EXPERIMENTAL INOCULATION OF MENINGO-COCCIC VACCINE

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While treating cases of epidemic cerebro-spinal meningitis by the vaccine method we were struck by the difficulties experienced in estimating the dosage. The following investigation, therefore, was undertaken to determine the limit of dosage for a normal adult, and in the hope that at the same time light would be shed on the degree of immunity produced.

Three points seem to be established by the experiments:—
(1) That a dose of 222 million organisms was as much as the subject could bear with safety. (2) That the inoculation of a vaccine is not likely to be of much value as a prophylactic. (3) That a vaccine three weeks old has not lost its potency.

Technique. A. Method of Preparing and Staining Slides for the Index.—Two volumes of corpuscles were mixed with one volume of emulsion and one volume of serum, incubated at 37° C. for ten minutes, and films stained in the following way:—Leishman's stain twenty drops for thirty-five seconds; distilled water ten drops added, carefully mixed and left for six minutes; film washed in three changes of distilled water for twenty seconds altogether, and dried high over a bunsen flame. The emulsion was on each occasion prepared from a twenty-four hours' sub-culture on 'nasgar'¹ of a strain 201 days old on the date of the first inoculation (December 9th, 1907).

B. Method of Counting.—The preparation of the films was always done by one person, and the counting also by one person;

1. For composition, see previous paper.

but before the counting was begun the slides were re-labelled by someone not concerned, and mixed with the slides of patients under treatment at the time. Every effort, therefore, was made to reduce any possible mental bias. One hundred leucocytes were counted on every occasion.

C. *Preparation of the Vaccine.*—The vaccine was prepared from twenty-four hours old sub-cultures on 'nasgar' of the same strain as that employed for the index. Normal saline was the diluting fluid, and after standardisation sterilisation was effected by heating in a water bath to 60° C. for thirty minutes. Sterility was confirmed by incubating for twenty-four hours on 'nasgar.' Until used, phials of vaccine were preserved in a cool dark place.

D. *Method of Standardising Vaccine.*—All the vaccines prepared in this laboratory are now standardised by this method.¹ A Thoma-Zeiss cell is employed, covered in by a thin slip chosen in the following way:—A series of ordinary thin (No. 1) 7/8 inch slips are carefully cleaned, dried, and applied lightly to the plate glass slip provided with the apparatus, after gently breathing upon the surfaces to be opposed. The least pressure that will cause them to adhere is used, and the slip chosen which shows when held up to the light the best distribution of Newton's rings. Only a few slips from each ounce are satisfactory in this respect. The slips so selected may, of course, be used many times over. The diluting fluid is sodium chloride 0.1 per cent., Giemsa's stain 5 per cent., in distilled water; filtered or preferably allowed to stand for a few days. Before use 5 per cent. of formalin is added. The organisms gravitate to the bottom, the formalin inhibiting movement, and the trace of salt assisting the staining. We are indebted to Major Harrison for the suggestion that formalin should be added. Either the red or the white corpuscle pipette may be used, according to the dilution required. A small plug of cotton wool is placed in the end, and the pipette is sterilised in the hot-air steriliser. The usual precautions as to the size and placing of the drop are observed, and the 1/12 oil immersion

1. The method was described by one of us at a Pathological Meeting of the Liverpool Medical Institution, on March 5th, 1908, and a short description appeared in the *Lancet* of March 14th, 1908, in its report of that meeting.

lens is applied after dropping excess of oil on the slip. It is advisable to first of all bring the scale into good position with the 1/6 lens. Counting, with the condenser somewhat lowered, may be commenced within an hour; and as a rule it is quite sufficient to examine three sets of sixteen small squares. It is well to focus through the *depth* of the cell, as an occasional organism may be found floating, but the vast majority lie placidly at the bottom and may be rapidly counted.

The method has been controlled by using for the same vaccine different dilutions in two similar pipettes, and by using simultaneously both the red and the white corpuscle pipette. That the error from irregularity of the coverslip or possible cupping from surface tension is negligible can be judged from the even distribution of the organisms in the small squares. Using a red corpuscle pipette and taking vaccine up to the second cross-marking from the point (*i.e.*, a dilution of 1 : 500), the average number of organisms per small square is five in a vaccine containing ten millions per cubic millimetre. By using the fullest dilution with the red corpuscle pipette it would be possible to standardise a vaccine containing 100 millions per cubic millimetre.

Control Experiments.—The index of the subject of the experiments was tested eight times against five different sera before commencing the inoculations, and five times against four different sera afterwards. On every occasion it was found to be normal—that is to say, between 0.9 and 1.1. Although we accept this range as a basis, in actual fact our results so closely approximate to unity that we feel it is advisable to give them for these thirteen observations—especially so in view of the criticisms that have been persistently levelled at the accuracy of the index:—193-214, 135-136, 229-228, 202-219, 62-59, 30-32, 70-72, 76-78, 80-81, 76-74, 98-97, 71-77, 77-80.

Experiment No. 1.—9th December, 1907. Four cubic millimetres (containing 32 millions) of a vaccine prepared four days previously were injected into the left forearm. At the end of one hour an index from each hand showed a very marked drop, which was recovered from one hour later. Till 13th December there was a well-marked positive phase, with a maximum of 1.5 (each hand) on 11th December. A second sub-normal phase was present at the next examination (17th December). One day later

the index was normal (normal also to staphylococcus aureus), and remained so on the following day, when the next injection was given.

Reaction.—There was no general reaction. Local swelling appeared one hour after inoculation, increased steadily during the next few hours to the size of a pigeon's egg. There was not much redness. Twenty-four hours later the swelling was still marked, but flatter and more diffuse; the skin for about four square inches was hard and tender to pressure, but otherwise painless; the edges were distinctly raised, and the colour bright red. On 11th December the redness around the puncture was less, but its area had increased by one-half inch all round; less hardness was present, except immediately around the puncture, where there was still a little swelling. On 12th December there was merely a little thickening and bronzing round the site of inoculation.

Experiment No. 2.—19th December, 1907. Eight cubic millimetres (containing 68 millions) of a vaccine prepared fourteen days previously were injected subcutaneously into the left forearm. One hour later the index of each hand had risen (1.3 L., 1.2 R.). Readings till 26th December were within the normal range, but at its upper limit; on the 2nd January, 1908, 1.2; and on the 7th and 8th January, normal. The index on the 23rd December was 1.1 to two strains of the meningococcus (215 and 112 days old respectively).

Reaction.—There was no general reaction. Local swelling about one inch in diameter appeared one hour after the inoculation; twenty-four hours later there was swelling and uniform redness for six inches around the puncture; degree and course very like the first inoculation.

Experiment No. 3.—8th January, 1908. Twenty cubic millimetres (containing 100 millions) prepared two days previously were injected subcutaneously into the left forearm. Twenty-four hours later the index had risen to 1.4, and it remained above normal on the 10th, 13th, and 14th (maximum 1.5 on the 10th). It was normal on the 20th, and then rapidly fell to 0.5, in which region it remained for four days. It then rose to normal, within a range of 0.2 of which it oscillated until the next inoculation on February 4th. During the second sub-normal phase indices taken against the staphylococcus albus and citreus were found to be normal.

Reaction.—There was some headache and malaise for twenty-four hours. On the day after the injection there had developed round the puncture a tender but otherwise painless area of brawny redness four by four and a half inches in extent. The axillary glands were unaffected. On the 10th the area of redness was larger, but the colour less intense; on the 11th the area of redness was only two inches in extent around the puncture. By the 21st January the area around the puncture had gone through stages very similar to calf lymph vaccination; on this day the scabs were removed. Local tenderness had ceased.

Experiment No. 4.—4th February, 1908. Thirty cubic millimetres (containing 222 millions) of a vaccine prepared eighteen days previously were injected subcutaneously into the left forearm. A negative phase appeared on the 5th, and was pronounced on

the 6th. On the 8th (ninety-six hours after the injection) the index was once more within the normal range. The indices on the 10th, 11th, 12th, and 14th showed a well-marked rise (highest 1.6 on the 10th and 11th).

To this again succeeded an oscillating but mainly sub-normal phase lasting at least a week. As confirmation that this oscillation was actually present, it is of interest to note that the blood of a patient under treatment at the same time gave, with a control common to both, a very even curve.

Reaction.—The injection was given at 5.30 p.m.; the subject had felt quite well all afternoon. At 6.15 p.m. there was a feeling of nausea accompanied by severe vertical headache and rigor. At 7.30 p.m. vomiting occurred, and some diarrhoea; the temperature was 103 F., and the pulse rapid; headache was very severe, and a restless night was spent. In the morning the temperature had fallen to normal, and the subject felt better except for the headache, which persisted for two days. There was considerable pain behind the eyes for thirty-six hours, and pain, but no stiffness, at the back of the neck for two days; this persisted to a slight extent for six days. There was no pain in the back. The skin over the head and neck was tender. On the second day after the inoculation severe herpes appeared on the right side of the lower lip and left ear, which were greatly swollen and covered with vesicles. Locally there was a tender area five inches in extent; redness appeared around the puncture very similar in appearance and course to the previous experiments. No local vesiculation occurred; the axillary glands were unaffected.

SUMMARY

These experiments form a series, with a longest interval during which no indices were taken of seven days. Succeeding inoculations were only given when the index change from the preceding inoculation had returned to normal.

Primary Negative Phase.—Apart from a drop in Experiment No. 1, lasting less than two hours but confirmed in each hand, there was no initial negative phase after the first three inoculations, but a very marked one (four days) after the fourth.

Positive Phase.—All four inoculations produced a positive phase, lasting in No. 1 not longer than a week—certainly four days.

No. 2 (? fourteen days), since a series of four consecutive indices at the upper limit of the normal range *may* be considered a rise, especially when preceded and followed by an undoubted rise, the former confirmed in each hand.

No. 3, not longer than twelve days—certainly six days.

No. 4, six days.

In every case the highest point was reached within two days of the development of the positive phase.

Second Negative Phase.—This followed the positive phase in three cases (Nos. 1, 3, and 4), lasting one to two days, four to five days, and a week or more respectively. With the larger doses this sub-normal phase showed a very distinct and somewhat prolonged oscillation, with, however, a sub-normal average—that is to say, the original positive and negative phases were merely the first in a series of which the amplitude steadily diminished. The number of days after each injection before the index had settled at normal worked out in—

No. 1—Ten days.

„ 2—Nineteen days.

„ 3—About twenty-four days.

„ 4—About twenty-six days.

Reaction.—The reaction, local and general, increased *pari passu* with the dosage of vaccine, and irrespective of its age, up to three weeks.

Agglutination.—This was tested on the 6th and 9th of December, and the 15th February, by the loop method, at laboratory temperature, 1/6 lens, time four hours. In dilutions of 1-10, 1-20, 1-40, the results were negative. Apparently the extent of saturation required to produce agglutination was much greater than our experiments reached.

CONCLUSIONS

Limit of Dose.—There can be little doubt that in this particular subject a dose of 222 millions even after three preceding inoculations was markedly excessive, leading to prolonged sub-normal phases, with a positive phase no longer than in the preceding experiment. Even the dose of 100 millions produced a second sub-normal phase to be avoided. It would seem, therefore, that the maximum useful dose for an adult is well under 100 millions; as by the smaller doses

the rise obtained was as good, and the sub-normal phases were less marked.

Immunity Produced.—It appears that the immunising power of a meningococcus vaccine so made and administered is not of much practical service as a prophylactic, and might, indeed, do harm from the production of supersensitisation. That the immunity conferred by an attack of the disease in a human subject is not of very long duration is shown by a case which we had under vaccine treatment in July, 1907, in collaboration with Dr. Rundle and Dr. Williams, of the Liverpool City Hospital, Fazakerley. During the illness a dose of 21 millions produced no local reaction. In November, 1907, the patient came to see us; the index then was 1·4 (tested once). During December, January, and February it fell to normal, and as the patient was complaining of variable but apparently increasing deafness a dose of 30 millions was injected subcutaneously.

The result obtained was in all respects comparable to a similar dose in the subject of these experiments. That is to say, six months after her illness the patient had returned to the normal state as tested by the local reaction after the inoculation of a moderate dose of vaccine. The patient's serum agglutinated her own organism once during the illness in a dilution of 1 : 25 at laboratory temperature, but on no occasion after recovery.