The Cameron Prize Lecture

ON

MICROBIC TRANSMISSIBLE AUTOLYSIS.

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BY

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AMONG the discoveries which in the course of recent years have most attracted the attention of biologists is the so-called phenomenon of bacteriophagy, or, as we should preferably say, the phenomenon of microbic transmissible autolysis. From the historical point of view it ought to be recalled that we are indebted to an English scientist for the first observations on this subject. Having inoculated vaccinia on agar media Dr. Twort observed the development of a micrococcus which occasionally showed a translucent or transparent change, starting as clear spots at the margin of the colonies. Under the microscope nothing but small granules could be seen in these glassy areas. Some of the colonies showing such a degenerative change could not be subcultured. Moreover, when a pure normal culture of the micrococcus isolated from vaccinia was touched with a small portion of a glassy colony the growth at the point touched soon began to become transparent or glassy; many of the micrococci were killed and replaced by fine granules. Killed microbes were not affected. When diluted with a large volume of water the transparent growth was found to pass through the finest porcelain filters, and one drop of the filtrate, deposited on the surface of an agar tube, was sufficient to make it unsuitable for the micrococcus—that is, the micrococcus inoculated on the surface would start to grow, but would soon become dotted with transparent points. Such a disease of the micrococcus could be conveyed to fresh cultures for an indefinite number of generations. Twort also made similar observations as regards microbes isolated from the intestinal tract of several patients.

One or two years later the Canadian bacteriologist, d'Herelle, took up the subject again. His fundamental findings are closely similar to those of Twort, but he made many complementary and interesting experiments. When a mixture of broth and faeces from patients recovering from dysentery is incubated for about twenty hours and subsequently filtered, a clear fluid is obtained, possessing the wholly unexpected property of clarifying a thick suspension of dysenteric bacilli. Furthermore, a trace of such a suspension which has thus been rendered clear, added to a second suspension of the same micro-organism, brings about the same effect, and so on. Similar results have been obtained by d'Herelle for other intestinal micro-organisms, provided, however, that the faeces came from individuals who had recently suffered from a disease of the digestive tract or who had been associated with such patients. For example, d'Herelle obtained fluids exerting a lytic influence on a micro-organism belonging to the group of the *Bacillus coli*, and which afterwards was very frequently made use of by ourselves in our own experiments.

It is thus very easy to obtain an indefinite quantity of lytic fluid, working, for instance, on the *Bacillus coli*. One has but to add some drops of a previously lysed *coli* broth culture to a rather thick suspension of the same microbe in broth. After several days in the incubator the now clarified suspension is heated at 58°. This temperature kills the microbes, but does not affect the lytic agent, which is only destroyed at from 67° to 70°.

To make apparent the presence of the lytic principle in a given liquid we have at our disposal two or three slightly different methods. We may add the investigated liquid either to a turbid suspension in broth of *Bacillus coli*, in which case the principle is detected by the supervening clarification, or to sterile broth which has been freshly inoculated with one drop of *Bacillus coli* culture, in which case the bacterial multiplication is prevented, the broth preserving during one, two, or even more days, when inoculated, its original limpidity. Another very simple and striking method of detecting the lytic power consists in depositing a droplet of the lytic fluid on the central part of a nutrient agar surface which, one hour before, has been inoculated at many points with *Bacillus coli*. The tube being subsequently kept in the incubator a microbic layer soon appears and thickens over the

whole surface, except at the points which have been touched by the lytic droplet, where, the growth having been inhibited, the surface remains bare. However, some colonies may appear later on this bare spot, consisting of resistant micro-organisms, but the aspect assumed by the tube is nevertheless quite demonstrative.

How is the lytic power to be interpreted? Since the lysis is capable of being indefinitely transmitted from one bacterial suspension to another, it cannot be doubted that the principle governing the process is regenerated while acting. A very simple and easy explanation has been afforded, and is still vigorously defended by d'Herelle, who considers the causative factor of the lysis to consist in a living organism, an invisible filtrable virus, which can live as a parasite in the inside of various bacteria, thus finally disintegrating them. The first hint of this theory had been put forward by Twort, but he suggested it as a mere possibility, without being convinced at all of its correctness.

According to this hypothesis the bacterium which shows the lysis, being merely the prey of a virus, only plays a passive rôle and takes no part in the determination of its own lesion. With my co-worker Ciuca I offered a quite different interpretation. We think that the lytic phenomenon, instead of being due to the agency of an invisible parasite, is in reality an autolysis, a process of autophagy. According to our conception the microbic lysis may be regarded as the consequence of a nutritive vitiation of the bacterial metabolism, primarily induced by some external disturbing influence. This vitiation would have as an effect the production of a lytic chemical principle, a very active substance determining the lysis, and, moreover, capable of communicating the same vitiation to new microbes belonging to the same species when brought into contact with them. Among the microbes thus touched, a certain number are not immediately destroyed, and even—being still capable of multiplying before undergoing the lytic process—reproduce the lytic substance. Thus the vitiation can be ceaselessly propagated, the lytic principle arousing in the living microbes the elaboration of a new amount of the same principle. If a comparison could help to the understanding of the phenomenon, we could perhaps detect a real similarity with the process of blood coagulation. Under certain circumstances—namely, when protected from the contact of foreign solid bodies such as glass, for instance, when kept in a vessel coated with oil or paraffin—a plasma may be kept fluid during a very long lapse of time without any appearance of clotting. But, if to such a fluid plasma even a slight quantity of serum is added, obtained from a previous and normal blood coagulation, the mixture quickly solidifies and liberates by the very fact a new amount of the coagulating principle, the thrombin. Thus the addition of thrombin induces the production of thrombin. Similarly, when subjected to the influence of the lytic principle, the microbe—at least, after a certain interval, during which it may be still capable of multiplying—not only shows the lytic lesion, but also, in consequence of being touched by the lytic principle, reproduces this. Assuredly, the comparison is not perfectly adequate, since the plasma cannot, whereas the microbes can, multiply, but the analogy still may be easily grasped.

Since, according to the theory, the principle is liberated by the microbes themselves, it may be anticipated that a very abundant regeneration of principle will not occur unless the microbes present be sufficiently numerous. In other words, where only a very small number of microbes are added to broth containing the lytic principle, the reproduction of the latter will be very weak if all of the microbes perish at once. As a matter of fact, unless the microbes be already very numerous at the very outset of the experiment—that is, when they are brought into contact with the lytic principle—the amount of the latter only increases considerably as a result of a certain multiplication of the microbes. But we must add that as a general rule this multiplication does take place. To be sure, when the amount of lytic principle, mixed with the microbes, is very large in proportion to the number of germs, the bacteria are, as may be expected, rapidly damaged, their multiplication being thus strongly inhibited. Still, in such cases one may frequently observe, after two or three hours at 37°, a very faint and transient opacity, which soon after wholly disappears. There are reasons to believe that in any case, when the germs are not numerous from the outset, a certain multiplication is requisite for an abundant regeneration of the principle. I shall refer to this notion again, when I shall have to deal with the resistance of the microbes and the changes that

may occur in the activity of the lytic principle. I shall merely add, just now, that when the lytic principle has been mixed with a broth suspension of microbes, thus bringing about a striking clarification of the liquid, many of the germs present, but not all of them, as a rule, perish. The lysis is not absolutely total. Being sufficiently resistant, some germs are spared. Then if a droplet of such a suspension is spread on the surface of an agar tube, a development of numerous colonies is most frequently observed, and the culture thus obtained is endowed with the lysogenetic property—that is, reproduces the lytic principle. Even a very slight quantity of those microbes, inoculated into a tube of broth, multiply in the liquid while communicating to it the lytic property. Indeed, the same broth, subsequently freed from the resistant microbes by sterilization at 58°, influences in the typical way normal microbes of the same species which are added to it. The notion is thus forced upon our acceptance that a culture may still develop, especially on solid media, which seems to be more favourable to the growth, even though capable of reproducing the lytic principle. This may be quoted as an example of adaptation, or, more likely, of an increasing selection of the most resistant microbic individuals. It is a firmly established fact that all of the germs present in the same pure culture are not altogether identical. Neither morphologically nor physiologically is a culture thoroughly homogeneous—a tendency to variation may always be detected. There is no need to insist on the well known phenomenon of mutation, which has been carefully studied during the past years in several countries. We are indebted to one of your countrymen, Dr. Arkwright, for a remarkable paper which may be quoted with reference to that subject, concerning especially the *Bacillus coli*. From the same strain different colonies may be obtained, which may be easily distinguished as regards their shape, some peculiarities in the morphology of the germs, and also respecting the motility and agglutinability. For instance, some colonies give cultures which are readily agglutinated under the mere influence of salt. Working on another species, de Kruif has even found that different colonies may be differentiated as regards their virulence. As a matter of fact, Gratia showed that different varieties, extracted from one and the same pure *Bacillus coli* culture, are not equally resistant to the lytic principle, the latter being thus capable of working as an efficient agent of differentiation, since it allows some types to grow to the exclusion of the others. Such is the reason why, as we observed from the very beginning of our work, the resistant cultures obtained on agar at the expense of lysed suspensions very frequently assume abnormal characteristics—for instance, a mucous appearance. Of course, it is rather difficult to define accurately the part played either by mere selection or by true adaptation. However it may be, there is no need to dwell on the theoretical considerations which perhaps could be evoked. It is quite possible that the lytic phenomenon may be only the excessive display of the pathological exaggeration of some normal function having as an effect permitting or favouring a certain variation of the living being from the original and primary type, thus actively contributing to the appearance of new races and allowing the phenomenon of mutation to be more readily elicited. Such is, perhaps, the biological significance of the transmissible autolysis. Perhaps the lytic power is nothing else than a weapon made use of by certain varieties in order to prevail over their competitors. Perhaps the property now openly displayed betrays the normally unperceived existence of a physiological function, the latter being the tool as well as the expression of bacterial antagonism. But I suppose you will wish me to refrain from philosophical dissertation, and to keep on the firmer ground of experimental investigation.

If one drop of a *Bacillus coli* suspension containing the lytic principle is placed on an agar surface a culture develops endowed with the lysogenetic property. But when performing this experiment we may attempt to obtain quite isolated colonies. We have but to apply the usual technique—that is, to dilute sufficiently the liquid containing the germ before floating it on the agar. But this procedure, by which the germs are sufficiently separated one from the other, has also the effect of washing them so as to free them from the liquid part of the suspension, and consequently from the principle which is dissolved in it. Being now deposited on a nutrient medium which does not contain any trace of the lytic agent, the microbes are henceforth altogether protected from its contact and grow without absorbing it. In such circumstances, if the dilution has been carried far enough, isolated colonies grow, the majority of which are not lysogenetic; in

other words, the lysogenetic property of a culture may disappear merely by freeing entirely the microbes from the surrounding fluid. This seems to indicate—and this notion is most important for the comprehension of the whole question—that when a culture grows in a fluid containing the principle, the young germs newly formed by the process of multiplication only feel the effects of the principle, and consequently become lysogenetic in their turn when they have absorbed a sufficient amount of it. If at an early stage of their existence they are removed from the contact of the principle they do not regenerate it.

The course of the phenomenon might be summarized as follows: Under some disturbing influence, the nature of which we remain quite free to discuss later on, a nutritive vitiation of the bacterium is primarily induced, testified by the appearance of the lytic agent. After this the interference of the external influence is no longer necessary. Henceforth the reproduction of the principle requires nothing more than the presence of living microbes, which, having absorbed a sufficient quantity of it, liberate new amounts of the same agent at a certain stage of their evolution. There is the nutritive vitiation, by means of the microbes themselves, perpetuated through an unlimited number of generations. However, a tendency to the restoration of the equilibrium appears more and more distinctly betrayed by a prevalence of more and more resistant germs. Some strains even may become absolutely refractory to the strongest principle.

But how is the nutritive vitiation primarily induced?

Taking into account the well known fact that dysenteric faeces are very rich in leucocytes, and that according to d'Herelle's observations the lysogenetic property of such faeces appears only at the period of convalescence, we felt entitled to imagine that perhaps the lytic phenomenon observed was merely the consequence of some immunological process, or, to speak more precisely, was the result of a special modifying influence exerted by the leucocytes on the micro-organisms. According to this hypothesis, the conflict between the leucocytes and the bacteria would result in inducing an hereditary vitiation of the bacterial metabolism, consisting chiefly in the production by the micro-organism itself of the autolytic ferment.

As a matter of fact, the experiment carried out in order to test this hypothesis gave in a satisfactory percentage of cases quite positive results. If a guinea-pig is given two or three injections of *Bacillus coli* culture into the peritoneal cavity, the cavity being finally punctured three or four days after the last injection, a few drops of leucocytic exudate may be collected, which may be capable of inducing the transmissible lytic property when mixed with a trace of *coli* culture. We have even obtained by means of this method very powerful lytic principles. In such circumstances the nutritive vitiation is brought about, in the bacterium, by the disturbing influence of the leucocytic exudate, and the autolytic property thus started may henceforth be indefinitely perpetuated through successive bacterial suspensions, without requiring any more the intervention of a leucocytic exudate.

The conclusion that the so-called phenomenon of bacteriophage is in reality an autolysis, the lytic agent being produced by the bacteria themselves, would be quite indisputable if the results of the above experiment were constant. But I must acknowledge they are not. Often positive, they are not infrequently negative, and it must be likewise acknowledged that, up to the present, the reason why they are inconsistent and contradictory has not been satisfactorily discovered. A special factor or condition interferes, which does not seem to be present in every case, which cannot be mastered, and the intimate nature of which is still quite enigmatic. Of course, many possibilities may be suggested. It may be that the lytic power is only started at a certain stage of the intra-leucocytic digestion of the micro-organism and only in the inside of a few cells. Perhaps, in some cases, the lytic agent is too weak to be made apparent. Unquestionably, as we shall see later, the potency of the lytic agent is liable to considerable variations.

However it may be, other observations have been made by several writers, as regards the appearance of the transmissible lytic property in leucocytic exudates or in pus. The vaccinia from which Twort extracted his antistaphylococcic principle is a leucocytic exudate. From a patient suffering from a still unopened abscess on the face, under the eyelid, Gratia extracted by puncturing a pus which was markedly endowed with the property of inducing the transmissible staphylococcic autolysis.

As could be expected, the objection was raised by the

adherents to the invisible parasite theory that this virus, being of frequent occurrence and widely distributed in the natural media, the possibility could not be excluded of its having penetrated into the humours, exudates, or abscesses, and afterwards been extracted by the investigators. Whatever may be the plausibility of such an objection, it must be confessed that it is very hard to remove it entirely. At any rate, the hypothesis of the invisible virus would be wholly refuted, and, on the other hand, the autolytic theory would be wholly unquestionable if certain facts, recently pointed out by several observers, were regularly confirmed. For instance, it has been asserted that the lytic power may start under the influence of bacterial antagonism, in a liquid containing merely two microbic different species such as *Bacillus coli* and dysenteric bacillus, or *Bacillus coli* and staphylococcus. Some observers even believe that the lytic agent may appear in a pure culture, kept during a long lapse of time under certain conditions. However, such findings are not sufficiently controlled to be unanimously accepted. Such being the case the question is not settled, and therefore we cannot avoid comparing both theories in the light of the firmly established facts, and discussing the data that can be alleged in favour of each of them.

When a tube containing some cubic centimetres of sterile broth and a moderate quantity of lytic fluid—for instance, 1/100,000 c.cm.—receives one drop of fresh normal *coli* culture, only a very faint and evanescent turbidity occurs after two or three hours, which is frequently overlooked. Practically the liquid keeps clear and remains such during two or three days or longer. This fact would be easily understood if due to a chemical substance—to a sort of antiseptic which, naturally, could not act otherwise than distributing its hindering influence over all of the germs present. Such being the case no wonder if all the microbes are equally touched. But were the lysis the consequence of a parasite penetrating into the bacteria and disintegrating them, then it should in all probability be expected that at least some bacteria would escape and develop normally. To be sure, as I mentioned before, a transient opacity is frequently observed, but even when quite easily perceptible it disappears as a rule very rapidly afterwards, almost suddenly. The capacity of attacking almost simultaneously the totality of the developed germs could hardly be ascribed to a living virus.

It may be mentioned besides that the phenomena observed are entirely the same, whether the lytic fluids under experiment be quite fresh or previously kept during some months. The inhibitive influence exerted on the growth is identical. One can hardly believe a virus to be always ready to attack the bacterium however long may be the lapse of time during which it has been starved—that is, has not been supplied with the living bacteria representing its necessary food. When kept inactive during a long period, the virus in all probability should require an appreciable delay before eliciting again its normal aggressive abilities.

It has been unquestionably ascertained that the lytic agent does not multiply in the absence of bacteria—in sterile broth for instance. Neither does it multiply when brought into contact with killed bacteria. It may assuredly be granted that bacteria when chemically modified by sterilization may no more represent an available food. But why does the virus still not develop when mixed with living *Bacillus coli* suspended in physiological salt solution? In such a medium the lysis does not occur nor the lytic power increase. But both phenomena soon appear if some meat extract or peptone is added to the salt solution suspension, a possibility of growing being thus afforded to the *Bacillus coli*. This corroborates the notion that a certain growth of the bacterium is necessary to the operation and to the reproduction of the lytic principle, and consequently does not plead in favour of the virus theory. In fact, though not representing indisputable proofs against it, these findings at any rate tend to show that the lysis is in reality the final consequence of a vitiated bacterial evolution occurring at a certain moment in the course of the bacterial life. Most probably the fact that a sudden dissolution of the microbes may be observed after a stage of multiplication must be similarly interpreted. Thus a certain period of bacterial multiplication, however short it may be, appears as a condition necessary to the reproduction of the lytic agent. When the broth shows a weak acid reaction, or only a very slight alkaline one—a condition which is not favourable to the lysis—waves of multiplication, as Gratia pointed out, may be observed, separated by periods of partial lysis, whereas the activity of the liquid increases as a consequence of the periodical destruction of the more sensitive micro-organisms.

Another fact which must be borne in mind is that the lytic agent, which is not impaired by time, is also capable, as Kabeshima first pointed out, of withstanding the contact of substances endowed with antiseptic properties—such as acetone, ether, sodium fluoride. Of course, it could be offered as an explanation that the immediate cause of the lysis, which is thus resistant to the antiseptics, is not the virus itself, but an actively dissolving substance elaborated by it. But chemicals like chloroform, which do not destroy the lytic property, leave also quite unimpaired the capacity of the lytic agent to multiply in the presence of the sensitive bacteria.

There is a fact upon which d'Herelle always very strongly insists. When a small quantity of lytic fluid is added to a bacterial suspension, a droplet of which is, after a short interval, floated on an agar tube, the microbic layer which develops is not continuous, but shows translucent disseminated spots where an obvious lysis occurs, such clear spots being more numerous the larger the amount of lytic fluid added to the suspension. According to d'Herelle, these spots represent colonies of bacteria which are infected by the virus. The bare fact that the number of spots is in connexion with the quantity of lytic fluid made use of is not to be doubted, but does not force upon our acceptance the conclusion that the lysis is due to a virus. Another quite plausible interpretation consists in assuming that all of the bacteria are not equally endowed with the capacity to anchor very rapidly the lytic principle. Then clear spots appear only at the spots where such bacteria have been deposited, and which have absorbed a sufficient quantity. Of course, a larger number of bacteria will absorb rapidly the principle if the latter is not much diluted. It has been proved that the bacteria, even when killed, possess the property of absorbing the principle from the surrounding medium. We know that in a suspension all of the germs present are not equal respecting their sensitiveness, which in all probability depends on their ability to absorb rapidly the principle. As a confirmation of the interpretation just suggested, and which has been defended, especially by Gratia, it may be shown that the number of clear spots appearing in the experiment quoted above is in connexion not only with the concentration of the lytic principle, but also with the sensitiveness of the bacteria. The same amount of the same principle gives less numerous spots when the resistance of the culture used has distinctly been raised. By adaptation the resistance of a bacteria may increase to such an extent that there is no production of spots even under the influence of a large amount of a very powerful principle.

Experiments performed concerning the antilytic serums obtained by Ciuca and myself do not readily agree with the virus theory. When intravenously injected into a normal rabbit, the lytic agent may be detected in the blood during a rather long lapse of time—twenty hours at least. But if the rabbit has previously received under the skin or elsewhere several injections of the same fluid, the latter injected into the blood immediately disappears and cannot be recovered. This is due to the fact that the immunized rabbit has elaborated an antibody which at once neutralizes the lytic factor. Such an immune serum, mixed with broth containing the lytic principle in suitable amount, abolishes its activity, the *Bacillus coli* subsequently added being thus allowed to grow without being affected and without reproducing the lytic agent. The latter cannot be recovered, even when passages (in the presence of *Bacillus coli*) are subsequently performed from broth to broth, by which passages the antibody is more and more diluted, so that it could no more neutralize the lytic agent had the latter been reproduced. Were the lytic agent a virus, the antibody could not work like that unless endowed with strong antiseptic properties towards it. But the serum shows the same properties when previously heated at 56°—a temperature which, as is well known, destroys, as a rule, the bactericidal activity.

The study of the antilytic serums procured furthermore interesting information as regards the specificity of the lytic agent, an aspect of the problem which obviously is in close connexion with the above discussed theories. As many writers have pointed out, a lytic principle acting on the *Bacillus coli* acts similarly on closely related species, such as the dysenteric bacillus, several paratyphoid or even typhoid bacilli. But it does not work on widely different micro-organisms, such as the staphylococcus. Conversely, a principle lysing the staphylococcus does not react with *Bacillus coli* and similar microbes. Thus Gratia and Jaumann found that an antilytic serum appropriate to the *coli* lytic

principle does not neutralize in the least the staphylococcal lytic principle, and vice versa. This result cannot be brought into harmony with the view of d'Herelle, that there exists only one type of lytic virus capable of adapting itself to different bacteria. Were such an opinion correct one and the same antilytic serum should annihilate this virus whatever might be the bacterial species under experiment. On the other hand, the result agrees with the opinion that the lytic principle is in reality derived from the bacteria themselves. In fact, principles elaborated by widely different bacteria should likewise be distinct and distinguishable by means of the corresponding specific serums; such is, indeed, the experimental result.

When two conflicting theories are to be tested, it is convenient to follow them in their logical consequences under certain experimental conditions. For example what is likely to occur when an extremely slight amount of lytic principle is mixed with a very large quantity of living *Bacillus coli*? Logically, if the agent be not a virus but a substance, it may be anticipated that in such a case it will not be regenerated. The very slight amount of lytic agent will indeed be distributed between so many microbic individuals that each of them, being not sufficiently touched, will not reproduce the principle. This is exactly what is shown by the adequate experiment—the principle wholly disappears and cannot be recovered. Were the principle a virus, it could not be satisfactorily explained why this living being cannot multiply under such conditions, when a large number of microbes are present—that is, when the virus is supplied with a large quantity of food. Of course, in a control experiment, the same amount of principle is mixed with a rather thin bacterial suspension; then its quantity obviously increases.

But what shall we observe if, in a similar experiment, the number of the microbes, although still considerable, is somewhat lessened, the dose of principle remaining the same? Then the quantity of principle absorbed by each microbe, although still very slight, is somewhat larger than before. Then the influence of the principle will be not strongly, but perceptibly, felt, the consequence being that the microbe, moderately touched, reproduces a principle which, correlatively, is endowed only with a moderate potency. The principle is reproduced, but the properties of this reproduced principle are not identical with those of the original principle. And the difference is not of a quantitative but of a qualitative nature. The new principle thus obtained may be unceasingly and very abundantly regenerated by means of passages through successive bacterial suspensions, but it shows special characteristics, and henceforth keeps them permanently. It is a weak principle. When added to broth inoculated with *Bacillus coli* it only prevents the growth during a short period; the liquid becomes turbid after some hours, and the opacity is not markedly clarified afterwards. The weakness of such effects is not due to the fact that henceforth the liquids obtained only contain a slight amount of lytic agent. Those liquids, indeed, still work when extremely diluted. In this respect the new principle is in no way different from the strong original principle. But it works always feebly, even when present in a large dose. It actually is attenuated.

Quite similar results are obtainable by means of somewhat different procedures. For instance, if a droplet of strong lytic principle is deposited on an agar surface which has just before been inoculated on all of its points with *Bacillus coli*, the spot touched by the lytic fluid remains bare, whereas around it a normal abundant growth occurs. But after several days one may perceive that through a slow process of diffusion traces of principle invade the margin of the thick bacterial layer, thus causing there a faint clarification, a partial lysis. Of course, the amount of principle thus brought by diffusion into this zone is extremely slight in proportion to the number of microbes. Now, if some of the latter germs are removed by means of the platinum needle and inoculated into broth, a very weak principle is obtained which subsequently remains such when reproduced. Were the lytic agent a parasite, it would appear that among those parasites only the less virulent ones are capable of penetrating gradually into the bacterial layer surrounding the clear spot. This is not very likely.

A weak principle may yet be obtained even when the amount of principle is somewhat larger in proportion to the number of microbes, provided that the contact of the microbes with the surrounding fluid containing the principle be only very short, a condition which is easily performed by removing after a proper interval a droplet of the mixture and spreading it on an agar surface. Some translucent spots showing a

partial lysis soon appear, from which a culture giving a weak principle may be obtained.

Without dwelling longer on the details, we may state that from the primary strong principle new types of principle may derive, different as regards the energy of their action, and that the virus theory does not account readily for the occurrence of such modifications, the conditions under which they appear being taken into consideration. From this point of view, as well as in many other respects, the virus theory is open to criticism. But far from asserting that the problem is solved, I frankly confess that the other views, even the one which I deem the most probable, still lack indisputable demonstration. Many efforts will still be necessary; the mystery is not disclosed. I hope that this interesting problem will attract the attention of many biologists, especially of British scientists, since the study of the subject originated in your own country.

MALIGNANT DISEASES OF THE BREAST:

WITH SPECIAL REFERENCE TO THE SUPRACLAVICULAR
EXTENSION OF THE OPERATION.

BY

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In 1902 I published, in the *Bristol Medico-Chirurgical Journal*, a paper on "The results of operation in 54 cases of cancer of the breast." The paper I now publish is based on a study of all my cases of operation for malignant tumours of the breast (including the first series of 54). Of the 251 cases, 242 were cases of glandular cancer, and most of them were of the scirrhus form; 3 were cases of the very much less malignant duct cancer; and 6 were cases of sarcoma. In order to ascertain the late results I have investigated the records of 120 consecutive cases, operated on during the ten years preceding October, 1921, so that any non-recurrence would have extended over at least three years.* There were 120 operations for ordinary glandular cancer of the breast in this period—October, 1908, to October, 1918. The 120 cases do not include one case of duct cancer which I operated on during this period, for the reason already mentioned.

How the Investigation was Conducted.

I found in my records of these 120 cases a note as to the late results in 66. I wrote to the remaining 54 (or their doctors) to inquire as to the late results. What I have endeavoured to discover is the proportion of cases with local recurrence, because it is only this form of recurrence which could be prevented by a more extensive operation. I regard as local recurrence—recurrence in the pectoral region† (including that area of the chest wall), the axilla, the supraclavicular region, and growth attached to, but not starting in, the sternum, and even growth in the skin of the back of the chest, on the side of the operation. I have never seen any recurrence towards the upper abdomen. Recurrence in the other breast, or the opposite supraclavicular region, or axilla, or one in the mediastinum, I have not regarded as a local recurrence, as no operation short of removing both breasts, or the fatty and glandular contents of both axillae, and supraclavicular regions, could prevent it.

I have been able to trace the late results as to local recurrence as above defined in 80 of the 120 cases. Of the remaining 40 cases some died with distant metastasis without local recurrence before the end of three years after the operation, others died also during this period of intercurrent disease, and in some cases the cause of death was unknown. In many cases, as I expected, the post office returned my letter of inquiry, as they could not trace the persons to whom they were addressed. This of course most often happened with the earlier cases in the ten years' series. But I was glad to find that a considerable number of those of whom I had no note of late results did receive my letter of inquiry, and were able to reply that they had been free from recurrence for many years. And I am grateful to several medical men

* The statistics on which this paper is based were prepared early in 1922.

† By pectoral region I mean not only the area originally occupied by the breast, but the infraclavicular, the lateral aspect of the chest below the axilla, and the region over the sternum.