A STUDY IN PYRETOTHERAPY.*

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The treatment of diseased conditions by means of induced pyrexia is now so frequently carried out that it is surprising how little we know of the biological processes involved. It is not an exaggeration to say that the beneficial effects of malaria in general paralysis are as gratifying as those of liver in pernicious anæmia, and of insulin in diabetes, but we still await a satisfactory explanation of its mode of action. An attempt in this direction was made by Eddison (1), who observed a failure of leucopoiesis in untreated cases of paralytic dementia, which he attributed to degeneration in the reticulo-endothelial system. He considered that, in general, pyrexia was associated with leucocytic reactions, and that this resulted indirectly in reticulo-endothelial stimulation. It is not proposed to consider here the arguments put forward by Eddison, but he should be given credit for being the first person in this country to draw attention to a very important aspect of pyretotherapy.

Reticulo-Endothelium.

The reticulo-endothelial system, in the strict sense of the term, is composed of the following elements:

(1) The endothelial cells which line the lymph sinuses of the lymphatic glands.

(2) The Kupffer cells of the liver.

(3) The reticulum cells of the marrow, which are stellate or spindle-shaped, and lie scattered about the myeloid tissue.

(4) The endothelial cells lining the sinusoidal blood-capillaries of the spleen and bone-marrow, and of the suprarenal and pituitary glands.

During the last few years this tissue has been subjected to a large amount of experimental investigation, and it is now regarded as subserving many important functions in the living organism. Its component cells are capable of acting as scavengers, and can remove

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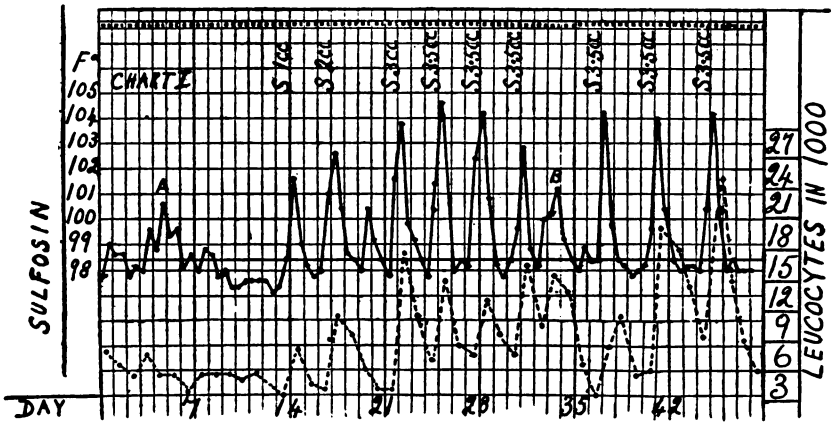
from the circulation all sorts of particulate material, and, under certain conditions, bacteria. There appears to be little doubt that the system is closely concerned with the protection of the body against disease. It is also held that this tissue is responsible, directly or indirectly, for both the formation and the disposal of blood-corpuscles. That it exercises a destructive function nobody will deny, but the published evidence as to its formative activities in post-natal life still requires confirmation. It does not by any means follow that because hæmatopoietic stimulation has occurred, the process must necessarily have involved the reticulo-endothelium. The bone-marrow contains a large stock of partially manufactured blood-corpuscles ready to meet emergencies, and these are capable of multiplication to a very high degree should necessity demand it. These cells consist, in the case of the white corpuscles, of polymorphonuclear leucocytes, myelocytes and myeloblasts, the latter being the most immature of all and containing no granules in their cytoplasm. Very possibly this hierarchy of cells is in itself sufficient to meet most of the leucopoietic demands of ordinary life, but it is possible that under exceptional circumstances blood production goes back to an earlier level of evolution, in which myeloblasts are derived from the reticulo-endothelium; and in this paper an attempt will be made to correlate the profound alterations in blood production, which characterize two forms of pyretotherapy, with changes in the activity of the more remote ancestral tissue.

Sulfosin.

During the past two or three years a large number of general paralytics, both in this country and abroad, have been treated by means of intramuscular injections of sulphur in oil. This method of inducing fever was introduced by Schroeder, who claimed that 57% of the patients treated by him had remitted (2). While my own experience of the efficacy of this substance in general paralysis has been disappointing, I am satisfied that it does, in a small number of cases, effect a definite improvement. Moreover, considered purely as a pyrexia-producing agent, sulfosin is probably the best substance that has ever been introduced for this purpose, and, since the periods of fever can be so accurately controlled, it becomes much easier to investigate the associated biological disturbances. We shall now proceed to consider some of these.

In a previous communication (3) I drew attention to the fact that in addition to the fever which they induce, injections of sulphur

in oil are almost invariably followed by a considerable degree of leucocytosis, which reaches its maximum in 24 hours, and often persists for as long as 48 hours. Eleven cases of general paralysis, which were undergoing treatment by sulfosin, were subjected for many weeks to daily white blood-counts under the most rigid experimental conditions. The samples of blood were withdrawn at approximately the same hour each morning, and the patients were at the time fasting and in bed. Over six hundred blood-counts were performed in this way, and the vast majority of them showed evidence of an increased concentration of leucocytes in the blood following sulfosin. Chart I shows very well what usually occurs,



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and refers to a case of general paralysis which was given nine injections of sulfosin over a period of five weeks. It records the daily leucocyte count and the maximum a.m. and p.m. temperature while the treatment was in progress, as well as during the fortnight prior to its inception. It will be seen that throughout the control period the number of leucocytes never rose above 8,000 per c.mm., the minimum being 3,500. The rise of temperature at the point A was associated with the performance of a lumbar puncture, and did not disturb the leucocytic curve. The first dose of sulfosin was followed next day by an appreciable increase in the number of white cells, and every succeeding dose met with a similar response. At one point, B, there were two leucocytic peaks, corresponding with the two rises of temperature which the preceding injection elicited. If the doses recorded on the chart are examined, it will be noticed

that a large dose is not necessarily followed by a high leucocytic peak, nor does the same dose, when repeated, always produce the same result, but in this, as in all my other cases, the reactions towards the termination of the course tended to be excessive. Sudden increases in the number of white cells in circulation, such as have just been described, may be due to a number of causes, prominent among which are contraction of the spleen, changes in blood volume, and accelerated production of cells by the bone-marrow. Consequently the next problem was to ascertain the cause of the leucocytosis in this particular instance.

Animal Experiments.

A rabbit was given a daily injection of sulfosin on 23 consecutive days, during which period a leucocyte count was performed every morning. After the fourth of these injections a leucocytosis was produced, and this was augmented and maintained throughout the whole period of inoculation. The leucocytosis was due to an increase of polymorphonuclear cells, which corresponds with what occurs, under similar circumstances, in man. This experiment was repeated on eight rabbits, with substantially the same results. The bone-marrows of these animals were submitted to microscopical examination, and, in every case, a marked degree of hyperplasia was found to have occurred. This varied in proportion to the amount of sulfosin administered, and in some cases was evident macroscopically as well as microscopically. The leucoblastic tissue was most affected, but the erythroblastic elements and megakaryocytes did not entirely escape. An illustrated report of the experiments has appeared elsewhere (4), so that no further reference to them is necessary. Although they show conclusively that sulfosin is a definite leucoblastic stimulant, it is obvious that the resulting polymorphonuclear leucocytosis cannot, of itself, exert any beneficial effect on paralytic dementia. It is further necessary to ascertain whether these leucocytes have developed from the reticulo-endothelium, or have merely arisen by a process of multiplication from the corpuscular reserves of the marrow.

To investigate this matter, it was decided to make use of the Indian ink method, which has proved so useful in the geographical exploration of the tissue in question. If a suspensoid, such as Indian ink, is introduced into the circulation of a living animal, the carbon particles are quickly ingested by the reticulo-endothelial cells of the liver, spleen and bone-marrow. The organs become jet

black in colour and remain so for weeks, but if appropriate dosage is employed the animal appears to suffer little inconvenience, and continues to live an outwardly normal existence for a considerable time. Fig. 1 is a photomicrograph of the bone-marrow of a rabbit treated on these lines. It illustrates very well a number of carbon particles which have been ingested by the delicate reticulo-endothelial cells lining one of the capillaries. From this photograph it will be evident that should reticulo-endothelium, which has previously been charged in this way, suddenly assume hæmato-poietic functions, there would be no difficulty in recognizing the process histologically.

A rabbit was now given, on three consecutive days, an intravenous injection of a 33% suspension of Indian ink in the proportion of 1·5 c.c. per kilogramme of body-weight. This was followed by 24 daily injections of 1 c.c. of sulfosin, after which the animal was killed and the bone-marrow examined. A considerable degree of hyper-plasia was found to have taken place, and the particles of Indian ink were still present within the cells of the reticulo-endothelium. The latter were clearly seen to exhibit hæmatopoietic activities. Primi-tive blood-cells could be discerned in process of formation within them, and whereas the endothelium lining the capillaries was mainly engaged in the production of red corpuscles, the scattered cells of the reticulum appeared to be responsible for the leucocytes. Myelocytes were observed to develop directly from ink-laden ancestors without the appearance of non-granular myeloblasts. Large nuclei were first formed which, in being extruded from their parent reticulum cells, became encircled by a granular cytoplasm. An attempt was made to photograph the process, but this was found to be impossible owing to the different planes occupied by the carbon particles. Fig. 2, however, illustrates the appearance of some of these primitive myelocytes shortly after their formation. Their nuclei are large, circular or oval in outline, and each contains one, two or three spots of basophilic chromatin. The cell marked A is generously surrounded by granular cytoplasm, while B has a narrower rim; the nucleus C has practically no cytoplasm around it, and has evidently only just been formed. In significant proximity to C lies an aggregation of Indian ink particles D, which in reality is enclosed within a reticulum cell, but the latter is not visible in the photograph. Although the myelocytes in the illustration are obviously of a very primitive type, the argument that they have arisen from the cells of the reticulum lacks force in the absence of the evidence supplied by the original sections.

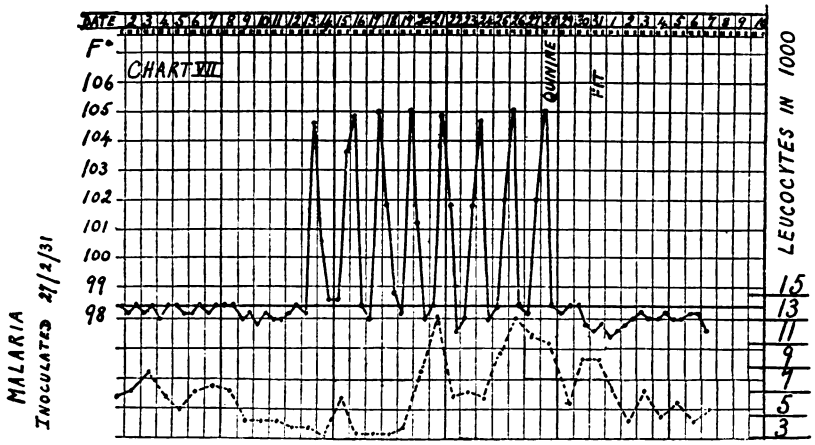
There is, however, a further indication that sulphur acts on the reticulo-endothelium. It was observed, during the course of the above-mentioned experiments, that when the drug was administered to animals which had previously received Indian ink, and whose reticulo-endothelium had thus been rendered more irritable, the resulting marrow hyperplasia was much more extensive than when sulfosin alone was employed. To test this accurately, two rabbits, Nos. 11 and 12, were given an intensive course of sulfosin for a period of a week, but prior to this one of them, No. 11, was injected with ink. The animals were approximately of the same age, and due regard was paid to the weight of each in deciding the doses to be administered. At the end of the week both rabbits were killed, and their bone-marrows examined microscopically. The hyperplasia was found to be very much greater in the case of rabbit No. 11, which had previously received Indian ink, than in that of the other animal. Figs. 3, 4 and 5 show respectively the marrow of a normal rabbit, and those of rabbits Nos. 12 and 11 referred to above. If the photograph of No. 12 (Fig. 4), which had been treated by sulfosin alone, is compared with that of the normal rabbit (Fig. 3), it will be observed that in the case of the former the fat spaces are definitely diminished in size, and the intervening cellular elements, which, of course, consist of developing red and white blood-corpuscles, are correspondingly increased. On the other hand, the marrow of No. 11 (Fig. 5), which had received the same doses of sulfosin as No. 12, but had previously been given Indian ink, shows a still greater degree of hyperplasia, the fat spaces being much smaller than those of the other animals. Indian ink, by itself has, however, the power of bringing about a degree of marrow hypertrophy, and therefore control experiments were carried out for a similar period of time on rabbits which had been given ink, but no sulfosin. With the doses of Higgins's ink employed very little hyperplasia was produced in a week, and the type of cellular response was totally different from that elicited by sulfosin, being predominantly erythroblastic in nature. We may conclude, therefore, that since the effect of sulfosin on the bone-marrow is enhanced by the presence of foreign particles within the cells of the reticulo-endothelium, the latter is either directly or indirectly influenced by this therapy.

Malaria.

An attempt will next be made to correlate the foregoing findings with the mechanisms at work in another form of pyretotherapy,

viz., malaria. The latter has stood the test of time as a most valuable therapeutic agent, and it seems highly improbable that sulfosin will ever supersede it.

The published reports of the leucocytic content of the blood in artificially induced malaria nearly all agree that in general there is a reduction in the number of white cells, although a pyrexial bout may be followed by a transient leucocytosis. Chart VII illustrates the course of the daily white count in one of my own cases, which was undergoing treatment with benign tertian malaria. These counts were performed at the same hour each day and under the



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same conditions as those of the sulfosin patients; they are, therefore, strictly comparable with these. On referring to Chart VII, it will be noticed that during the last week of the incubation period there was a definite lowering of the leucocytic level, which, except for one intermission, persisted throughout the period covered by the first three rigors. After this there was some evidence of leucocytic stimulation, which disappeared when convalescence had been established by the administration of quinine. In spite of what has been written to the contrary, the occurrence of a fit after the termination of the fever failed, in this case, to affect the daily white count.

The leucocytosis exhibited in this chart is of a wholly different nature from that which follows sulfosin, being much smaller in degree, and bearing no evident relation to the pyrexial paroxysms.

It is extremely doubtful if such a relatively mild leucocytic stimulation could affect the bone-marrow to any marked extent, and one may conclude that, in this respect at least, the two modes of pyretotherapy have very little in common. There are, however, other aspects of malaria which deserve the most careful consideration.

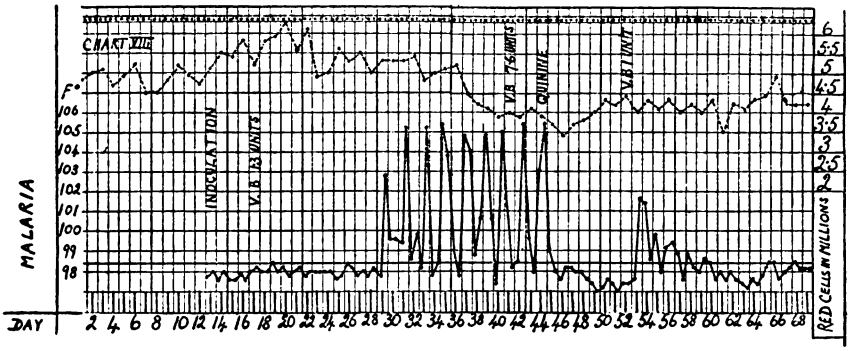
(a) *Anæmia.*

Malaria is a disease which primarily affects the red blood-corpuscles, and its effect on these is one of the most striking features of the condition, both clinically and pathologically. Nobody who has treated general paralytics in this way can fail to have been struck by the profound anæmia which is produced, and this is not uncommonly seen in those cases which never develop pyrexia. Pijper and Russell (5) report an actual increase in the number of red blood-corpuscles during the incubation period, before the onset of the anæmia, in 9 cases examined by them, while Rudolf and Ramsay (6), who investigated 4 cases, found that an erythrocytosis preceded the anæmia, and often persisted for a considerable period throughout the fever. It would appear from this that during the early stages of the infection, regeneration actually exceeds corpuscular destruction, and Pijper and Russell conclude that "an injection of malarial blood is a direct stimulus for the bone-marrow towards increased activity."

The anæmia associated with this therapy may be of a considerable degree of intensity. James (7) states that in the primary attacks of naturally acquired malaria, the loss of blood-cells during each febrile paroxysm may be from a quarter of a million per c.mm. to a million or more. Among the above-mentioned cases examined by Rudolf and Ramsay the lowest cell-count was 1,300,000, and in one of these the anæmia reached its most profound form after the quinine had stopped the rigors.

The results of my own investigations in this direction are similar to those which have just been quoted. I have been able to confirm the observation that the anæmia of malaria is preceded by a definite erythrocytosis, which persists during the first few pyrexial attacks. This seems all the more remarkable when one considers that the bone-marrow is already manufacturing a large number of additional red cells to replace those destroyed by the parasites. One must not forget, however, that part of this erythrocytosis may be due either to contraction of the spleen, or to a diminution of blood volume brought about by sweating.

Chart VIII furnishes a record of the daily red count in one of the cases treated by me. These examinations were performed at approximately the same hour each morning for a period of ten weeks. Enumerations were made by two observers from the same pipette, and the mean between the two results recorded. If the two counts differed by more than 3%, further enumerations were made until the experimental error was sufficiently reduced. In spite of these precautions, serious oscillations appear on the chart, and although some of these are possibly attributable to technical inaccuracy, sudden variations of over half a million cells should be regarded as having a deeper significance. The truth of the matter is that in general paralysis, as in a number of other diseases,



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the erythrocytes are rarely in a state of equilibrium, and consequently a single blood-count is unreliable.

Examination of Chart VIII reveals the fact that a few days after malarial inoculation there was a distinct rise in the erythrocytic level, and that this elevation was maintained, with a few minor intermissions, for about eighteen days. After the fourth rigor the curve began to fall, and the maximum degree of anæmia, 3,500,000 cells per c.mm., occurred three days after the administration of quinine. In connection with this period of anæmia, two points of great interest present themselves, and they refer not only to this particular case, but to four others in which I have been able to undertake a detailed study. Firstly, we would expect in a rapidly developing anæmia, such as has just been described, a substantial rise in the reticulocyte count to occur, similar to that manifested in pernicious anæmia after the administration of liver. Examinations of this nature were carried out at frequent intervals, but the

response was very small indeed and not in the least in keeping with the anæmia. Secondly, the bone-marrow should be able to make good, to a far greater extent than it does, the corpuscles lost during sporulation; in other words, the intensity of the anæmia is not sufficiently accounted for by the corpuscular destruction. This is a matter on which the leading authorities on malaria appear to be in agreement. I would suggest as a feasible explanation that, following the preliminary erythrocytosis, there is an actual inhibition of red blood-cell formation in the marrow due to interference with reticulo-endothelial activity. This would account not only for the excessive anæmia, but also for the absence of an adequate reticulocyte response. On this hypothesis, the hæmatopoietic tissue would be subjected, in malaria, to two opposing influences, one stimulative and the other inhibitory. Both of these would affect, either directly or indirectly, the reticulo-endothelium.

(b) *Phagocytosis.*

It has been shown experimentally that if a suspension of bacteria is injected into the circulation, the great majority of them are taken up with extraordinary rapidity by the endothelial cells of the spleen, liver and bone-marrow. Aschoff considers that this holds true, not only for the ordinary bacterial infections, but also for protozoa. In malaria many of the parasites are engulfed by the reticulo-endothelial cells of the spleen, and possibly also of the bone-marrow. Moreover, during each period of sporulation, a quantity of pigment is released into the circulation by the breaking up of the blood-corpuscles, this pigment can be demonstrated histologically to have been ingested by the cells of the reticulo-endothelium. Here are two additional factors which must influence the activity of this tissue, and it may well be that they are responsible for the inhibition of corpuscular regeneration referred to above. At all events, this process of phagocytosis in malaria must be of importance, although it is never referred to in discussions on this form of therapy.

(c) *Bilirubin Formation.*

It is now very widely held that the process of bile-pigment formation is carried out by the Kupffer cells of the liver, which form part of Aschoff's reticulo-endothelial system. It is thought that these cells transform the hæmoglobin of destroyed red blood-corpuscles

into bilirubin, and that the latter substance then passes through the liver cells to make its way into the bile-capillaries. When hæmolysis is excessive, as in pernicious anæmia, the Kupffer cells manufacture an extra quantity of bile-pigment and the latter is found in considerable amounts in the circulating blood. This phenomenon is detected by means of the *indirect* Van den Bergh reaction. In malaria, as previously pointed out, large numbers of blood-corpuscles are destroyed by the parasites, and theoretically this should result in an increase in the bilirubin content of the serum. In order to investigate this matter I examined at frequent intervals during their treatment the blood of four general paralytics. Unfortunately, it is by no means easy to perform the quantitative Van den Bergh reaction in malaria, as the other pigments in the circulation interfere with the colorimetric matching of the serum. Moreover, general paralysis is a disease in which there is not infrequently a strongly positive indirect reaction quite apart from any effect of treatment. In spite of these difficulties, a fairly reliable series of observations was obtained, and a few of these are recorded on Chart VIII. On referring to the latter, it will be seen that during the preliminary period of erythrocytosis 1·3 units of bilirubin were present; this rose to 7·6 units about the time of the eighth pyrexial peak, and a week after the administration of quinine the amount was only 1 unit. Normally the serum contains about half a unit of bilirubin, so that in malaria this pigment is definitely increased. According to modern teaching, this would involve activity on the part of the Kupffer cells, so that here again we have evidence of reticulo-endothelial stimulation. It should be noted, however, that in none of the four cases examined in this way did the serum contain a greater quantity of bilirubin than eight units. This seems to be a further indication that the anæmia is not entirely due to blood destruction, but that in addition, the formative activities of the bone-marrow are inhibited.

Discussion.

In the foregoing observations and experiments, attention has been focused on the part played by the reticulo-endothelium in the reactions of the organism to two forms of pyretotherapy, *viz.*, sulfosin and malaria. It has been shown that the leucocytosis which follows sulfosin involves a profound stimulation of the bone-marrow, and that many of the newly-formed corpuscles originate from the reticulo-endothelium. In malaria, on the other hand,

the hæmolysis brought about by the parasites leads to hæmatopoietic demands of a totally different nature. If it is conceded that the reticulo-endothelium is the ultimate source of the red blood-corpuscles, then it will follow that with malaria as well as with sulfosin this system is affected. Many, however, deny that in post-embryonic life any process of this sort occurs, and would limit the formative activities of the marrow to the multiplication and maturation of pre-existing cells. On the other hand, Sabin and her associates (9) have clearly shown that both red and white corpuscles can arise from the more primitive ancestral tissue. By means of depleting the marrows of pigeons by a period of starvation, and subsequently administering food in small quantities, they were able to observe the formation of nucleated red cells from the endothelium of the marrow capillaries. In my own experiments with Indian ink I have frequently witnessed the same phenomenon, and the matter will be dealt with on a future occasion.

There is no reason why a similar process of regeneration should not occur in malaria. Numbers of red blood-corpuscles are destroyed by the sporulating parasites, and this must seriously impoverish the existing reserves of the bone-marrow. It is conceivable that, in order to make good the loss, fresh cells have to be formed by the reticulo-endothelium. The latter, as we have already seen, exercises a phagocytic function whereby its component cells ingest both parasites and pigment. In addition, it is engaged in the manufacture of bilirubin from the hæmoglobin of the destroyed red corpuscles. Consequently, malaria exerts a threefold influence on this most important system.

Stress has been laid on a single aspect of sulfosin and malarial therapies in an endeavour to find a factor which is common to them both, and which might, in addition, throw some light on pyretotherapy in general. So many methods of inducing pyrexia have been introduced—all, apparently, with a measure of success—that it is surely time that an effort was made to formulate some principle which might be applicable to them all. There are reasons why a theory which embraces the reticulo-endothelium should be particularly attractive. In the first place we have seen that malaria, which achieves such excellent results, affects this system very intimately. Secondly, all forms of pyretotherapy tend to produce obesity after the termination of the treatment. This deposition of fat is noticeable even in patients who exhibit no mental improvement, and in some cases is very striking indeed. Since there is a fair amount of evidence that the reticulo-endothelium plays a

prominent part in the metabolism of fat (*vide* Gaucher's disease), the former may be responsible for the production of the obesity. Thirdly, the intimate relationship that exists between this system and the hæmatopoietic structures places it in a peculiarly favourable position for being affected by a variety of therapeutic agents. But even if this involvement of the reticulo-endothelium be granted, the question arises as to how the process can result in any improvement in general paralysis. In this connection a number of possibilities present themselves.

A study of the literature reveals the fact that this system is closely concerned with the protection of the body against disease. In addition to its undisputed powers of phagocytosis, it is alleged to be able to manufacture antibodies, as well as to form protective tissue reactions in the region of chronic inflammations. It is, however, quite unnecessary to assume that the curative effects of pyretotherapy in general paralysis are due to a direct bactericidal action. After all, malaria is useless in the treatment of secondary syphilis, and there is no reason why, after a sojourn in the body of fifteen to twenty years, the spirochæte should become more susceptible to its influence. There is an equal possibility that the improvement in paralytic dementia may result from a general toning up of the patient's system. The disease is one which is prone to appear at a period of life when bodily vigour is past its zenith, and metabolic changes of importance are taking place. Moreover, in this, as in all other illnesses, the clinical picture represents the reaction of the complex human organism to a number of different influences, of which, in the present instance, the spirochæte is only one. Heredity, environment and co-existing abnormal bodily states also play their part, and the modification of certain of these influences often effects an improvement in the patient's condition. This is frequently noted in those mental hospitals which draw their clinical material from the slum districts of big cities. General paralytics who have been admitted in a deplorable state of mental and physical deterioration, will often improve in a surprising way after a few weeks of good feeding and careful nursing, quite apart from the institution of any specific remedy. Consequently there is no need to attribute the good effects of pyretotherapy to a lethal action on the spirochæte, for the soil is just as important as the seed. The argument, therefore, that is advanced in this paper is that both sulfosin and malaria strongly affect the reticulo-endothelium, and that possibly other fever-producing agents act in the same way. Clinical improvement, whenever this occurs, is far more likely to

be due to changes in bodily metabolism of a general nature than to the development of specific influences inimical to the pathogenic organism.

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