RAPID PRODUCTION OF AUTOGENOUS VACCINE FOR TREATMENT OF PNEUMONIA

WITH NOTES ON ITS USE

BY

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As the result of an investigation into the type constitution of pneumonia in Kenya during the years 1930 to 1935 a striking preponderance of infections due to the "Group IV" pneumococci over the standard American Types I, II, and III was observed; and this appeared to be the rule in lobar pneumonia among European and Indian residents as well as among African natives. The following table shows the results of typing a series of 776 cases of lobar pneumonia, mainly among natives, the figures including a small proportion of cases of pneumococcal meningitis, in which the commonest causative organism was Type I:

Pneumococcus type ..	I	II	III	Group IV
Type incidence ..	22.0%	5.6%	8.3%	64.1%

Serological analysis of the Kenya Group IV, begun in 1931, produced twenty-one distinct types up to October, 1935, when I was obliged to retire from work. While the majority of the very severe and fatal cases of the series were due to Type I, many were attributable to the Group IV pneumococci, of which the Kenya types designated as Kw.D, Kw.J, Kw.L, Kw.M, Kw.O, and Kw.V were found to be especially important. It is of interest that some of these were identified with types of the American series. But this picture suggested that the most rational means of specific biological treatment of pneumonia, where the causative pneumococci were so largely diverse, should be found in vaccine made from the actual infecting type cultivated from the patient to be treated.

The Case for Vaccine Treatment of Pneumonia

It cannot reasonably be doubted that lobar pneumonia is essentially a localized infection for which vaccine treatment is well suited, provided that, whatever the vaccine chosen, it is used in a sufficiently early stage of the attack—preferably within the first forty-eight hours. In this connexion W. H. Wynn (1934) says: "When the patient is seen within the first three days a vaccine or serum should be given. A vaccine has the great advantage that it is immediately available." The point here in regard to the immediate availability of the vaccine is, of course, that no preliminary typing of the patient's pneumonia is needed as for serum treatment. The vaccine Wynn uses "contains equal numbers of pneumococci, streptococci, and *B. influenzae* (P.S.I. vaccine)." The dose for an adult is "200 millions of each organism—that is, 600 millions in all"—and the object of the vaccine is "to stimulate the production of non-specific antibodies in adequate amount; the specific effect is not seen for some days." The only criticism I offer is the obvious one that the patient's infecting type of organism might not be represented in the vaccine bottle, so that no specific effect might follow at all. Reliance is therefore evidently placed mainly on the non-specific stimulus of the vaccine employed. If the vaccine used is an autogenous one, the specific effect can be looked for with certainty, and may play an extremely valuable part.

It has been urged against the principle of vaccine treatment of pneumonia that, since antibodies are already present, injection of vaccine is likely to induce a dangerous "negative phase." Here it is presumed, apparently, that antibodies are already present in adequate amount, though such could hardly be the case in a severe illness. And when the clinical signs indicate that the antibody concentration is below that needed for victory over the infecting antigen, the question to be answered is if it is legitimate to risk by vaccine injection this postulated "negative phase" with a mind to its being followed speedily by an abnormal rise in the antibody concentration and a consequent improvement in the condition of a patient who otherwise is likely to die—or, at least, to undergo a very distressing illness which might be cut short by the vaccine. As a result of my experience in vaccine therapy I regard the "negative phase" as a bogey from the pedantry of which a modern pathological outlook seems largely to have freed itself: in every case treated by means of specific or autogenous vaccine I look for an immediate "non-specific" effect in stimulation of antibodies, and doubtless my experience in this matter is shared by others.

With regard to the use of chemical substances in treatment of pneumonia, the following quotations from a letter by Alexander Fleming (1938) are apposite. In reference to the results of his experiments with "T 693" he writes, "I have come to the conclusion that chemotherapy and immunotherapy in pneumococcal infections are not competitive but complementary." He says, also, that "the more immune the patient is the better will be the killing of the pneumococci, and the ultimate result will depend on whether the patient has sufficient resistance to deal with the pneumococci even after they have been in contact with the drug." Further, he continues, "Pneumonia is a serious disease, and should be treated by all methods which have the effect of increasing the immunity of the patient or which are detrimental to the pneumococcus." And to quote from this valuable communication again, the writer says: "We must not forget that it is possible to immunize patients actively by means of vaccines. It has been shown that in animals which have received pneumococcal vaccine protective antibodies can be demonstrated within three days, and the same thing has been demonstrated in patients suffering from pneumonia." The foregoing is a useful corrective to that sceptical attitude which is met with too often in regard to vaccine treatment of pneumonia—an arbitrary scepticism not founded on any special knowledge and experience. There is no doubt that in Kenya many people die of pneumonia whose lives might have been saved by vaccine, and doubtless this is true elsewhere.

Preparation of "M.P.W." Autogenous Pneumococcal Vaccine

The obvious disadvantage of an autogenous vaccine for pneumonia, where vaccine injections must be begun in the earliest possible stage of the illness, is the length of time taken to prepare it by even the most rapid and certain ordinary methods. The procedure below described for the preparation of autogenous pneumococcus vaccine, designated as "M.P.W." vaccine, which consists of the rather obvious expedient of washing out the peritoneal cavity of a sputum-inoculated mouse and using the heavy pure suspension of pneumococci thus obtained as vaccine, enables injections of it to be made on the morning following the day or evening on which the first specimen of the patient's sputum is collected, thus securing a very impor-

tant saving of time. It only rarely happens that a satisfactory specimen of sputum cannot be obtained in the first two days of a pneumonia attack. It may be objected that a mouse inoculated intraperitoneally with an emulsion of genuine pneumonia sputum may produce cultures of casual pneumococci that have come to adhere to the sputum during its rapid transit of the upper respiratory passages and throat. This objection is scarcely valid or warrantable by experience: the infecting type of pneumococcus being extremely abundant in the sputum and of maximum virulence for mice, the chance that a mouse would, so to speak, ignore these in favour of a few adventitious pneumococci must be negligible.

TECHNIQUE OF PREPARATION

The procedure adopted for making M.P.W. vaccine is as follows:

1. Inoculate a large mouse, or preferably two mice in case of accident, intraperitoneally with 0.5 c.cm. of a rather thick saline emulsion of a satisfactory specimen of pneumonia sputum. The specimen should have been collected direct into a sterile tube from the patient's mouth. There is no need for preliminary washing of the specimen.
2. The mouse should be chloroformed when moribund rather than be allowed to die. It is then pinned out on a board that immediately before has been dipped in a strong solution of disinfectant, and the whole of its anterior surface painted liberally with iodine. The skin is deflected, and the exposed surface of the abdominal wall painted with iodine again before opening the peritoneal cavity. Before each stage of the dissection the scissors and forceps are dipped in spirit, which is flamed off.
3. A smear is made of the peritoneal exudate and Gram-stained to verify pneumococcus culture of normal abundance. With a sterile wool-plugged pipette and teat the peritoneal cavity is then first washed into about 1 c.cm. of plain saline, giving a dense suspension which may be used if desired for bile-solubility tests or agglutination tests with standard type sera. But this preliminary wash may be dispensed with.
4. The main peritoneal wash for the vaccine is then made into about 4 c.cm. of 0.5 per cent. phenol saline in a sterile wool-plugged centrifuge tube. This is suitably closed or covered (I use a cut-off segment of a rubber stopper, sterilized by flaming) and spun at about 6,000 revolutions for thirty seconds to throw down cells and flocculi.
5. The supernatant pneumococcus suspension is pipetted off into a wool-plugged tube, which is then covered with a rubber cap and immersed up to the cap in a water bath at 60° C. for thirty minutes.
6. The killed suspension is then introduced into a small vaccine bottle, labelled with the dose to be given. This is 1,000 millions of cocci, generally contained in an injection volume of 0.5 c.cm. The vaccine is administered to the patient immediately after bottling; the usual tests of its sterility may be safely dispensed with.

A full-grown mouse generally yields 4 to 5 c.cm. of suspension of a density of 1,000 to 3,000 millions per c.cm., corresponding to opacity representing from 0.3 to 0.6 mg. Burroughs Wellcome's opacity tube scale was used for estimating the strength of these vaccine suspensions; to obtain the value in millions the mean was taken of the two correlations with Wright's and the haemocytometer methods of estimation. The dose in cubic centimetres of the vaccine is, of course, adjusted according to the strength of the suspension obtained from the mouse peritoneal wash. A too heavy suspension should be diluted with carbol saline so that between 1,000 millions and 1,500 millions of cocci may be contained in a convenient volume for injection of about 0.3 to 0.5 c.cm. It is preferable that the vaccine should be considerably over the strength of 1,000 millions; the extremely low toxicity of killed pneumococcus suspension should be borne in mind.

Dosage of M.P.W. Vaccine, and Therapeutic Trials

This type of vaccine was first produced and experimented with by me seven years ago, with the clinical collaboration of Dr. P. C. C. Garnham (1931) of the East African Medical Service, in a series of cases of pneumonia exclusively among natives. Two factors here militated against success: one was that natives with pneumonia seldom come to hospital until their illness is in an unfavourably late stage for vaccine treatment; the other was that the dosage then employed—only 100 millions of pneumococci—was far too low to be likely to be effective with patients in such circumstances. The only apparent effect of the vaccine in these first trials was a slight reduction of the average duration of the febrile period from about eleven days to about nine days (pneumonia in Kenya is apt to be remarkable for the long period between onset and crisis).

It became evident that the best opportunities for testing the vaccine method would be afforded by European patients, who were more commonly under medical care in the earlier phases of their pneumonia. Accordingly in 1933 further trials were made with this vaccine in heavier dosage, in the first instance with a European severely ill with lobar pneumonia, the sputum yielding the Kenya Group IV type Kw.D (Type VIII of the American series), from which M.P.W. vaccine was produced containing 400 millions per c.cm. Encouraged by the obvious success of the vaccine in inducing an early crisis in this case and by the absence of toxic reaction to the injections, trials were made with higher dosage on native cases of pneumonia, more with a view to acquiring further experience of tolerance than in the hope of good therapeutic results with patients who, as has been said, come for admission to hospital in unfavourably late stages of the attack. Doses up to 3,000 millions were thus tested. No definite evidence of toxic reactions to such dosage appeared. Therapeutically the results were variable: sometimes the desired effect of a pronounced fall of temperature or early crisis on the day following injection was secured, but more often results were indefinite—this generally with patients whose treatment had to be begun too late. To test the vaccine with early cases among Europeans it was necessary to win by slow degrees the confidence and interest of the local practitioners in this method of treating pneumonia. Consequently, the number of European cases treated early in the disease with properly high dosage of M.P.W. vaccine had only reached the very inadequate total of eight before I was obliged to discontinue work finally in 1935. Brief details of these cases and one native case are given below, partly for their interest, but largely in the hope that some of the many pathologists whose laboratories are attached to a general hospital may be induced to give the method a searching test in the medical wards.

Illustrative Cases

Case 1.—A middle-aged male European, two days ill with lobar pneumonia. Cardiac and respiratory embarrassment was very marked. M.P.W. vaccine was given at midday. Six hours later there was a very slight rise in the temperature, and the patient said he felt as though a cold in the head were coming on. The next day he was more comfortable, and had greatly improved in the evening. The following morning the temperature had fallen by crisis, and the patient said he felt well. A second injection was given three days after the first, and a third injection after another three days. Convalescence was rapid and uneventful from the day of the crisis. The nursing sister in charge said she had never seen a severe case of pneumonia get well so quickly. The infecting organism

was the Kenya Group IV type Kw.D. (Type VIII of the American series).

Case 2.—An elderly male European with lobar pneumonia; severe Type II infection. M.P.W. vaccine was given on the third day of illness. The temperature was 101.5° the same evening but dropped to 97.5° at 6 o'clock the next morning, the patient feeling better and being less cyanosed; the temperature rose to 99.6° the same evening. Vaccine was given again the following day, when the temperature was subnormal. Convalescence was regular from that date. The doctor in charge of the patient wrote, "One of the most successful cases of lobar pneumonia I ever had."

Case 3.—A female European with lobar pneumonia; severe Type III infection. M.P.W. vaccine was given late in the illness. The only notes available are the doctor's report that it was a good case and the patient made a quick recovery, the crisis occurring forty hours after the vaccine injection.

Case 4.—A female European with lobar pneumonia; extremely severe Type I infection. M.P.W. vaccine was given—2,000 millions. The patient was said by the doctor in charge to be moribund when the first injection of vaccine was made, and he regarded this as the last hope. The next morning her condition was reported satisfactory. A second injection was given forty-eight hours after the first, and the crisis occurred within the next twenty-four hours. The doctor said that the vaccine "worked a miracle for the patient."

Case 5.—A male African with lobar pneumonia; severe infecting pneumococcus type "Kw.S." The mouse peritoneal exudate from which the vaccine was made included also a strain of Friedländer's bacillus, in numbers exceeded by the pneumococci, so that the vaccine necessarily contained both types of organism. M.P.W. vaccine was given; there was no toxic reaction. Crisis occurred the following day and convalescence was easy. This native case is included here because it was the only one in which a mixed vaccine was used, and it is typical of the few satisfactory native cases that were collected.

Case 6.—An elderly male European who had lobar pneumonia, Type I infection, which proved fatal. He was given 0.08 mg. of M.P.W. vaccine. The only report received on this case was that the vaccine had "no effects."

Case 7.—An elderly male European suffering from a second attack of pneumonia, Type I infection, which ended fatally. M.P.W. vaccine was injected on the fourth day of the illness, the doctor first seeing him on the third day. His temperature dropped to normal about thirty-six hours after injection and the pulse to 106, and he appeared much less toxic. The temperature rose again, however, after a few hours, and he died four days later. Only one dose of the vaccine was given; if it had been repeated the illness might perhaps have run a different course. The vaccine was reported to have had no apparent effect in increasing the toxæmia, but to have been followed by a temporary improvement in the patient's general condition.

Case 8.—A European woman with very severe lobar pneumonia, Type I infection, ending in death. M.P.W. vaccine, 3,000 millions, was given. The temperature fell to 99° the day following injection, but rose again, and the patient died about eighty-four hours later. Only one dose of the vaccine was given by the doctor, but it seemed that this effected a temporary improvement, and should have been repeated.

Case 9.—A male European aged 46, with lobar pneumonia which was extremely severe and involved both lungs. The infecting pneumococcus was a type of Group IV, unclassified. M.P.W. vaccine, 1,000 millions, was first injected about forty-eight hours after onset. During the next two days the patient appeared to be moribund; the Arneth count showed a marked shift to the left, the second lung became involved, and the doctor in charge was nervous of giving another injection of the vaccine. He was encouraged, however, to try a second dose of 1,000 millions seventy-two hours after the first. The next day there was general improvement in the patient's condition, speedily followed by the crisis, and he made a good recovery.

Scheme of Dosage

It is recommended that the injection be repeated on the following day if the first dose of vaccine has not lowered the temperature by that time; in cases where a good effect had resulted within about thirty hours after the first injection the vaccine might be repeated after forty-eight hours, and subsequently, if thought advisable, at intervals increasing from forty-eight hours to seventy-two, ninety-six, and one hundred and twenty hours. The vaccine should be repeated even when the patient's condition is worse on the day following the first dose: there need be no fear of increasing the toxæmia. It is noteworthy that in the handbook of the South African Institute for Medical Research the dose of 8,000 millions of Lister's prophylactic group pneumococcal vaccine is advised for treatment of pneumonia; and experiments in treatment with the same dose of a similar prophylactic vaccine issued in 1934 showed that the 8,000 millions were well tolerated by pneumonia patients.

Summary

An account is given of the types of pneumococcus found in lobar pneumonia cases in Kenya.

A case is stated for vaccine treatment of pneumonia.

A method for the rapid production of autogenous pneumococcus vaccine ("M.P.W.") is presented.

Details of nine cases of pneumonia treated with M.P.W. vaccine are appended.

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2-SULPHANILYL-AMINOPYRIDINE (M & B 693) IN TREATMENT OF GONORRHOEA

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Of the 102 cases of gonococcal infection included in this preliminary report, seventy-nine were in men, nineteen in women, and four in young girls. No adjuvant treatment—for example, irrigations—that might be of material assistance was given during the use of the drug under consideration—M & B 693. The results indicate a high proportion of apparent cures (over 91 per cent.).

Toxic effects were encountered in 29 per cent., and their nature and incidence are given later in tabular form. The commonest manifestations of intolerance were nausea and headache, and in most cases these symptoms were slight and the patients were able to continue with the drug in reduced dosage. The discontinuance of the drug was quickly followed by the subsidence of all the undesirable symptoms, and no prolonged ill effects were observed.