British Medical Journal.

SATURDAY, DECEMBER 31ST, 1910.

THE STATISTICS OF OPSONINS.

IN a recent number of *Biometrika* appears a further contribution by Drs. Greenwood and White[1] to the statistical study of phagocytic distributions as obtained by the ordinary opsonic method. Their former study[2] was based on samples of cells not exceeding 2,000 in number, and from such material they had deduced the important fact that the frequency-curves of the total distributions, as well as those of the means of small samples, exhibited a pronounced asymmetrical or skew character. In the present work the material employed was a count of 20,000 cells, made by one of the authors (J. D. C. W.) at the Inoculation Department of the London Hospital, the greatest care being taken to ensure the homogeneity of the bacterial emulsion (tubercle bacilli) and its thorough incorporation with the leucocytes and serum. The strength of the emulsion was calculated to give an average of 3 to 4 bacilli per cell, and the incubation time was fifteen minutes. The actual counting of the 20,000 cells, with the aid of a mechanical stage, involved about a month's work.

The frequencies of the leucocyte contents were then classified in the ordinary way, and it was found that a Pearson frequency-curve of skew type could be fitted with approximate accuracy to the results. The closeness of fit was not all that could be desired, however; but the discrepancy, which was due largely to a paucity of cells containing one bacillus and an excess of those containing three bacilli, was not very appreciably remedied either by including cells with bacillary clumps, or by excluding from the count those groups of 500 cells whose curves presented the greatest individual irregularities.

The authors then attacked the main problem of the investigation—namely, the distribution of means of samples of 25, 50, and 100, taken from the total series of 20,000 cells. Thus three skew curves were obtained in which the ordinates represented the number of samples (of 25, 50, and 100 cells respectively), and the abscissae the respective indices in terms of the mean of the whole sample of 20,000 cells. The odds against the occurrence of various deviations from the mean were then calculated for the three curves, and it was determined that, taking a 10 to 1 chance as the limiting value for evidence of differentiation, the index limits were 1.3 to 0.7 in the case of samples of 25, 1.25 to 0.75 in the case of samples of 50, and 1 2 to 0.85 in the case of samples of 100. In other words, for single determinations of the index, any variations between these limits were of no significance.

An interesting problem arose out of these calculations. As has been mentioned, the various indices were calculated on the basis of the mean of the whole series of cells, but in actual practice it is usual to form indices from two small samples of not more than 100 cells, and the question therefore arose what amount of qualification of their results would be rendered necessary by taking into consideration the large number of possible indices which could be derived by dividing the count of any one sample of, say, 100 cells by that of any other in the whole series of such samples. The authors attempted an empirical solution of this problem, which agrees closely with that developed from purely mathematical considerations by Professor Karl Pearson,[3] to whom the data were submitted.

The method adopted by Drs. Greenwood and White was briefly as follows: Packs of cards each having on it the number of bacilli, for example, in a sample of fifty cells, were shuffled (that is, 400 cards altogether); one was then drawn and not replaced; the remaining cards were reshuffled and another card drawn, and so on, till all the cards had been drawn. One series of numbers was thus obtained. All the cards were again shuffled, and the process of drawing repeated.

A second set of numbers was thus furnished. Each number of the first set was then divided by the corresponding number of the second set, and the various indices analysed in the usual way. The result showed that a considerable reduction of the odds previously calculated was demanded when the index was determined from a comparison of two small samples. Thus, taking for example the odds against a second sample of the same material giving an index outside the limits 1.2 to 0.8, these were reduced from 3 to 1, 6 to 1, and 15 to 1 (in the case of samples of 25, 50, and 100 respectively) to 1.5 to 1, 2 to 1, and 4.6 to 1.

The general conclusion arrived at by the authors is that no reliance can be placed on single determinations of the tuberculo-opsonic index which fall within the limits of 20 per cent. of the mean. Unless the index-determination can be repeated several times under comparable conditions, little value can be attached to the result. In diagnosis, therefore, such multiple determinations, though time-consuming, would always be practicable; but in the process of vaccination the results of successive determinations would, as the authors rightly point out, be falsified by the rapidly varying antibody content, and the labour and time which would be involved in order to obtain reasonably comparable conditions would militate against the method as a useful guide in controlling the injections.

It may be pointed out, in conclusion, that the authors' results hold good only for the tuberculo-opsonic index as it is at present practised; and it would be well if all who carry out such estimations in tuberculosis were to reflect carefully on the limitations to which Drs. Greenwood and White have shown that the method is liable, and, before drawing far-reaching conclusions, to contrast their results with the elaborate tables of calculated chances which accompany this important paper.

[1] M. Greenwood, jun., and J. D. C. White (1910): A Biometric Study of Phagocytosis, with Special Reference to the "Opsonic Index." Second memoir. On the Distribution of the Means of Samples (from the Statistical Laboratories of the Lister Institute and the London Hospital). *Biometrika*, vol. vii, p. 505.

[2] Vide *Biometrika*, vol. vi, p. 377, and this JOURNAL, 1909, i, p. 1562.

[3] Karl Pearson (1910): On the Constants of Index-distributions as deduced from the like Constants for the Components of the Ratio, with Special Reference to the Opsonic Index. Ibid., p. 531.

ANAEMIA DUE TO PARASITIC WORMS.

THE relation between parasitic worms and anaemia is one which has engaged considerable attention, both clinically and experimentally. Many of these worms induce changes in the blood—for example, eosinophilia—without causing obvious symptoms of anaemia. As far as observations go, eosinophilia seems to be much more frequently associated with the presence of nematode worms than with tapeworms or flukes, and there is no doubt that it is an almost constant symptom of miner's worm anaemia, due to *Ankylostoma duodenale*. So constant, indeed, is it in this disease and to such an extent does its degree indicate the severity of infection, that it has been recommended by Boycott as the most important diagnostic sign, next to the presence of ova in the faeces, in endemic areas. The explanation of the phenomenon of eosinophilia, as well as the other blood changes due to parasitic worms, is still quite obscure. Many are the attempts that have been made and varied the methods employed to probe the mystery, but the measure of success has not been great. A suggestion not infrequently made is that in the case of "biting" worms, such as *Ankylostoma*, the anaemia is mainly due to continued small losses of blood.

Possible enough as this may be, it is not supported by experimental evidence, and it is far from explaining the blood condition. In the case of tapeworms it is entirely inapplicable. No loss of blood occurs in "*Bothriocephalus* anaemia," and yet *Dibothriocephalus latus* is accredited with the production of one of the most profound forms of anaemia. This fact has led to search for a cause in other directions, and has naturally strengthened the present toxic theory of the etiology of the graver anaemias. It is evident that if *Dibothriocephalus* and *Ankylostoma* are to be regarded as the cause, direct or indirect, of the anaemias with which they are associated, some link must be found connecting the worm with the blood condition. The fact that certain toxic substances when injected into the blood can give rise to profound anaemia, and the demonstration that analogous substances can be isolated from the bodies of the parasites establish this link. In the case of *Dibothriocephalus latus*, which from its bulk is more suited for experimental work than the small *Ankylostoma*, Tallqvist has definitely shown that such substances are present in the tissues of the tapeworm. By suitable means they can be extracted and their nature to some extent determined. Amongst them are found a haemolysin and a haemagglutinin, which act not only *in vitro*, but also when injected into a normal animal. By such injection Tallqvist was able to reproduce an anaemic condition resembling to some extent true *Bothriocephalus* anaemia. The haemolysin is of a lipoid nature, and substances of this kind can be extracted from various normal organs of the body, in particular the intestinal mucosa, as well as from certain malignant neoplasms. The theory is, therefore, very tempting that the anaemia is the direct result of the absorption of these substances by the blood. Under what conditions they are absorbed and in what manner is still a matter of great doubt. It is of some significance, in support of this view, that no other human tapeworm is known to give rise to anaemia of a pernicious type, and that repeated attempts have failed to isolate from these a haemotoxic substance in demonstrable quantity.

The case of *Ankylostoma* anaemia is somewhat different. The anaemia is of a different type, resembling more closely the hydraemia of chlorosis. That the condition is actually hydraemic has been demonstrated by Haldane in one case. The occurrence of repeated small haemorrhages is, as already mentioned, not without some importance in the etiology, but attempts have not been wanting to ascertain the existence in *Ankylostoma* of haemotoxic substances resembling those found in *Dibothriocephalus*. The difficulties in this direction are somewhat great, for a large amount of material is necessary for a continued series of experiments. More than one observer, however, has stated that a haemolytic substance can be extracted from the tissues of *Ankylostoma*. On the other hand, the latest contribution to the subject by Loeb and Fleischer[1] contains a direct negative statement on this point. Using worms which had been dried and ground to powder, these workers were quite unable to detect the presence of any direct haemolytic substance, or of any substance which could be activated by combination with lecithin. At the same time they were able to isolate from the anterior end of the worm a substance possessing a remarkable power of inhibiting coagulation of blood; the substance is extremely stable, and is not destroyed by boiling nor by being preserved in a dried condition. It behaves very much like an analogous anticoagulin obtained by Weinberg from the sclerostome of the horse, but it appears to be of quite a different nature from the corresponding substance in the leech. The authors draw a further interesting comparison between it and the anticoagulating principle in cobra venom. In how far it is to be regarded as effecting the blood changes of *Ankylostoma* anaemia does not appear, and to determine this point much further work is necessary.

THE MEDICAL REPORT OF THE LOCAL GOVERNMENT BOARD.

THE Medical Supplement to the thirty-ninth annual report of the Local Government Board, which relates to the year 1909–10, has just been issued. It makes a volume of over 600 pages. Only some thirty-five are occupied by the report of the Medical Officer to the Board. These Dr. Newsholme devotes to a general review of the work of his department for the year, and gives up the rest to publishing in full the reports of his various assistants on special pieces of work conducted by them. Certain of these have been issued previously and been noticed at the time in our pages, but others make a first appearance. The work covered by these appendices as a whole falls into two classes, of which the first is made up of literary research in the form of reviews of the manifestations of various diseases throughout the world during the year under consideration. It is work which could be done anywhere, but nowhere better, and probably nowhere so well, as in the offices of such a centre of information as the Local Government Board. The other class consists of investigations of a purely scientific but thoroughly practical character. The reports on these will, we have no doubt, be the subject of frequent reference in our own columns and elsewhere, and meantime, as they all alike form good examples of the admirable work which the Medical Department of the Local Government Board is performing, we are unwilling to pick out any of them for

[1] *The Journal of Infectious Diseases*, vi[1], No. 5, October 25th, 191 pp 625–31.