtered. The residue, after having been freed from alcohol by placing it *in vacuo*, is extracted with distilled water. The water extract is at once filtered. It is a limpid liquid, which, on injection into the circulation, proves to be pyrogenous. It contains 1.2 per cent. of solids. The dose required to produce a characteristic access of fever in a moderate sized dog is five cubic centimetres, that is, three-quarters of a grain of solid matter.

From this experiment, two conclusions suggest themselves—1. That the agent, of whatever nature, is soluble in water, insoluble in strong alcohol; 2. That it cannot consist of bacteria, or have anything to do with them. Both of these conclusions would be erroneous; and this leads me to what I regard as the kernel of the experiment, viz.: We have an apparently homogeneous and transparent solution which is pyrogenous. Are its constituents really in solution? Is it really homogeneous? If I had not from other experiments learnt that no "animal poison" is really soluble, I should have answered the first question unhesitatingly, Yes. To test it, we apply a method first introduced into pathological investigation by Professor Klebs of Prague—namely, filtration under pressure through porous cells. By applying this method, I obtain a second filtrate. I test it physiologically, and find that it is not pyrogenic. Pyrogen, therefore, is a substance which passes through filtering paper, but not through porcelain. Further, I find, on microscopical examination, that the first filtrate, although at first it contains no bacteria, is not entirely free from particles; and, if I repeat the examination after an hour, bacteria are present in considerable numbers. If I examine the second filtrate in the same way, even twenty-four hours later, it remains barren. The porcelain has, therefore, removed from the liquid, along with the pyrogenous agent, that on which the development of bacteria depends.

We can scarcely overlook the bearing of these facts on the question of the mode of origin of bacteria; for, considering that bacteria in their ordinary state are destroyed, as Dr. Bastian and others have shown, at the temperature even of boiling alcohol, and that, without reference to temperature, they are inevitably killed by immersion in absolute alcohol, it is clear that our fever liquid as used contains no active bacteria. It is equally clear that it contains that particulate material out of which bacteria spring, for otherwise it could not be deprived of its fertility by filtration. It must, therefore, contain particles which, although they resist alcohol and heat, yet are endowed with a latent capacity of development. I may add that the existence of such particles has been inferred on other grounds by mycologists, who have compared their condition to that of the winter-spores (*Dauersporen*) of the fungi.

LECTURES

ON THE

EXPERIMENTAL INVESTIGATION OF THE ACTION OF MEDICINES.*

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VI.—RESPIRATION.

Position of the Respiratory Centre; chiefly situated in the Medulla Oblongata, but extends also to the Spinal Cord.—Effect of Strychnia upon it.—Influence of Nerves upon it.—Influence of Vagus.—Vagus contains two sets of Fibres, Accelerating or Inspiratory, and Retarding or Expiratory.—Cause of Rapid Breathing in Pneumonia.—Influence of the Superior and Inferior Laryngeal Nerves.—Nasal and Cutaneous Nerves.—Local Action of Vapours when inhaled.—Action of Ammonia.—Methods of Registering Respiratory Movements.—Acceleration of Respiration by Drugs.—Is it due to (1) Excitement of the Voluntary Nerve-Centres, (2) Increased Temperature, (3) Increased Venosity of the Blood?—Increased Venosity may be due to (A) Prevention of Blood from reaching the Air; (B) Prevention of Air from reaching the Blood. Blood may be prevented from reaching Air—(a) By Stoppage of the Heart: Action of Quinine.—(b) By Embolism of the Pulmonary Vessels; Action of Condurango.—(c) By Contraction of the Pulmonary Capillaries; Action of Muscarine.—Observation of the Pulmonary Capillaries in the Frog under the Microscope.—Effect of Heat and Cold upon them.

It used to be supposed that the respiratory centre was not only situated in the medulla oblongata, but was confined to it. Legallois found that the cerebral hemispheres, cerebellum, and even a part of the medulla itself, could be removed without arresting respiration; and thus showed that the respiratory centre was either in the medulla or in the spinal cord. (*Expériences sur la Principe de la Vie*. Paris, 1830, tome i.) Floreus noticed that injury to a point named by him *neud vital*, at the lower end of the calamus scriptorius, instantly arrested respiration, and thus caused death (*Comptes Rendus*, vol. xxxiii, page 437); and all experimenters have found that division of the cord just below the medulla also arrested breathing. These experiments seem to show most conclusively that the respiratory centre is situated in the medulla, and does not extend to the spinal cord; but the recent researches of Prokop Rokitsky (Stricker's *Medicinische Jahrbücher*, 1874, p. 30) on this subject show how careful we must be in drawing conclusions from experiments. Like all others, he has found that, under normal conditions, breathing ceases as soon as the influence of the medulla is destroyed by division of the cord just below it. But if strychnia be given to the animal, so as greatly to increase the excitability of its respiratory centres as well as of other reflex centres before the cord is divided, respiration will go on after the section has been made; and strychnia injected into the veins after the section will restore the respiratory movements, which the cut had arrested. This shows that the respiratory centre is not confined to the medulla, but extends into the spinal cord. The part contained in the cord is, however, too weak to keep up respiration alone under ordinary circumstances, though it can do so when its power is increased by strychnia. These remarkable effects of this poison give promise of future benefit from its use as a restorative in cases of death from drowning, etc.; but further experiments on animals are necessary before we dare employ such a powerful remedy in man.

Rokitsky's experiments enable us to demonstrate the presence of a respiratory centre in the spinal cord, as well as in the medulla of adult animals; but it is only fair to say that this was shown long ago by Brown-Séquard in the case of young ones. In young mammals and adult birds, he found that the thorax continued to execute rhythmical

* The request of a correspondent for a reference to Hering's experiments has, fortunately, directed my attention to a mistake of some importance in my last lecture. I there stated that Hering found that respiratory movements ceased when arterialised blood was passed through the head and venous blood through the body; while, on the contrary, apyrexial convulsions took place when venous blood was passed through the head and arterial blood through the body. I ought to have said that movements occurred in the blood-vessels such as would have taken place had the same sort of blood which circulated through the brain been passing also through the body. The experiments, however, were made on curarised animals, so that respiratory movements were impossible. Hering had previously ascertained that the arterial movements were synchronous with the respiratory movements, and might, indeed, be regarded as caused by impulses proceeding from the respiratory centre. They might thus serve as indications of the condition of the centre, where respiratory movements had been paralysed by curara. This paper is to be found in the *Wiener Acad. Sitzungs-ber., Math.-naturw. Classe*, vol. ix, Abth. 2, pp. 829-856.