REMARKS

ON

PREVENTIVE INOCULATION AGAINST BUBONIC
PLAGUE.

BY

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PREVENTIVE inoculation against the plague is the only means by which this disease may be rationally combated when it has manifested itself in an epidemic form. The numerous preventive measures suggested by the general rules of hygiene are not sufficient to overcome the epidemic, especially in India, where it is difficult to apply such measures with all due rigour and precision. Now that the plague has so widely and obstinately established itself in India, there is little hope of being able to eradicate the disease otherwise than by preventive vaccination. Of the truth of this the Government authorities and all intelligent people in India are so firmly convinced that very many energetic means are adopted to this end.

Experiments on animals show that it is comparatively easy to produce immunity from the plague. Those made on man show that it is possible also in the case of man himself after spontaneous infection. In fact, the results obtained by Haffkine are to a great extent satisfactory and lead us to hope that if for his method were substituted one more easily applicable on a large scale these results would be completely satisfactory, and the average mortality due to plague would be reduced to an absolute minimum.

Two years ago we proposed a method of preventive vaccination which gave excellent results upon animals. This method was brought under the notice of the public through various publications in different European periodicals, and also through the columns of the BRITISH MEDICAL JOURNAL of April 24th, 1897. After the experiments made by those who first turned their attention to the subject, we very soon came to the conclusion that it was possible to confer immunity from the plague by means of lifeless vaccines; that is, by means of toxic substances extracted from the bacteria themselves or from their products. And since we immediately foresaw the inconveniences arising from the employment of cultures of plague bacilli—inconveniences which have been afterwards verified in the practical application of the method on a large scale, as we shall have occasion to show—we directed our attention to the isolation of their active substances, to which immunity is due. And this object, based on the experiments made on animals, seems to us to have now been fully attained.

After numerous preliminary experiments, which it is unnecessary here to record, we succeeded in extracting from the plague bacilli a substance belonging to the group of the nucleo-proteids. This substance, which is decidedly poisonous, was shown to be capable of conferring considerable immunity on animals of different species, even though injected in very small doses. Although the method of preparation of the vaccinating substance used by us for preventive inoculation against plague was made known to the public through the above-mentioned publications, still we think it desirable to describe this method here in a few words.

We cultivate the plague bacilli in large glass dishes containing a stratum of common agar-agar. After some days' development we scrape with a bone spatula the colonies which have formed, and dissolve the mass in a 1 per cent. solution of caustic potash. We then add a very dilute solution of either hydrochloric or acetic acid until a slight acid reaction is introduced, and we collect in a filter the precipitate form. After careful washing the precipitate itself is dried *in vacuo*, and in the presence of sulphuric acid, or else immediately re-dissolved in a 0.5 per cent. solution of carbonate of soda. The dried substance, which has lost none of its chemical and biological properties, is easily redissolved in a solution of carbonate of soda when required. The solution of this substance may also be passed through a Chamberland filter for greater guarantee of sterility. The precipitate is composed solely of a nucleo-proteid in a state of relative purity. It possesses all the general reactions of nucleo-proteids, is

soluble in alkalies, insoluble in dilute acids, gives on digestion an insoluble product and a peptone, and on dissociation by sulphuric acid gives nucleinic bases. It is extremely toxic for several animals, and is able to produce the intravascular coagulation of the blood.

The experiments made during the year 1897 in the Laboratory of General Pathology in Florence were directed to proving the immunising efficacy of the above-mentioned nucleo-proteid. According to the publications already mentioned, they were performed on black rats, white rats, and mice to the number of 110, on 30 rabbits, and 15 guinea-pigs. The nucleo-proteid was injected (dissolved in a solution of carbonate of soda), sometimes under the skin, and sometimes into the peritoneal cavity of the animals under experiment. These injections were in some cases given only once, in others they were repeated two or three times in order to give greater certainty of immunity. The infection of the vaccinated animals was carried out only when they had quite got over the local effects of the injection, and we adopted sometimes the peritoneal sometimes the subcutaneous method, but more frequently the former, for reasons which we have stated elsewhere, and which would take too much time to repeat here.

The cultures adopted were always highly virulent, and capable of killing rats in 18 to 24 hours. The results were constant. The animals vaccinated always resisted the infection produced by large doses of virulent cultures, whilst the control animals always died. The experiments left us no doubt as to the method proposed by us for producing immunity.

In July, 1897, whilst at Bombay, we had occasion to repeat along with Dr. Malenchini similar vaccination experiments on monkeys. We employed the grey monkeys so common at Bombay, and sufficiently sensitive to the plague. In this set of experiments 6 monkeys were vaccinated as follows:

No. 1	received	1.705	cg. of active substance.
No. 2	"	1.705	"
No. 3	"	1.470	"
No. 4	"	1.410	"
No. 5	"	1.750	"
No. 6	"	1.750	"

Two monkeys were vaccinated twice, and received altogether 1.41 cg. each of active substance; and finally in two other monkeys, only one injection with 1.88 cg. of active substance was made. In these two latter experiments the dose injected at one time was too large; both monkeys fell ill, and one died in a short time (before infection); the other revived slowly, but completely. All of the remaining nine monkeys sustained very successfully, without becoming even slightly ill, the intraperitoneal or subcutaneous injection of a considerable quantity of virulent plague bacilli, small quantities of which killed the animals kept for control. The infection was made three times in the peritoneal cavity and six times under the skin. In 2 cases we infected the inoculated animals eighteen days after the last vaccination, in 1 case twenty-four days after, in 3 cases twenty-two days after, and in 3 others sixteen days after. These experiments show very strikingly that the immunising substance employed by us as a preventive against plague is effective also in the case of monkeys. It confers a few days after its injection an active immunity, and it is effective also if the infecting germs are introduced in considerable quantities into the animals under experiment, and if the conditions of the experimental infections are so serious that without this preventive treatment a rapidly fatal issue would result.

Finally, other similar experiments, producing immunity by means of the nucleo-proteid prepared as above, were recently repeated by us on rats and monkeys in the municipal laboratory of Parel at Bombay. The object of these was to establish the maximal duration of the immunity and to determine the minimal doses of active substances necessary, and to compare the different methods of producing immunity suggested for the purpose of combating the plague. These experiments are not yet, however, complete, and therefore the problems now propounded are not yet solved. They, however, clearly set forth what we have said, and we may now with sufficient certainty affirm that the nucleo-proteid extracted by us from the plague bacilli is capable of conferring on the animals experimented upon by us a complete immunity from bubonic infection.

Notwithstanding our desire to experiment largely on man by means of this method of vaccination it was not possible, owing to the numerous obstacles which we were unable to surmount and which only allowed us to try the vaccinating substance in question upon ourselves and on the operators in the Laboratory of General Pathology in Florence. We injected the nucleo-proteid, of which we have already spoken, after having dissolved it in a solution of carbonate of soda, under the skin of the arm and in quantities varying from 1 to 2 mg. In all the persons thus treated fever, general discomfort, headache, and a strong local reaction (hard swelling, redness, and pain) at the point of injection set in. The general disturbances passed away after twenty-four hours, the local reaction lasted four or five days and then disappeared without leaving any trace.

In February, 1898, when Dr. Polverini and one of us (Dr. Galeotti) had to go to Bombay, we were anxious to repeat the experiments. Dr. Polverini, who had never been inoculated before, was injected with 3 mg. of nucleo-proteid, dissolved in the usual alkaline solution. The injection was made in the morning, and as the day advanced he began to shiver and to feel generally uncomfortable. During the night the temperature rose to 101.3° F. By the following morning all these disturbances had come to an end and there only remained a strong local reaction, which lasted three or four days, which however did not in any way prevent him from pursuing his usual occupations. At the same time Dr. Galeotti was inoculated under the skin of the arm with 3 mg. of the same substance. The general and local reaction was in him much less, and his temperature scarcely reached 100.1° F. After three days he received another injection of other 3 mg., and this time the reaction was still less; and finally after seven days a last injection was made containing the same dose, and the reaction was absolutely insignificant, inasmuch as no elevation of temperature was observed.

The facts which these few experiments disclose are the following: First of all, it follows that the nucleo-proteid prepared by us, though endowed with strong toxic properties, is, within certain limits, innocuous even in the case of man, and produces only general disturbances of a transitory character. The local reaction, which manifests itself by infiltration and inflammation of tissues, disappears quickly and spontaneously, and does not give rise to suppuration or necrosis. Secondly, the phenomena observed after the injections show that man is very susceptible to the action of the nucleo-proteid in question, inasmuch as a dose of even 3 mg.—an extremely small one in comparison with the weight of the human body—is sufficient to produce a notable general reaction, sufficient to raise the temperature to 101.3° F., as in the case of Dr. Polverini. Man is, with regard to this substance, infinitely more susceptible than all other animals. In order to obtain in monkeys a reaction similar to that obtained in us after the injection of 2 or 3 mg., we had to inject 1 to 2 cg. of nucleo-proteid. This sensibility leads us to presuppose a greater facility in man for acquiring immunity. If the degree of immunity is in proportion to the reaction produced during the process of immunisation, it is a very favourable fact that we obtain strong reactions with small quantities of toxins. This is specially advantageous in the present case, in which the nucleo-proteid used by us is endowed with necrotic properties when injected in very large doses. Thirdly, we must note that the diminution of reaction in successive injections—a fact which was sufficiently well shown in the case of Dr. Galeotti—speaks well in favour of the capacity which the human organism would have of acquiring insensitiveness against the toxins of the plague bacilli.

And now, after having set forth the results of these experiments of ours, which demonstrate the efficacy on animals of the method of vaccination suggested by us and its complete innocuousness in man, we wish to compare this method of ours with Haffkine's.

We must first record some experiments made in the Municipal Laboratory of Parel (Bombay) with the object of comparing the efficacy of the various methods proposed for producing immunity from plague. The result of one set of these effected on monkeys, with the object of determining the immunising value of Haffkine's liquid, as used on man, shows:

(1) That considerable quantities of lymph (40 to 60 c.cm.) for

a monkey weighing 2 kilog. are necessary to produce a sufficient degree of immunity. (2) That this state of immunity lasts about one month. (3) That in Haffkine's liquid only the deposit—to a large extent composed of bacteria—is active, whilst, if filtered, it has no toxic or immunising power. (4) That in any case the toxic power of Haffkine's liquid, even if injected in its entirety, is, in the case of monkeys, very slight, since we were able to inject in monkeys weighing 2 kilog. as much as 160 c.cm. without their suffering from it.

On the other hand, experiments made with filtrates of bouillon cultures gave results different from the above. About a litre of culture, a month old, was taken, made in ordinary broth containing only a very small quantity of albumoses. The culture was very virulent for rats and monkeys. This culture was filtered through a Chamberland candle, and the filtrate, which was decidedly alkaline, was divided into two portions, one of which was used just as it was, and from the other we obtained, by acidification, a substance identical with the nucleo-proteid extracted by us from the plague bacilli. Leaving aside this precipitate of nucleo-proteid, the toxic properties of which were known, the toxic power of the two liquids, that is, of the original alkaline filtrate, containing some nucleo-proteid in solution, and of the acid filtrate deprived of the nucleo-proteid, was tried on rats and monkeys. These experiments, which have been described in detail elsewhere, and need not be repeated here, show that the toxic power of the plague germ filtrates is mostly due to the nucleo-proteid, which such filtrates, being alkaline, contain in greater or less quantities in solution, which proceeds from the partial decay of the dead and degenerating bacteria. Unfortunately such researches could not be carried out, whereas the intention of the experimenters was to see if the immunising properties of the cultural liquids had the same lot as the toxic properties, and especially to see if they were inseparably connected with the existence in the cultural liquids of quantities of nucleo-proteid in solution, but we may with every probability give an affirmative answer to such a question. Taking this into consideration, and also the fact that the nucleo-proteid, which goes to constitute the body of the plague bacilli, is very capable of producing by itself an active immunity, it is allowable to conclude that, if the cultures attenuated or filtered, or killed (therefore also Haffkine's liquid) possess some immunising property, this property is due to the nucleo-proteid dissolved or still contained in the bodies of the microbe, a nucleo-proteid which was isolated by us by means of a chemical process.

Haffkine, too, concurs in this, when he tries to test the strength of his liquid by the quantity of deposit produced in the cultures during the development of the micro-organisms.

After this we may readily see the advantages to be derived from the use of an active substance in a state of relative purity, in comparison with the use of liquids, which contain the substance, mixed with many heterogeneous elements, which have nothing to do with the production of immunity, and by their presence create a considerable amount of reaction in the organism, a reaction in itself hurtful to the individuals injected, and possibly interfering with the specific reaction due to the vaccinating substances, to which alone the establishment of immunity is due.

In the broth used by Haffkine for the cultivation of the plague bacilli there are considerable quantities of albumoses and products of the further disintegration of the elements which constitute muscular fibres, all substances decidedly toxic and endowed with a considerable pyogenic power. To these must be added the substances produced by the proteolytic action of the bacteria upon the albumose itself, and to all these substances, which have nothing to do with the immunity, must be attributed the numerous inconveniences which are observed with more or less frequency after a Haffkine's inoculation—inconveniences which represent a general intoxication of the organism, and sometimes attain a considerable intensity. Such facts have in a great measure contributed to the unpopularity of this method of prophylaxis.

We must also note that if we inject into the tissues of the organism the entire bodies of the plague bacilli with the idea that the bodies may become dissolved, and its component substances (nucleo-proteid) utilised in order to produce immunity, we are proceeding by a more slow and difficult way than when we inject these substances themselves already

separated and dissolved, and ready to be immediately absorbed. We may understand how in this second case the reaction of the organism under such bacteric poisons (in proportion to which the immunity is all the more assured) is more rapid, intense, and efficacious, inasmuch as immunity is all the more certain in proportion to the amount of this reaction. In order to kill the bacilli contained in his cultures, Haffkine heats them to 65° or 70° C. Now it is certain that at such a temperature the very delicate proteic substances to which in general is due the immunising power change very considerably, and may become entirely inactive. And nucleo-proteids especially (on which we have seen depends the power of the cultural liquids) suffer from the action of a temperature which almost reaches the point of coagulation of albuminoids, and lose after heating many of their chemico-biological properties. In fact all the experiments already mentioned show that the filtrates obtained from Haffkine's lymph are infinitely less toxic than the filtrates of cultures similar to those used by Haffkine, but not heated.

Another disadvantage, and a purely technical one, of the Haffkine method is the facility with which its prophylactic fluid may become contaminated. One would think at first sight that in so simple a matter it would be possible to eliminate entirely this danger, but in reality this is not so when it is a question of preparing hundreds of litres of culture and submitting this liquid so easily contaminable to several processes, of which one of the most dangerous is that of bottling. In many bottles of Haffkine's liquid were found micro-organisms of different kinds, and also sometimes pyogenic germs still capable of producing suppurations and pyæmia in the animals inoculated. In consequence sometimes abscesses and necrosis set in in the case of man, and these facts, when they came to the knowledge of the public, increased the general diffidence in regard to preventive inoculation.

With our method these difficulties are entirely eliminated, since we can reduce the immunising nucleo-proteid to the dry state, and in such a form it may be kept indefinitely and with perfect certainty of sterilisation. The most scrupulous care has to be taken only in the preparation of the cultures. The later operations may indeed be carried on with a certain amount of freedom (naturally only objects and liquids perfectly sterilised must be used), since even if it happens that some saprophytic germ gets in contact with the substance in preparation, it has no means of developing in the dried substance, and remains absolutely innocuous. It must also be noted that the nucleo-proteids possess, at least until disintegration sets in, a certain bactericidal power. In short the preparation of this immunising substance requires no greater antiseptic precaution than is necessary in the chemical preparations of other substances (such as alkaloids) destined for subcutaneous injections, and the dangers of infection liable to present themselves in the inoculations with our vaccine dried and redissolved in a sterile liquid are not greater than those which present themselves in the administration of ordinary remedies hypodermically. Besides, one of the most serious objections which have been made to the method recommended by Haffkine is that there is no dose criterion. The cultures of the plague bacilli develop in a very irregular manner, and sometimes very slight differences in the composition of the "cotton mediums" are sufficient to considerably alter the development and the virulence, and consequently also the immunising power of the prophylactic agent to be produced. Haffkine attempted to estimate the strength of the liquid contained in each series of bottles by the deposit which these bottles contained, but that this method of measurement is fallacious is self-evident. Instead, however, the dose of the immunising nucleo-proteid prepared by us in a state of considerable purity may be exactly determined by weight, since it is in the dry state, and thus we can obtain quantitative solutions just as with other medicines, and once the fundamental dose is established it may be reasonably varied according to age, sex, and constitution, and we may inject in any individual the precise quantity of active substance necessary to produce immunity without any of the inconveniences arising from an excessive dose of the toxic substance.

One other advantage, due to the fact of it being possible to preserve the immunising substance in the dry state, is the facility with which it may be distributed and sold; and this

advantage ought not to be neglected in the event of preventive inoculation requiring to be performed on a very large scale. Another great advantage which our method possesses in the event of its being applied in India, is that it in no way offends the religious sentiments of the native population of India. The difficulties which Haffkine encountered in this respect are well known, as his liquid was considered to be impure owing to its being composed, in the first place, of peptone and flesh, and even after the modifications made by him in the preparation of the broth the mistrust has not diminished. In our case, however, no objection can exist, as the active substance adopted by us contains nothing of the culture medium, and is an extract of the bodies of the bacteria, which, as is well known, are vegetable elements. Consequently, inoculation by our method would in no way offend the Vedic prescriptions. What is more, the suspicion (unjustly entertained against Haffkine's method) that the immunising liquid contained living micro-organisms could not occur. To sum up then:

1. The efficacy of our method of preventive inoculation against the plague by means of the nucleo-proteid extracted by us from the plague bacilli has been proved from the experiments made on animals—rats, rabbits guinea pigs, monkeys.

2. The immunising substance used by us is innocuous in the case of man. No troubles of any kind arise from its use, either local or general.

3. If the cultural liquids used in their entirety possess an immunising power, they owe this power to the nucleo-proteid which they contain and we have isolated.

4. The advantages of using an active substance isolated and pure in place of liquids (cultures) which contain this substance, mixed, however, with heterogeneous elements of different kinds, are at once evident.

5. The advantages which our method of inoculation possess in comparison with Haffkine's method are the following:

- (a) In using Haffkine's liquid along with the active substance are injected other toxic substances, which produce a reaction harmful to the organism and useless for producing immunity; with the use of the pure nucleo-proteid prepared by us, all the reaction produced is specific and useful for the production of immunity.

- (b) Haffkine's liquid has a very low toxic and immunising power, since it has to undergo heating so that the active substances contained in the cultures are, to a certain extent, altered. With our method this does not take place, and the active substance retains its highest immunising power.

- (c) Haffkine's liquid easily becomes contaminated, and there is no guarantee of the sterility of each separate bottle, while the active substance used by us is prepared in a thoroughly sterilised condition, and is perfectly preserved in the dry state.

- (d) It is impossible to determine an approximate dose of Haffkine's liquid; the nucleo-proteid prepared by us can be administered in perfectly well defined doses.

- (e) By the use of our method the religious feelings of the native population of India are in no way offended.

AN UNDESCRIBED FORM OF PLAGUE PNEUMONIA ;

WITH FIVE CASES.

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I DESIRE to bring to notice an indefinite and obscure form of plague, which, if attention is not called to it, may readily escape the recognition of the most careful and experienced physician. The ordinary classical form of plague pneumonia is known to all: its clearly marked physical signs; its rapid onset and course; its initial gravity; the severity of its general symptoms; and its rapidly fatal termination render its diagnosis all too easy. Its character, in short, is fulminant.

But there is a plague pneumonia which presents quite a different clinical character, quite another combination of general and local symptoms. Its onset is not fulminant, but