Section of Therapeutics and Pharmacology.

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A New Specific Antituberculous Medicament.[1]

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THE waxy envelope of the tubercle bacillus is an evident means of protection to the bacillus. Experiment shows that the acid-fast bacilli are neither reabsorbed nor destroyed by the phagocytes [1]. The phagocyte disseminates the bacillus in the organism, but never destroys it. Resistance to tuberculous infection is, therefore, the greater according as the blood contains a larger quantity of lipolytic ferments.

Carrière [3] and Clerc [2] have carried out interesting researches dealing with the richness in lipase of the serums of many animals.

Carrière [3] shows that the serum of the guinea-pig, the extreme susceptibility of which to tuberculosis is well known, contains the smallest quantity of this lipase.

Metalnikoff [4] proved that the mite attacking beehives is quite refractory to the action of tuberculosis because of the very great lipolytic power of its serum.

Noel Fiessinger and P. L. Marie [5], who repeated the experiments of Metalnikoff, insist on the fact that the waxy or fatty envelope of the tubercle bacillus is a true envelope of protection; the organism must, therefore, lipolyse and then bacteriolyse those bacilli in order to defend itself against penetration by microbes. The lipase "sensitizes," as we may say, the tubercle bacillus by destroying its waxy envelope [6]. Those animals, the lipase of which is particularly active, phagocytose and destroy the injected tuberculous bacilli very quickly. These authors consider the lipase of the mite to be the cause of its immunity.

[1] At a meeting of the Section, held April 15, 1919.

E. Schulz [7], then one of us [8] mentioned by Grasset [9] state that in the case of tuberculous subjects, whose defensive reaction is sufficient, the bacilli which can be stained by Ziehl's method become fewer in number, and are replaced by the lipolysed granulous forms. Therefore, "we think that the increase of the local lipasic activity constitutes one of the most important means of defence for the tuberculous lung" [10].

Metalnikoff [4], Deycke and Much [11], have isolated the bacillary waxes by chemical means, and have tried, by dissolving them in oils (nastin, tuberculo-nastin dissolved in olive oil) to manufacture a vaccine which produces the appearance of that specific lipase.

Deycke [11], Citron, and Müller [12], showed that the injection of the wax of the tubercle bacillus produced some lipolytic antibodies in the bodily organism. But these authors could do no more than obtain insufficient results in the treatment of tuberculosis.

But, as Metalnikoff rightly said [13], the tubercle bacillus wax, though as in the case of the tuberculin, endowed with certain immunizing powers, is quite unable, of itself, to confer perfect immunity. In order, therefore, to confer that immunity, or at least to combat the invasion by the bacillus successfully, we must *immunize with the sum total of toxic substances contained both in the bacillus itself, in its waxy envelope and in the medium in which it lives.*

The organism must be able, not only to neutralize the toxins but also to bacteriolyse the tubercle bacilli by first destroying their waxy protective envelopes, that is by lipolysing them, thus making it possible for them to be easily reabsorbed or destroyed by the phagocytes.

For many years we have done our best to fulfil those indications, and to find out the way to extract *directly* from the tubercle bacilli, the waxes and the substances that naturally adhere to those waxes [14] without transforming them or destroying them by manipulation, or by treatment with acids, ethers, chloroforms, xylol, heat, &c., which neutralize the greater part of their antigenic powers.

Relying on the work of Professor A. Borrel, of the Pasteur Institute (whose valuable and kindly help we gratefully acknowledge here) with reference to the dissolution of waxes of the tubercle bacillus in oils and animal fats [15] we have discovered a method which will not in any way alter the treated bacilli that may afterwards be shown to be alive by their culture in glycerinated bouillon. We, therefore, absolutely respect the constitution of our antigen, thus preserving its powers in the highest degree.

Our specific medicament contains, therefore, the bacillary waxes and all that may be dissolved by that process out of the toxins and the bacillary substances contained in the bacillus itself. Their presence is proved by the local and general reactions which follow the subcutaneous injections.

Waxes being, as Macé says [16], "the impregnated substrates of true active products of tubercle bacilli," their dissolution in our medium and by our process, which does not alter them directly, embodies them in the medicament. Our medicament is then really *specific* because it both contains and sets in action the sum total of active principles. By means of its use, we induce the organism to produce antibodies in *quantity* and *quality* necessary and sufficient for preservation as a therapeutic agent of a preceding infection.

The experiments carried out on animals have proved to us the innocuousness of that product and the power acquired by the blood of treated animals to lipolyse and to bacteriolyse the tubercle bacilli *in vitro* [17]. In our first experiments we did our best to obtain a vaccine producing only very little or better still, no local or general reaction; this vaccine may be administered subcutaneously or intravenously. We shall refer to this at a future date in a fresh series of studies yet to be published relating to the preventive vaccination of bovine animals; these studies and experiments appear, so far, to have yielded excellent and encouraging results. But, being aware of the difficulty of the passage of antibodies through the fibrous and not very vascular strata that surround tubercular lesions, a difficulty which Wright and Sahli have already emphasized, we carried out a fresh series of experiments and endeavoured to inject our medicament directly at the level of the tubercular lesions themselves, that is to say, in the case of pulmonary tuberculosis we made the injections by the intratracheal route. We have been encouraged in our researches by the works of La Jarrige [18], mentioning the experiments of C. Bernard, the thesis of Delor [19], Guisez and Stodel [20], G. Rosenthal [21], Berthelon [22].

One of us (Balvay), who introduced Forlanini's method into France [23], benefiting by that author's great experience in the treatment of cases of pulmonary tuberculosis, applied that method in the exhibition of our specific medicament. He obtained positive results; the proof of which is supplied by observations hereafter to be mentioned.

First of all, we made sure of the penetration of our medicament into a healthy and into a tuberculous lung. We were then enabled to

demonstrate [24] with the assistance of our friend, Dr. Guieysse, professor of histology, that not only are the fatty bodies which compose it, the presence of which is easily made evident by the osmic acid, found throughout the whole extent of the lung, but that they also penetrate into the tubercular tissue.

(Section of the lungs of a tuberculous rabbit killed twenty-four hours after an intratracheal injection of ½ c.c. of medicament were shown.)

We do not think there is any need to insist on the importance of such a statement from the therapeutical standpoint. In the face of such results, and with a view to making the injection by the intratracheal route, which has been proved to be without danger in experiments on tuberculous animals, we have modified the composition of our medicament, chiefly by increasing the quantity of waxes and toxins contained in it. We have noticed that the subcutaneous injection of 1 c.c. has always brought about general and local reactions (erythema, nodules, distinct elevation of temperature); such reactions may supervene after the first or after the third or fourth injection; this was shown long ago to be the case by Levene [25]. On the contrary, when subjects of pulmonary tuberculosis are treated by intratracheal injections, we have never noticed any reaction no matter how great the dose injected.

We must, therefore, seek to discover whether the vaccine is not modified at the level of the broncho-pulmonary mucous membranes, or whether the antibodies produced by the vaccine at the level of the lesions themselves are immediately taken up by the antigens, and the reaction fails to take place merely because they are not utilized. Relying on that hypothesis, we injected 1 or 2 c.c. of our vaccine into the trachea of six patients suffering from surgical tuberculosis, but whose lungs were healthy. In each case the elevation of temperature was the same as that which follows subcutaneous injections. The reaction has always been positive (Balvay, Godlewsky, Bouis). It was evident therefore (1) that the vaccine did not undergo any modification at the level of the broncho-pulmonary mucous membrane; (2) that when the antibodies produced *in situ* were immediately taken up by or fixed on the antigens there was no reaction. The practical deduction is that the vaccine must be injected directly to the level of the lesions. The method is applicable also in the case of surgical tuberculosis. Breslauer [26] appears to have had some idea of the application of this method.

These facts will perhaps enable us to supply the explanation long

sought for, as to the absence of reaction when tuberculin is injected into a healthy animal. We think this absence of reaction in healthy animals can be explained by the fact that according to well established evidence the healthy organism never produces tuberculous antibodies. The antibodies produced in a tuberculous person far from the lesions and, in consequence, not immediately utilized, would be, on the contrary, the cause of these reactions. Our experiments dealing with that question will soon be published.

As many as eighty patients suffering from pulmonary tuberculosis have been treated for more than thirteen months by intratracheal injections of our specific medicament; their ages vary from 17 to 47. We treated patients suffering from various lesions (fibrous cases, exhibiting recent evolution of the lesions, fibro-caseous, caseous, those in the cavity stage; all of them with open lesions, and most of them in the febrile stage). The result of treatment has shown that our specific medicament produces its maximum effect in patients whose lesions are entirely tuberculous and whose expectoration is very small. Hence the necessity for getting rid of the microbic associations and of lessening, as much as possible, the expectoration of certain patients. That result has been obtained by the method of intratracheal injections of gomenol oil at 5 per cent. In the case of patients whose expectorations are abundant, we daily inject into their trachea 5, 10, or 20 c.c. of gomenol oil. Those injections are made under the control of the laryngeal mirror, and have never given rise to the least accident. During the days following the first injections they usually set up an abundant expectoration, which diminishes rapidly in quantity until, after two or three weeks of treatment, it ceases. That period of intratracheal injections in reality constitutes a period of pulmonary cleansing.

Serial examinations of the sputum of these tuberculous persons prove that the bacilli have first a tendency to lessen in number, afterwards that their number diminishes but very little, and that the bacilli never disappear entirely from the expectoration. When the expectoration is greatly reduced, no matter whether the result has been obtained by intratracheal injections or by the fact of the sputum not being originally abundant, we commence the intratracheal treatment by our vaccine. Each patient is daily injected intratracheally with 2 or 4 c.c. of the medicament.

The observations noticed after those injections are the following: Progressive diminution of the temperature, a better general state of health, regular increase of weight, and upon auscultation, a tendency of the pulmonary lesions to a fibrous transformation.

The most striking result of these injections is that they are followed by constant modifications supervening in the morphology and the number of the bacilli of patients treated in that way. They consist in the fact that nearly all the bacilli are lipolysed and bacteriolysed, and in the usual and transitory increase in the number of bacilli without any increase in the expectoration.

Those modifications and that elimination are very quickly followed by the disappearance of bacilli from the sputum.

The first modifications usually make their appearance after the third week, and the disappearance of bacilli after six weeks or three months of treatment.

Conclusions.

(1) In pulmonary tuberculosis our specific medicament must be brought up to the level of the pulmonary lesions, into which we have proved that it penetrates. That result is obtained by the method of intratracheal injections. In the case of abundant expectorations, preliminary cleansing of the lung is necessary.

(2) When introduced by the intratracheal route, the specific medicament seems to act very satisfactorily on the temperature, on the general condition, the weight, and on the lungs. Invariably it produces changes in the morphology of the tubercle bacilli (lipolysis and bacteriolysis), a transitory increase in their number, and, after that, their elimination; that is, the disappearance of the bacilli from the sputum after a treatment of from one to three months. *This last stated fact indicates the important part played by the medicament in the social struggle against pulmonary tuberculosis.*

(3) Its employment has never given rise to the slightest untoward symptom. We would specially call attention to the fact that we have had the opportunity of following the case histories of a certain number of our patients for more than ten months. In all cases, the good results noticed when they left the hospital have been maintained, although they have started work again, and sometimes very hard work. These results are recorded down to October, 1918. It is our intention shortly to furnish a further account, with notes of observations, on many other cases equally demonstrative of the value of our method of treatment.

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DISCUSSION.

Dr. HALLIDAY SUTHERLAND: The interest of the paper, to which we have listened, is mostly a speculative interest, because the composition of the medicament, for which considerable claims have been made, has not been announced. It is claimed that the remedy is absorbed into diseased areas of the lung, but I am bound to say that the photographs passed round fail to demonstrate that assertion. In these photographs of sections of a tuberculous lung, we see irregular masses of an oily substance, stained black with osmic acid. Now if the medicament were absorbed, either by osmosis or by phagocytosis into the diseased tissues, it ought to be evenly distributed and finely subdivided. In reality it is not even within the pulmonary tissues, but lies within the lumen of these bronchi or bronchioles which are not occluded by disease. The same appearance could be reproduced by the intratracheal injection of any vegetable or animal oil or fat. It is also claimed that the remedy contains a portion of the waxy envelope of the tubercle bacillus in solution. If that be so, the preparation of this substance marks a most extraordinary advance in bacteriological chemistry. The exhaustive researches of Bullock proved the difficulty of finding a suitable solvent for the protective envelope of the tubercle bacilli. For my own part I have boiled tubercle bacilli for six weeks continuously in chloroform, without obtaining complete solution of the wax. On the other hand, we do know that tubercle bacilli can be grown in oil, and presumably the oil in which they are grown will contain some of the exotoxins. In an oily medium the bacilli revert to the streptothrix form, and possibly the composition of the exotoxins might also undergo alteration. If that be the nature of the medicament, as I speculate it may be, then the results could be explained without difficulty. The patient's nutrition would benefit by the absorption of a certain amount of a nutritive oil from the respiratory tract; and his resistance would be increased by a reaction to the tuberculin contained in the oil. I have used tuberculin for the past thirteen years, and I still hold it to be our most valuable adjunct to general treatment, when properly administered. That was also the view of Trudeau, and is to-day the opinion of those who have used tuberculin most extensively. So far as the medicament is concerned, all I have said is perforce sheer guessing, but if the guess be right then I think as good results could probably be obtained by giving cod liver oil by the mouth, and tuberculin subcutaneously. If it be an oil containing tuberculin, then intratracheal injection is a very crude method of judging dosage, as this would depend on the absorbing power of diseased tissue. Moreover, intratracheal injections, unless absolutely necessary, are not advisable, as they certainly do not add to the patient's comfort. In conclusion, may I ask Dr. Bossan, who comes from a school to which we are all indebted for much clinical inspiration, to forgive the critical nature of my remarks, which are of necessity, speculative, and may I also support the request from the chair that we might have more exact information about this very interesting therapeutic experiment.

Dr. BOSSAN (in reply): (1) The preparation of the vaccine is not a secret. It is common knowledge that waxes, fat substances and oils in combination are mutually dissolved. Professor Borrel stated in his paper, already quoted from, that the waxes of the tubercle bacillus are dissolved in oils and animal fats. This fact is easily proved by the loss of acid-fastness in the treated bacilli. To prepare the vaccine, place the bacilli in well sterilized castor oil or poppy oil or olive oil, and leave the emulsion thirty days in the incubator. Then filter it through a porcelain filter and the vaccine is ready for use. Great care must be taken to prevent the access of any source of contamination. (2) It is easy to see from the photographs that the masses

FIG. 1.

Small cavity twenty-four hours after injection. The walls are seen to be lined with the vaccine reduced by the osmic acid.

FIG. 2.

Tubercular nodule twenty-four hours after injection. The vaccine is seen to be reduced by the osmic acid which has penetrated into the nodule.

of oil are within the pulmonary tissues, some of them even in the epithelial cells. And this will be still better seen from the examination of preparations under the microscope. With the aid of my friend, Dr. Guieysse, I shall shortly be enabled to demonstrate to the Fellows microscopic preparations showing cells full of oily granulations. (3) The intratracheal injections are easily carried out and well tolerated by the patients. (4) I must again repeat that the administration of tuberculin alone is not sufficient, and that "in order to confer perfect immunity, or at least to combat the invasion by the bacillus successfully, we must immunize with the sum total of toxic substances contained both in the bacillus itself, in its waxy envelope and in the medium in which it lives."