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Penicillin

Nobel Lecture, December 11, 1945

I have recently had the honour of lecturing in Sweden on the way in which the properties of penicillin came to be revealed from laboratory experiments and the development in the clinic of the application of the knowledge so acquired. It occurred to me that it might be more interesting today not to repeat much of what I then said, but to endeavour to show how the present great activity in the investigation of antibacterial substances is due to the development of appropriate methods and their co-ordination. One can also indicate the many directions in which work on these substances is now proceeding.

It is becoming more widely recognized that during the last sixty years an immense amount of work has been done on the subject of antibiotics, and that numbers of observers have reported a wide diversity of antagonisms occurring between micro-organisms. Even the earliest observations were

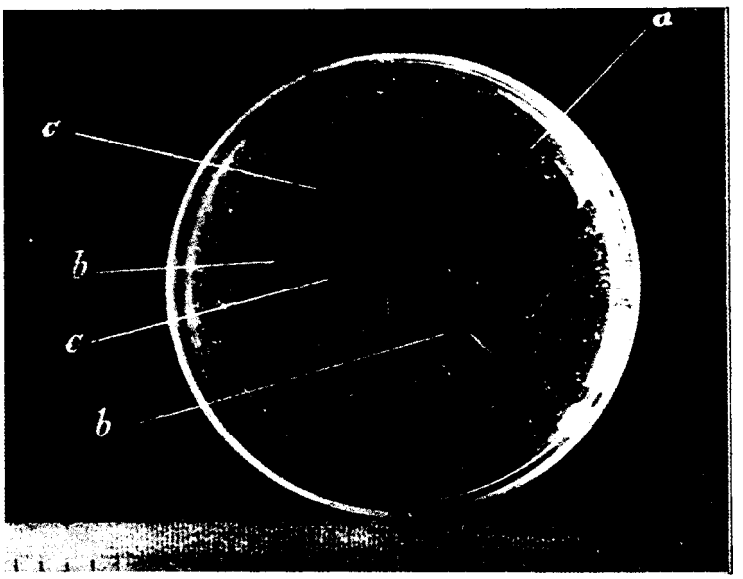

Fig. I.

conducted with a bacteriological technique which in essentials differed little from that frequently employed at the present time. I may recall to your attention that as long ago as 1889 Doehle illustrated the inhibition of the growth of anthrax bacilli by a coccus (Fig. 1). Again in 1887 the method of alternating streaks of two species of bacteria was introduced by Garré (Fig. 2). This method is essentially the same as that which is employed

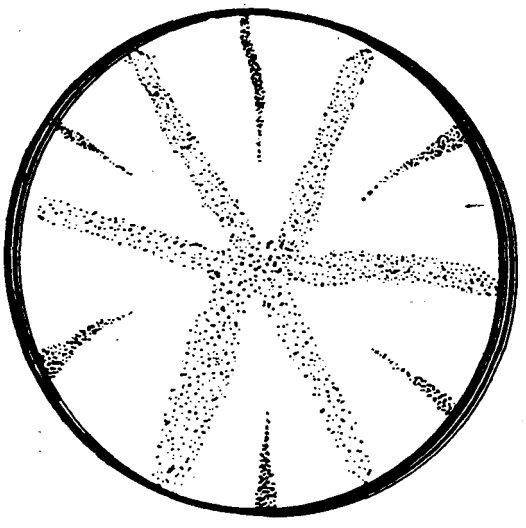

Fig. 2. Drawing illustrating Garré's method of studying antagonism by alternate streaks. The large streaks of *Ps. fluorescens* were made and allowed to grow for twenty-four hours, and then streaks of *B. typhosus* were made, which grew only at the circumference of the plate.

today, for example when the bacterium whose inhibitory power is being studied is placed across the diameter of a petri dish containing agar and other species are placed at right angles to it. Frost in 1904 described no fewer than seven methods for the investigation of the antagonistic properties of bacteria. One of the most interesting was that in which he grew one bacterium in liquid medium in a flask and another in medium contained in a collodion sac suspended in the flask. An antibiotic produced by the bacterium in one medium could diffuse through the collodion and inhibit the bacterium in the other (Fig. 3).

Botanists also early elaborated methods for observing the interaction of bacteria and fungi in culture. Reinhardt, for example, in 1892 described the inhibition of a fungus, *Peziza trifoliorum,* by a bacterium at a distance of 25

mm, as well as antagonisms between *Penicillia* and *Aspergilli* when these were grown on solid media. Many general technical procedures and observations were also furnished by Porter (1924), who among other instances noted that a penicillium inhibited the growth of *Pestalozzia* (Fig.4). An interesting example of fungal inhibition utilizing the technique of growth on agar was described by Nadson and Jolkevitch in 1923. They came on a contaminating fungus, which they later called *Spicaria purpurogenes,* which had the capacity to kill the common baker's yeast. This they showed clearly by planting the two organisms in alternate rows on agar. The fungus killed the yeast by secreting a purple dye which stained the killed cells differentially. They were able to show that the dye was produced in greater quantity when the yeast was present than in its absence.

These are but a very few of the large number of examples of the successful application of relatively simple techniques for the demonstration of antagonisms between micro-organisms. As a result of these observations nu-

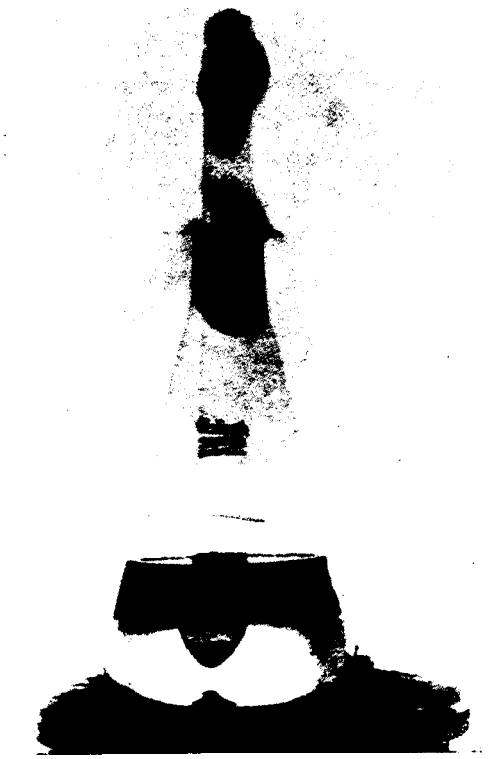

Fig. 3.

merous suggestions are to be found in the literature for using the antagonistic properties for therapeutic purposes against bacterial disease in man, and for the control of certain forms of fungal disease which take an enormous toll of food plants. In spite of this great accumulation of knowledge it is only in the last five or six years that any antibiotic appears to have attained a place of real importance in medicine. Perhaps the most useful lesson which has come out of the work on penicillin has been the demonstration that success in this field depends on the development and co-ordinated use of technical methods. In spite of the numerous first-rate bacteriological and mycological observations on antagonism, the work up to 1940 was conspicuous by the relatively poor quality of much of the chemical work, when indeed this side of the matter was considered at all. Particularly during the last 30 years or so, biochemistry as we know it, has been acquiring new techniques, often of great delicacy, suitable for dealing with many substances which occur naturally. I have never forgotten the remark made by Szent Györgyi, a Nobel

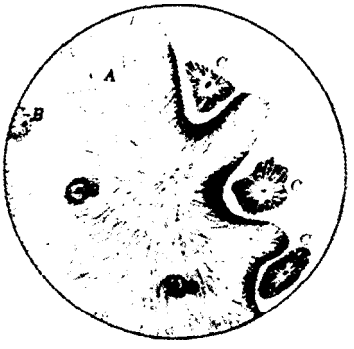

Fig. 4. *Pestalozzia* (A) inhibited by the colonies of one species of *Penicillium (C)* but not by those of another *(B).*

Prize winner, in 1929, when I had the good fortune to work with him in Cambridge. He said that biochemical methods were then sufficiently good to enable any naturally occurring substance to be extracted, provided there was a quick test for it. It is true that two mould products which are antibiotics, mycophenolic acid and penicillic acid, were isolated many years ago in the crystalline form, but their properties were only recognized incidentally in the course of an investigation on pellagra, and their discovery was not followed up by any extensive exploration of their properties. The work on pyocyanase and the products of *Psedomonas pyocyanea,* pursued intensively

at the beginning of this century, displayed all the ideas which we are working out at the present time, but the biochemical technique was quite inadequate, and as a consequence the biological work suffered greatly.

It was the botanists who in the 1930's first followed through in a thoroughly satisfying way the whole process, from detecting the antagonism of one fungus towards another to isolating and investigating the responsible chemical substance. In 1932 Weindling reported the antagonistic effect of a strain of *Trichoderma lignorum* on such fungi as *Rhizoctonia solani* and *Phytophthora parasitica.* This antagonism he found was due to the secretion of a "lethal principle" which was isolated by Weindling and Emerson in 1936 in a crystalline form. This substance is now known as gliotoxin. Gliotoxin has now been extensively investigated by chemists who are interested in its structure, but investigation of its biological properties has shown that it is far too toxic to the animal cell to be employed therapeuticall. in man, and its instability when slightly alkaline probably bars it from use in dealing with plant pathogenic fungi.

In 1939 Dubos, after long study and preparation of soil bacteria, described the isolation of a powerful antibacterial substance from a spore-forming soil bacterium known as *Bacillus brevis.* In collaboration with others he pursued the investigation of this substance, now known as tyrothricin, both from a chemical and biological point of view and in the clinic. Tyrothricin was found to consist of two polypeptides, gramicidin and tyrocidine, which were crystallized. These have now been thoroughly examined and, though of great interest from many points of view, they have proved too toxic to act as chemotherapeutic agents, though they have had some use as local applications. They have proved to be of great interest to the crystallographers, who find them useful for the study of protein structure by X-ray methods, as they and other substances from similar organisms are some of the simplest crystalline polypeptides known.

At the time the first results of Dubos were announced, preliminary experiments had been performed at Oxford on penicillin. The combination of bacteriological, biochemical and general biological techniques led first to the isolation and then to the detailed study of this product, from which it was clear that the substance belonged to the chemotherapeutic group. Thus at about the same time comprehensive investigations were being pursued on two antibiotics. Both these investigations were characterized by successful collaboration between chemists and those with biological training and knowledge, and that, to my mind, was the crucial point, and explains why up to

that time so little had resulted from the examination of a multitude of known inhibitors. The nearest previous example had been the work on pyocyanase at the beginning of the century, but at that time biochemical technique was in a relatively crude state. Fortunately one of the first substances to be studied in a comprehensive way was penicillin, and as soon as its chemotherapeutic properties were recognized there followed a mounting volume of work, which had as its primary object the possibility at the best of finding other chemotherapeutic agents, at the worst of isolating substances of biological and chemical interest. The procedure is now fairly clearly established. First the production of an antibacterial substance must be demonstrated. Usually moulds and bacteria are grown on liquid media and the liquid examined by some such method as Heatley's cylinder-agar plate technique (Fig. 5) - which

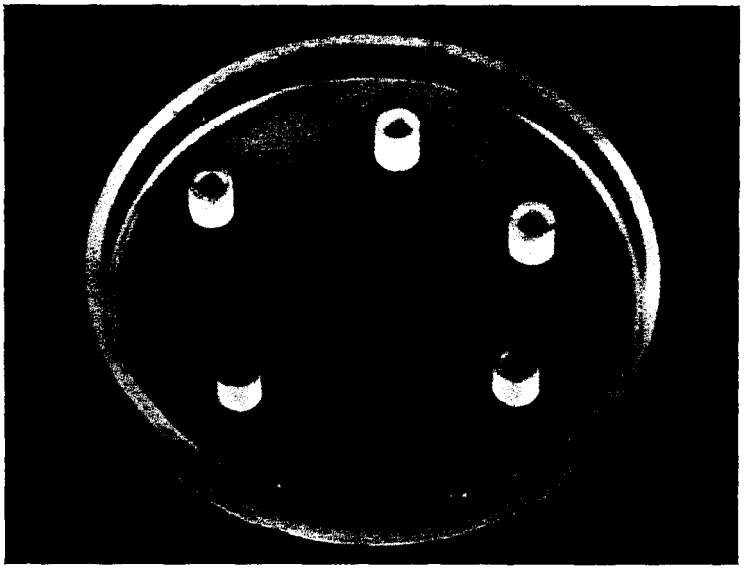

Fig. 5.

proved so useful in the penicillin work - for inhibition of such organisms as *Staphylococcus* and *Bacterium coli.* This method of testing has also been applied to the examination of many other possible natural sources. Thus Burkholder and his colleagues have examined a large number of lichens, which are a symbiosis of fungus and alga, by grinding them up with saline or other fluids and testing the liquid extract. A large number of lichens were found to yield inhibitory substances, some of which it was suggested might be lichen acids,

but as yet no real chemical examinations are reported and certainly no further biological examinations. In a similar way many thousands of plants have been examined by Osborn and others, with the discovery of many with an antibiotic action.

A number of methods have been employed, especially by Waksman and his colleagues, for the examination of antagonistic organisms present in the soil. A large number of antagonistic spore-bearing organisms have been isolated and attention is now being paid to the *Actinomyces* group. Besides these

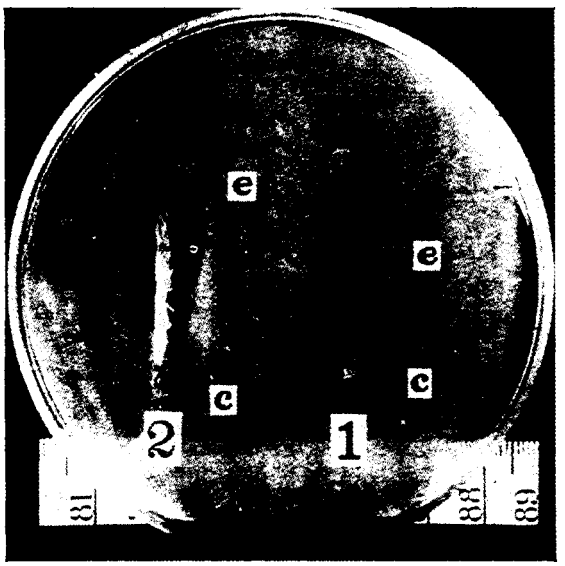

Fig. 6.

groups, which are prolific in antagonists, many of the numerous *Basidiomycetes* have been shown by Wilkins and Harris to produce antibacterial substances. These fungi as a rule grow very slowly and in artificial culture do not produce spores, so the initial detection of their antibacterial action presents some difficulties. Wilkins and Harris grew the fungus on a solid medium and then with a sharp tool cut out a section passing through the centre of the colony. The section was embedded in a seeded agar plate and if inhibitors were present they diffused out and inhibited the bacteria in the plate (Fig. 6). It was possible by this means to see whether the younger or older part of the fungus produced the antibiotic. From the surveys of known collections of fungi and higher plants, as well as the examination of stray contaminants, one recognizes that literally hundreds of producers of antibacte-

rial substances are now known and that there is a considerable prospect that hundreds of new compounds await a comprehensive investigation. As a first step to the further investigation of any substance the best conditions must be found for the production of the antibiotic substance - in the case of fungi it is a matter of finding the best cultural conditions and the best composition of the culture medium. The latter is often of the greatest importance, for a fungus may grow luxuriantly and yet produce no antibiotic if the medium is not suitable, whereas on another medium the antibiotic will be produced

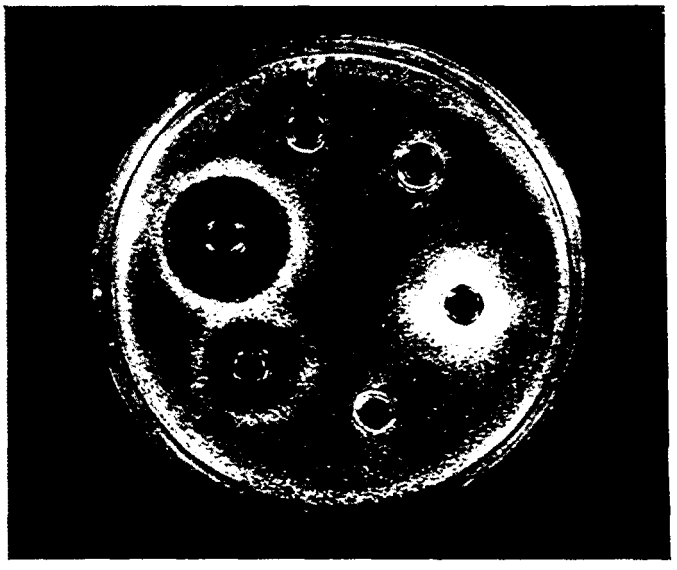

Fig. 7.

(Fig. 7). With plants the production of the antibiotic has to be considered in relation to the season of the year, the phase of growth of the plant, the different parts of the plant, and so on.

Then comes the determination of the way to extract the substance. Though each extraction presents a new problem, many substances are soluble in organic solvents and most of these are relatively easy to purify, especially by means of chromatography. Neutral substances which are insoluble in organic solvents however, still offer great difficulties in purification, but methods are gradually being evolved to deal with this class of substance, though much remains to be done.

Having extracted the substance in a highly concentrated or pure form, the range of organisms - usually pathogenic - against which it is active, is de-

termined, together with such data as its possible inactivation by serum or body fluids. The toxicity to whole animals, usually mice, is determined and it is particularly important to determine the effect of repeated injections, for in chemotherapeutic practice repeated doses will always be necessary. Some antibiotics, for example, which on a single injection are well tolerated, produce liver damage when the injection is often repeated. Toxicity to tissues is determined by *in vitro* observations on leucocytes or tissue cultures and by injections into the cerebrospinal cavity of animals. Pharmacological investigations into the effect of the substance on the most important physiological functions follow. Of these, the observations on the heart, blood pressure, and respiration are the most important. If at the end of all these observations a substance appears to be both sufficiently powerful antibacterially and sufficiently non-toxic, then protection experiments on an appropriate animal such as the mouse or, for the tubercle bacillus, the guinea pig become justifiable. Infection must of course be caused by an organism sensitive in *vitro* to the antibiotic under test.

This scheme of procedure, which was elaborated for the investigations on penicillin, is unfortunately not always carried out systematically on new antibiotics, largely, I suspect, because the urge to claim "priority" of discovery leads to premature publication. This undoubtedly accounts for the publication of many far from complete investigations. In any case, before serious trials on man are carried out, comprehensive laboratory work is necessary.

What has been the harvest from the large number of investigations which have been done during the last few years, using methods similar to those just outlined? We now know of many antibiotics, some of which have been obtained crystalline, but nearly all are too toxic for therapeutic use. A few which are too toxic for chemotherapeutic purposes have been used as local applications. However, one at least shows every promise of proving of use in the clinic. I refer to streptomycin, prepared first by Schatz, Bugie and Waksman from an actinomycete from the soil. This substance is a base and it is much more effective in media on the alkaline than on the acid side of neutrality. It is remarkably non-toxic and acts *in vitro* on a range of bacteria, including the tubercle bacillus, which are relatively or completely unaffected by penicillin. Complete protection can be afforded by subcutaneous injections of the substance, to mice infected intraperitoneally with such organisms as *Brucella tularensis* and *Pseudomonas pyocyanea.* One characteristic which streptomycin seems unfortunately to share with many antibiotics is that of

rapidly inducing in susceptible organisms a high resistance to the drug. This is a subject which obviously offers interesting prospects for analysis. There are indications that the clinical results with streptomycin will not be quite so straight forward as one could hope, but nevertheless the substance is of the greatest importance.

Previously Waksman and his colleagues had isolated a different antibiotic, streptothricin, from another soil actinomycete. It was more toxic than streptomycin but was shown to have chemotherapeutic effects in animals. It is not, however, likely to find a use in medicine.

Then there is proactinomycin which we have studied in Oxford. This is even more toxic than streptothricin but nevertheless we have been able to protect mice against intra-abdominal infections with streptococci by administering it by mouth. The distance which chemotheraphy has advanced in the last few years can perhaps be measured by the fact that this protective action might ten years ago have been looked on as almost miraculous, while today it means little, as the substance involved is much more toxic than other effective agents. These compounds already extracted from the *Actinomyces* are stimulating great interest in this group of organisms and it is probable that the next few years will see considerable additions to knowledge.

Again much interest is now being bestowed on the antibiotics produced by bacteria. Though they have been known to produce antibiotics for a longer time than any other class of micro-organism few substances have been extracted from them except tyrothricin and various products of *Ps. pyocyanea.* Much interest therefore attaches to the recent announcement by Meleney and his colleagues that a strain of *B. subtilis* had yielded a new substance which is to be called bacitracin. This substance, which appears to have true chemotherapeutic properties, acts against a range of susceptible organisms similar to that of penicillin. It is much more stable than penicillin and appears to belong to the class of neutral substances generally insoluble in organic solvents, though it is stated to be soluble in butanol. Little has been so far published about it, but it is stated to be relatively non-toxic, to protect mice against streptococcal infection when administered subcutaneously and to behave in a way similar to penicillin when used for local application to wounds. Sufficient has been administered to man to demonstrate its antibacterial power in the blood. This work forms an excellent example of my main thesis of the necessity for comprehensive investigation, for it has been clearly known since 1907, when Nicolle made his first observations, that *B. subtilis* produces an antibiotic, but it is only now that an appropriate com-

bination of technical procedures has revealed some of its real properties and potentialities.

The greatest focus of interest at present is of course the search for an antibiotic with chemotherapeutic properties against the tubercle bacillus. There is no reason to despair of such being found, for the experimental success obtained with streptomycin clearly indicates that the possibility exists. Though this drug will probably not itself be the solution for the treatment of tuberculosis, its effects both in animals and man give grounds for hope.

Many hundreds of flowering plants are now known to produce antibacterial substances but as yet few of the active substances have been extracted and so far, none promise to have therapeutic importance. Nevertheless one or two points of interest have emerged from their study. It was recommended even in Roman times that the wallflower *(Cheiranthus cheiri)* be applied to wounds received in battle and in the mediaeval herbals such statements are frequent. It is therefore of interest that large zones of inhibition of *Staphylococcus* were found by Osborn when flowers, leaves or, in particular, seeds were extracted and tested by the cylinder-plate method. Dr. Chain extracted the active material and showed that it was in fact cheiroline, a crystalline isothiocyanate, which had been known for many years without recognition of its antibacterial powers. There can be no doubt that our ancestors used a good antiseptic dressing when they applied crushed wallflower leaves to wounds.

My colleague Heatley, in investigating the antibiotic produced by a small wild flower, *Crepis taraxacifolia,* elucidated an interesting phenomenon. He found that an inactive precursor was present in the plant and that the yellow petals of the flower contained an enzyme which, by acting on the precursor, produced the antibiotic. This he crystallized and called crepin. As an antibacterial substance it is of little interest, but it has a powerful inhibitory action on the mammalian heart. It is interesting to speculate in what way, if any, this enzyme-substrate reaction can benefit the plant, and whether its antibacterial powers have any significance.

Antibacterial action is only one aspect of the potentialities of these substances, for their possible effects on protozoa and other non-bacterial causes of disease, particularly in the tropics, and on the fungi which cause skin infections, are as yet little explored. In this connection there is interest in a substance which it now appears was first isolated by Dutch workers in 1938 in the course of an investigation of *Penicillium expansum.* They eventually extracted a crystalline antibiotic which they called expansine and which is the

same as that which has later been obtained from several other moulds and goes under a variety of names - clavacin, clavatin, claviformin, patulin, penicidin. This substance has a powerful antifungal effect and was used by the Dutch observers for treating fungus diseases of the skin. Sanders has found that many pathogenic fungi are inhibited by it at a dilution of at least 1 in 160,000, but it is toxic even when applied locally and its possible place in therapeutics remains to be seen. The interest of antibiotics is not confined to human or animal therapeutics, for penicillin has been used successfully and apparently economically to treat the galls on apple trees caused by a susceptible bacterium, by injecting crude metabolism liquor containing penicillin into each gall.

These are the very interesting practical aspects of this form of study, but there are in addition many interesting theoretical points. For the chemist there is the investigation of the structure of substances which are often of quite novel types. The antibiotics so far known are mostly complex substances of large molecular weight and the structure of few of them has so far been elucidated. As Dubos has insisted, much biochemical interest attaches to the clarification of the mechanism by which these substances produce their effects, for a thorough knowledge of this may bring with it the ability to construct, as it were, tailor-made chemotherapeutic agents suitable for every infection. From a still wider point of view the clear definition of these antibacterial substances may help us to understand the ceaseless struggle for existence which is being waged by microscopic organisms everywhere.

The stimulus of finding that one at least of the antibiotics could play an important part in medicine has resulted in such an outburst of investigation in this field that there can indeed be few countries now in which some investigations of the type we have been discussing are not being actively pursued. One has thus the prospect that this intensive study must result in the next few years in a great accumulation of knowledge, some of which may be immediately applicable to medicine and some of which will contribute to theoretical knowledge. In any event those engaged in the work can look forward to many happy hours of investigation.