PART III.

SPECIAL REPORTS.

REPORT ON OPSONINS.

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THE July number of the "Johns Hopkins' Hospital Bulletin" for 1907 contains a series of five papers on the subjects of opsonins and vaccine therapy. The subjects are of so much importance, and have attracted such wide attention on the part of the profession, that the views from a new body of workers must be received with attention, and be subjected to careful study. We, therefore, think it advisable to summarise here the main points of the more important of these papers.

The first paper deals with "The Treatment of Gonorrhœal Arthritis by Vaccines." The writers selected this disease for their experimental study owing to its frequency and the fact that treatment, as at present carried out, is unsatisfactory. Another point of importance they considered is that the disease usually runs a chronic course. The strains of gonococci employed in the estimation of the index were two in number, and were obtained from a case of gonorrhœal arthritis and from a case of gonorrhœal peri-ostitis. It was found impracticable to isolate the special organism present in each individual case. An emulsion was made from cultures not more than twenty-four hours old made on blood agar. As regards vaccines, four made from different strains were employed, but it is stated that no distinct difference could be demonstrated in the clinical results or effects on the opsonic index when the patient was vaccinated with a vaccine made of his own organism, or when a different vaccine was used. The usual initial dose was 300 million

gonococci, and this was gradually increased up to 1,200 million. A local reaction was observed in most cases, but only once was at all troublesome. No negative phase was observed as far as the index was concerned. The number of injections varied in the different cases from one to eight—fifteen cases in all being treated. Looking over the charts and protocols of these cases, one finds that the index was below normal in all of them prior to vaccination, and that after the injection the index invariably rose. In one or two cases vaccination seemed to do good after other means had failed, but the writers by no means feel assured that the good result can be attributed to vaccination alone. They feel convinced that there is no real danger of cumulative negative phases, and that, therefore, the vaccines may be given at intervals of seven to ten days, uncontrolled by an estimation of the index. The technique for determining the opsonic index, indeed, they regard as too inaccurate at present to be of any real value. No brilliant results and no immediate cures were obtained, but the general experience of the writers seems to be favourable to the method. They strongly, and rightly, object to the publication of isolated miraculous cures, and maintain that the treatment must be tested like any other, by persons uninfluenced by enthusiasm for theoretical considerations. With these opinions we are in thorough agreement.

The second paper deals with "The Tuberculo-Opsonic Index and Treatment by Tuberculin." In making a bacillary emulsion the writers have found that the most satisfactory method is to emulsify the living bacilli with salt solution in the same way as a staphylococcus emulsion is prepared. The organisms are then killed by exposure to direct sunlight for ten hours, and an emulsion is obtained free of fragmented bacilli and of clumps. The limits of error in determining the index were next considered. It is pointed out that the same worker will obtain very different counts according as he counts the bacteria in fifty leucocytes found at the beginning, the middle, and the end of the smear, and that this occurs even when the worker has had prolonged and regular experience in

the general technique. It is possible, they think, to obtain a correct estimate by counting from 300 to 500 cells, but with the usual count of 50 cells they have found the index to vary from .4 to 2.34, by making a series of counts at different parts of the same two slides. They, therefore, boldly state that the present technique is too inaccurate to be of value. This deliberate opinion, based on careful work, is one with which we believe many honest workers will agree, and is in favour of doing away with the determination of the index as a guide to treatment. The writers also point out that the variations to be seen in opsonic charts can partly be accounted for by the laws of chance, and that the charts of cases that do well often do not differ materially from the charts of cases that do badly. Although the control by the opsonic index is untrustworthy, the writers think that the method of treatment by tuberculin R. in doses of from .001 to .0025 mgs. is of decided value in cases of localised tuberculosis. Injections were given to nine reported cases every eight or ten days, and in the majority of these improvement was noted, though no miraculous cures were obtained. The importance of general hygiene in addition to the tuberculin treatment is insisted on. As a diagnostic aid, Wright's method of heating the serum for the detection of immune opsonins has not proved of value.

The third paper, entitled "Studies on Opsonins," was primarily undertaken to determine to what extent the opsonic content of the blood could be increased, as it seems *à priori* strange that such immune substances as agglutinin and bactericidal anti bodies can be so enormously increased, while opsonins can only be comparatively slightly increased by the injection of opsinogens. The organism employed in the study was *Staphylococcus aureus*, and the animals worked upon were rabbits. In addition to taking the opsonic index the writer also determined the phagocytic index—i.e., the index obtained by dividing the percentage of cells phagocytizing in any given slide with the number phagocytizing in a control slide. The index was taken, moreover, with not only the undiluted serum but also with a series of dilutions up to

one in a hundred. As far as dilution was concerned, it was found that in a general way the largest percentage of cells phagocytizing occurred in the undiluted serum, and progressively diminished as the dilution was carried on. This was, however, by no means constant. Only in a general way do the phagocytic and opsonic indices run parallel. In fact, in using bacterial emulsions of different strengths the same percentage of cells may phagocyte, while the number of bacteria taken up may be widely different. The index in normal rabbits prior to inoculation was found to vary widely, and the ratio of one serum index to that of another was not found to vary proportionately when dilutions were made. As regards the main object of the inquiry, it was found impossible to raise the index as determined by Wright's methods to more than about five times the normal. The writer lays great stress on the variation of the index in normal serums, and also upon the great liability to error in making the estimation. He holds that a count of less than 600 cells is of little value, and maintains, like the writer of the last summarised paper, that the count depended on the part of the slide from which the cells were chosen. This could easily occur, for the larger polynuclear cells loaded with bacteria may reasonably be regarded as of a different consistence and bulk to those that contain few bacteria. In making the smear, therefore, they would be likely to be spread out at a different place to that of the lighter cells.

The fifth paper deals with "The Specificity of Opsonins in Normal and Immune Sera." Bulloch and Western, working with *B. pyocyaneus*, the tubercle bacillus, and with staphylococci concluded that opsonins were specific. Simon and others, working with *B. coli*, *B. typhosus*, and staphylococci come to the opposed conclusion. The present writer, in numerous experiments, found that serum saturated with staphylococci was also deprived of its opsonin for *B. coli*. On working with *B. pyocyaneus*, however, it was found that this organism was exceptional in many ways in that saturation of serum with staphylococci did not remove the opsonin for *B. pyo-*

cyaneus, and in that heating the serum does not remove the opsonin for that organism. It was also found that *B. pyocyaneus* undergoes spontaneous phagocytosis in the absence of serum more readily than other organisms. Some further experiments confirmed this writer in his view that the opsonins of normal serums are not specific, but suggested that the increase of opsonin following the injection of a bacterial vaccine is a true specific increase.

The opinions expressed above concerning the specificity of opsonins were confirmed by the writer of the fourth paper, who, working with typhoid bacilli, found that the opsonin of normal rabbit serum was largely non-specific, but that the opsonin developed during the process of immunisation of rabbits was definitely specific for the typhoid bacillus. This writer determined the amount of opsonin present by determining what dilution of the immune serum would produce phagocytosis as compared with normal serum. By this method he has demonstrated that the opsonin may increase enormously during the process of immunisation, although Wright's method of estimating the index may only show a fractional increase. The mathematics of this paper cannot, however, be regarded as very sound.

Regarding the series of papers as a whole we find some very definite expressions of opinion on the opsonin question. The more important of these appear to be as follows:—(1) Treatment of disease by vaccines, as suggested by Wright, seems in many cases to produce moderately favourable results; (2) Wright's method of determining the amount of opsonin present, although better than the method of taking the "phagocytic index," is too inaccurate to be of any real value; (3) the amount of opsonin present in the serum of normal animals seems to vary enormously from day to day; (4) in treatment of disease by vaccines it is unnecessary, and, indeed, useless, to follow the opsonic index curve as determined by Wright's methods; (5) the opsonins of normal serum are non-specific.