INVESTIGATIONS ON THE PROBLEM OF IMMUNITY AGAINST *SPIROCHÆTA PALLIDA* IN GENERAL PARALYTICS TREATED WITH MALARIA.

By A. Beck, M.D.,

Rockefeller Research Fellow.

(From the Central Pathological Laboratory of the London County Mental Hospitals and the Devonport Laboratories, Seamen's Hospital, London.)

The improvement of general paralysis by treatment with malaria is known to be accompanied by—and may be said to be due to—the disappearance of the spirochætes from the brain. The problem of the mode of action of this therapy therefore is centred in the question to what factor the antispirochætal effect of malaria is due.

It is natural that the most impressive of the changes produced by malaria —the fever—should have first suggested itself as the possible curative agent. This assumption was strengthened by experiments on animals, in which it was found possible to prevent the development of syphilitic lesions or to cause them to heal quickly by the application of heat, either in the form of hot air (Weichbrodt and Jahnel) or hot water (Schamberg and Rule, Bessemans and collaborators, Kolmer and Rule), or the high-frequency current (Carpenter, Boak and Warren). Although these experiments make it probable that the raised body temperature participates in the curative effect of malaria, there are facts known which do not bear out the assumption that the beneficent action of malaria is due solely to the heat.

Thus, cases of general paralysis are known that have had only mild pyrexia—the temperature never reaching the height found necessary in the animal experiments—or in whom the fever never developed at all (Herrmann), but who nevertheless were cured by malaria. Furthermore, if it be assumed that heat is the sole curative factor, it is difficult to explain why spirochætes in other parts of the body should be much less or not at all influenced by it, if the long persistence of the positive Wassermann reaction in the blood and the occasional appearance of tertiary lesions after malaria treatment be recognized as evidence of continued infection. The supposed deleterious effect on the spirochæte of a body temperature of 40° or 41° C. is not in accordance with the statement of Truffi that spirochætes are able to survive for 5 hours in birds' blood, which has a normal temperature of 45° C.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

The rise of body temperature is not the only expression of fever. It is known from clinical observations and animal experiments that fever—no matter of what origin—leads to an increase of normal and immune antibodies. It is conceivable that such a non-specific increase of possible syphilitic antibodies is responsible for the curative effect of malaria. Although hitherto there has not been much evidence in favour of a humoral defence mechanism in syphilis (see Mulzer for summarized literature), it is still possible that whatever syphilitic antibodies exist might become detectable only after their increase in response to the fever. Indeed some authors claim to have found antibodies in the blood and cerebro-spinal fluid of malaria-treated general paralytics. Gallinek found that the serum of such cases had a distinct immobilizing effect on spirochætes, which was absent in untreated general paralytics and normal persons. Similar results have also been reported by Benvenuti. Scharnke and Ruete observed a similar effect also with the cerebro-spinal fluid of untreated cases, whilst the sera of these cases behaved like normal sera. Hoff and Silberstein found that a prompt immobilization of spirochætes occurred in the presence of leucocytes and cerebro-spinal fluid of general paralytics under treatment by malaria or relapsing fever. They also demonstrated the appearance in the cerebro-spinal fluid of antibodies enhancing the phagocytosis of the spirochæte (spirochætotropins). This was accompanied by the rise of the normal bacteriotropins against *B. coli*, staphylococci and streptococci. Caldwell found that the serum of malaria-treated patients exerts a bacteriolytic effect on culture spirochætes. But since—according to recent experience (Kolmer and Kast, Jahnel, Plaut)—the true *pallida* character of these spirochætes has become questionable, Caldwell's results may only be the expression of a possible increase of non-specific bacteriolysins.

As the demonstration of antibodies against *Spirochæta pallida* in malaria-treated general paralytics would not only be of theoretical interest—as explaining the mechanism of malarial therapy—but might possibly also have practical value as a prognostic sign, a further elucidation of the immunity problem in general paralysis seemed to us desirable.

The experiments which—at the suggestion of Dr. Golla—were undertaken for this purpose comprised: (1) Examinations of the behaviour *in vitro* (immobilization, eventual agglutination) of *Spirochæta pallida* in presence of serum and cerebro-spinal fluid of malaria-treated general paralytics; (2) examinations *in vivo* on the existence of a bactericidal substance (spirochætocidin), and of (3) a phagocytosis-enhancing substance (spirochætotropin) in the serum and cerebro-spinal fluid of these patients.

The samples of blood and cerebro-spinal fluid were derived from patients who had undergone treatment with malaria 2–6 months previously. As antibodies would be most likely to be present in improved cases we chose for our experiments patients who had already shown some clinical evidence of improvement.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

I. The Behaviour *in vitro* of *Spirochæta pallida* in Presence of Serum and Cerebro-spinal Fluid of General Paralytics.

Technique.—One drop of serum or cerebro-spinal fluid was mixed on a previously warmed slide with one drop of the exudate of a rabbit chancre (Truffi or Nichols strain*). The paraffin-sealed preparation was observed immediately, and at intervals of 10 minutes, 1, 2, 3 and 5 hours by the dark-ground illumination. In order to maintain a constant temperature of 37° C. the microscope was provided with a hot stage, and the preparation was kept during the intervals of the observation in the incubator. As a control, sera of known non-syphilitic persons were examined simultaneously. The spirochætes were examined as to their mobility, the speed and type of their movement, and the eventual appearance of agglutination-like phenomena.

When totally unaffected, the spirochætes showed the well-known lively rotatory type of movement. The diminution of their vitality first manifested itself as a slackening of this movement. This was followed by a phase of alternating immobility, with now and again rather lively movement. Later only sporadic, slow, undulating movements involving a part or the whole body of the spirochæte were to be remarked. Finally there was a complete standstill, only now and then interrupted by a short convulsion. With the onset of immobility the spirochætes often became broader, their silver-white appearance turned into grey and their contours became less distinct.

In this way 9 sera and 2 fluids of general paralytics as well as 3 sera and 1 fluid of known non-syphilitic persons were examined. The results of this experiment are given in Table I.

This table shows that in the majority of cases the spirochætes often exhibited a considerable diminution of their motility after one hour. Only rarely was good motility sustained after two hours and later.† Comparison of the serum and cerebro-spinal fluid of treated general paralytics and normal controls did not reveal any regular difference in their influence on spirochætal movements. These movements sometimes persisted even longer in the serum of treated general paralytics than in the control serum (Cases Su— and Ro—). The longest duration of spirochætal motility was found with the serum of an untreated general paralytic (Case Ve—), which showed after 5 hours a ++ motility, and after 7 hours still a + motility. The other untreated case behaved like the controls. Thus, contrary to Gallinek's results, our experiments did not suggest any inhibitory effect on spirochætes by the serum or

* We are indebted to Prof. Schulemann, I. G. Farbenindustrie, Leverkusen, who kindly put these strains at our disposal.

† It may be pointed out that the motility was estimated from the behaviour of the spirochætes in the entire preparation. Sometimes single spirochætes were observed to maintain an unaltered motility up to 48 hours. They were usually found near small aggregates of cells (derived from the chancre exudate), and their long survival may have been due to the vicinity of this nutritive source.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

TABLE I.—*Showing the Immobilization of* Spirochæta pallida *in Serum and Cerebro-spinal Fluid.*

Date of experiment.	Patient.	Material examined.	Time (in months) after treatment.	Result after— 10 min.	1 hr.	2 hrs.	3 hrs.	5 hrs.
28.v.35	Bl—	Serum	6	++++	o	o	o	o
,,	Ba—	,,	6	++++	+	o	o	o
,,	Su—	,,	4	++++	++	+	±	±
,,	Be—	,,	Control	++++	o	o	o	o
29.v.35	Ve—	,,	Untreated G.P.I.	++++	++	++	++	++
,,	Pa—	,,	2	++++	∓	o	o	o
,,	Fa—	,,	Untreated G.P.I.	++++	+	o	o	o
,,	Ph—	,,	2	++++	∓	o	o	o
,,	No—	,,	Control	++++	∓	o	o	o
19.vi.35	Br—	,,	6	++++	++	o	o	o
,,	Ro—	,,	4	++++	+++	++	+	o
,,	Ra—	,,	Control	++++	++	+	o	o
,,	Br—	C.S.F.	6	++++	+	±	±	o
,,	Ro—	,,	4	++++	+	+	+	o
,,	Ra—	,,	Control	++++	++	±	±	o

++++ = All spirochætes mobile.
+++ = More than half of the spirochætes mobile.
++ = About half of the spirochætes mobile.
+ = About a quarter of the spirochætes mobile; movement slower, sometimes intermitting.
± = Sporadic motility.
∓ = Only sporadic convulsions.
o = Complete immobility.

cerebro-spinal fluid of malaria-treated general paralytics compared with that of non-syphilitic persons.

Some authors (Hoffmann, Zabolotny and Maslakowetz), Jeanselme and Touraine) claim to have observed an agglutination of chancre spirochætes in the serum of syphilitics. We have not been able to satisfy ourselves as to the existence of this phenomenon. Sometimes, under prolonged observation, several spirochætes were seen to mat together in small starlike clumps, as described by Touraine, but these aggregations were only sporadically distributed amongst a majority of free spirochætes. Furthermore they were not regularly found in the sera of general paralytics, and were observed as frequently in normal sera. It is most unlikely, therefore, that these aggregations had any relation to the activity of an antibody.

The sera were also examined for the antibodies recognizable by the adhesion test (Brown and Davis). This reaction—a modification of the Rieckenberg phenomenon—was applied by its authors for the demonstration of antibodies against *Spirochæta ictero-hæmorrhagica*. Its principle is the adsorption of small particles (*B. coli*, staphylococci, blood-platelets, etc.) on spirochætes

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

the surface of which has been altered by the action of an immune serum. Krantz reports that he obtained a positive Rieckenberg phenomenon with chancre spirochætes and the serum of syphilitic rabbits. Following the technique of Brown and Davis the action of several sera from syphilitics, general paralytics and normal persons on chancre and culture spirochætes (Reiter strain), was examined but no positive adhesion reaction was obtained.

II. Investigatoins on the Presence of a Spirochætocidal Substance in the Serum.

Although the observations *in vitro* did not reveal any difference between the serum of general paralytics and normal persons, this cannot be regarded as sufficient to exclude the possibility of the occurrence of syphilitic antibodies in the former. Immobile spirochætes are still able to produce a syphilitic infection (Truffi), so that immobilization cannot be taken as a sure criterion of death. A clear demonstration of any possible spirochætocidal antibody could therefore only be carried out by an animal infection experiment.

Technique of the examination for spirochætocidins.—Four c.c. of active serum were mixed with 0·2 c.c. of an emulsion of spirochætes prepared from rabbit chancres (Truffi or Nichols strain). The mixture was kept for 2 hours in the water-bath at 37° C. and repeatedly shaken. One c.c. of the mixture was then injected into each testicle of two rabbits. For the preparation of the emulsion the excised chancres were cut into small pieces, ground in a mortar with about 4 c.c. of saline and filtered through eight layers of gauze. The number of spirochætes in these emulsions varied between 5 and 15 per field (magnification 950). As a control, sera from known non-syphilitic persons were examined in the same way. The injected animals were kept under observation for at least 3 months and were inspected weekly. In each case the syphilitic nature of the lesions which developed after the injection was confirmed by the microscopic demonstration of spirochætes.

In this way 7 sera of general paralytics, treated 2–6 months previously with malaria, 1 serum of an untreated general paralytic and 2 sera of known non-syphilitic persons were examined. As a control of the infectivity of the spirochætes a mixture of the emulsion with saline instead of serum was also injected into two rabbits.

Any inhibitory effect of the tested sera on the spirochætes might manifest itself by a prolongation of the period of incubation, or by either the complete suppression of the syphilitic lesions or a diminution of their intensity and duration. In Table II the results of these experiments are recorded.

Table II shows that there never was a complete suppression of syphilitic lesions by the addition to the spirochætes of serum of malaria-treated general paralytics. Nor was there any evidence whatsoever of a mitigating influence of the serum on the course of the syphilitic infection. The period of incubation,

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

TABLE II.—*Giving the Results of the Examination of Spirochætocidins in the Serum.*

Patient.	Kind of malaria treatment.	Time (in months) after treatment.	No. of animal.	Result of inoculation.	
Pa—	Benign tertian fever	2	B 17	+++ 46–73	The emulsion contained about 5 spirochætes (Truffi strain) per field.
			B 18	*d*	
Su—	*Pl. Knowlesii*	4	B 19	+++ 26–82	
			B 20	*d*	
Ba—	Benign tertian fever	6	B 21	++++ 46–80	
			B 22	*d*	
Be—	Non-syphilitic control	—	B 25	++++ 46–80	
			B 26	*d*	
—	Saline control	—	B 27	++++ 46–95	
			B 28	*d*	
Ph—	Benign tertian fever	2	B 56	+ 22–50	The emulsion contained about 15 spirochætes (Nichols strain) per field.
			B 57	+++ 41–70	
Hi—	Quartan fever	4	B 50	++++ 26–76	
			B 51	+++ 26–74	
Bl—	Benign tertian fever	6	B 48	++++ 26–88	
			B 49	+++ 30–67	
Fa—	Untreated G.P.I.	—	B 44	++++ 26–60	
			B 45	*d*	
No—	Non-syphilitic control	—	B 52	*d*	
			B 53	++ 33–74	

Size of chancre:
++++ = Plum.
+++ = Walnut.
++ = Cherry.
+ = Hazel-nut.

d = Prematurely died.*

The first number attached to the + signs denotes the number of days which elapsed between the inoculation and first appearance of the lesion; the second number indicates the number of days which elapsed between the inoculation and the beginning of the regression.

the intensity and the duration of the syphilitic lesions varied to a certain extent between the animals, but these variations occurred equally in both groups of animals—the rabbits inoculated with the serum of general paralytics and the control rabbits. Thus the experiment *in vivo* also did not reveal any spirochætocidal power of the serum of malaria-treated general paralytics.

III. INVESTIGATIONS ON THE PRESENCE OF SPIROCHÆTOTROPINS IN THE BLOOD AND CEREBRO-SPINAL FLUID.

It is known that in some diseases caused by spirochætes, immunity may partly depend—as in bacterial diseases—upon antibodies enhancing the phagocytosis of spirochætes. This has recently been impressively shown in the case

* These animals were lost by an outbreak of enteritis.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

of fowl spirochætosis by Levaditi and Stoel, and by Himmelweit. It has been asserted by Ehrmann, Levaditi, Gierke and others on the strength of histological examinations that *Spirochæta pallida* may also undergo phagocytosis. Nyka reports that he has observed phagocytosis of syphilis spirochætes taking place *in vitro*. The occurrence of an antibody enhancing the phagocytosis of *Spirochæta pallida* (spirochætotropin) has been described by Hoff and Silberstein in their above-mentioned experiments with the cerebro-spinal fluid of malaria-treated general paralytics. In order to verify these assertions the following experiments were undertaken with the serum and cerebro-spinal fluid of two malaria-treated general paralytics and one non-syphilitic person.

Technique.—Leucocytes were obtained by injection of a thick starch suspension into the peritoneal cavity of a guinea-pig. The animal was killed 17 hours later and the exudate washed out with a 1% sodium citrate saline solution. After having been washed once the leucocytes were suspended in saline, so that a thick emulsion was obtained.

One c.c. of this emulsion was mixed with 2 c.c. of serum or cerebro-spinal fluid and 0·2 c.c. of an emulsion of chancre spirochætes (strain Nichols, about 7 spirochætes per field) and kept for 2½ hours in a water-bath at 37° C., the mixture being repeatedly shaken. Then 1·5 c.c. of this mixture was injected intratesticularly into a rabbit. For the control of the infectivity of the spirochætes a mixture of saline (instead of serum), leucocytes and spirochætes was also injected into an animal.

TABLE III.—*Giving the Results of the Examination of Spirochætotropins.*

Patient.	Kind of malaria treatment.	Time (in months) after treatment.	Kind of examined material.	No. of animal.	Result of inoculation.
Ro—	Benign tertian fever	4	C.S.F.	B 60	*d*
				B 61	Ch ++++ 25–80
Br—	Benign tertian fever	6	,,	B 58	Ch +++ 32–85
				B 59	Ch ++++ 32–111
Ra—	Non-syphilitic control	—	,,	B 62	Ch. +++ 32–85
				B 63	o.i. + 46–100
Ro—	Benign tertian fever	4	Serum	B 5	*d*
Br—	Benign tertian fever	6	,,	B 4	o.i. + 46–85
Ra—	Non-syphilitic control	—	,,	B 65	o.i. + 25–85
—	Saline leucocytic control	—	—	B 64	o.i + 38–117

Ch., chancre with sore. o.i., deep-seated orchitic infiltration, no sore.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

Table III shows that even the combined effect on spirochætes of serum or cerebro-spinal fluid and leucocytes never succeeded in preventing the development of the syphilitic infection. There was a greater variability in the time of incubation, the intensity and duration of the syphilitic lesions than in the foregoing experiments. But the variations affected equally the animals injected with the material from general paralytics and the control animals. In some rabbits the injection was only followed by a deep-seated orchitic infiltration, which regressed without developing into a chancre with sore. In view of the relatively small number of animals showing this diminished form of infection, it is not justifiable to make conjectures about its possible cause. But its equally frequent occurrence in the "general paralytic animals" and the control rabbits excludes the possibility of these attenuated lesions being due to the action of an antibody.

Discussion.

The experiments described give no evidence of the participation of a humoral defence mechanism in the curative effect of malarial fever. It must, however, be admitted that the search for antibodies could only be carried out by rough methods. For an exact examination a quantitative titration of the hypothetical antibody with varying doses of spirochætes would have been necessary. In view of the very great consumption of animals which such an experiment would involve we desisted from it.

The action of an antibody being with great probability excluded, and that of the heat factor not being in accord with all the facts of malarial therapy, the assumption is strengthened that the destruction and subsequent disappearance of the spirochætes from the brain is due to the activity of the cells themselves. The observation of an intensified inflammatory reaction in the brain (Sträussler and Koskinas), the evidence of increased cell destruction from the increase of the amino-nitrogen content in the cerebro-spinal fluid (Donath and Heilig, Wiechmann), as well as the increase of the signs of inflammatory reaction in the cerebro-spinal fluid during malaria (Benvenuti), all point to a greater cellular activity in the brain and tend to support this conception. But even if the increase of the local inflammatory process be assumed to be the basis of the curative effect of malaria, the mechanism by which the fight between the spirochætes and the cells is decided in favour of the latter ones has yet to be determined.

I wish to thank Dr. Nicol, Medical Superintendent of Horton Mental Hospital, for affording me every facility for obtaining material, and Dr. Hutton, who very kindly obtained the blood and cerebro-spinal fluid from patients at Horton. I have further to thank Dr. Golla, Director of the Central Pathological Laboratory, who rendered this work possible, and Dr. A. Davies, Director of the Devonport Laboratories, Seamen's Hospital, for the hospitality given in his laboratory.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press

Conclusions.

1. The serum and cerebro-spinal fluid of general paralytics treated with malaria and of normal persons do not differ in their influence on the motility of *Spirochæta pallida*; there is no evidence of agglutinins against syphilis spirochætes or of antibodies recognizable by the adhesion phenomenon (Brown and Davis).

2. The virulicidic test on the animal did not reveal the existence of spirochætocidins in the serum.

3. No evidence was found of an antibody enhancing the phagocytosis of *Spirochæta pallida*.

4. The mechanism leading to the improvement of general paralysis is not of humoral, but of cellular nature.

References.—(1) Benvenuti, " Il mecanismo di azione della malariaterapia," Tozzi, Rome, 1933, quoted by Wagner-Jauregg, *Klin. Wochenschr.*, 1935, xiv, 2, p. 481.—(2) Bessemans, A., Potter, F. de, and Hacquaert, R., *C.R. Soc. Biol.*, 1928, xcix, pp. 1610, 1613; *ibid.*, 1929, c, p. 757; ci, p. 163.—(3) Brown, H. C., and Davis, L. J., *Journ. Exp. Path.*, 1927, viii, p. 397.—(4) Caldwell, W. A., *ibid.*, 1930, xi, p. 1.—(5) Carpenter, C. M., Boak, R. A., and Warren, S. L., *Amer. Journ. Syph.*, 1930, xiv, p. 346; *Journ. Exp. Med.*, 1932, lvi, p. 751.—(6) Donath and Heilig, *Wien. klin. Wochenschr.*, 1926, xxxix, 1, p. 353.—(7) Ehrmann, S., *ibid.*, 1906, xix, p. 828.—(8) Gallinek, A., *Monats. f. Psych. Neurol.*, 1931, lxxix, p. 292.—(9) Gierke, E., *Münch. med. Wochens.*, 1906, liii, p. 393.—(10) Herrmann, G., *Med. Klinik*, 1925, xxi, 1, p. 395.—(11) Himmelweit, F., *Zeitschr. f. Hyg. u. Infektionskr.*, 1933, cxv, p. 710.—(12) Hoff, H., and Silberstein, F., *Zeitschr. f. ges. exper. Med.*, 1925, xlviii, p. 619; *ibid.*, 1926, xlix, p. 294.—(13) Hoffmann, E., *Derm. Zeitschr.*, 1906, xiii, p. 565.—(14) Jahnel, F., *Klin. Wochenschr.*, 1934, xiii, p. 550.—(15) Jeanselme, E., and Touraine, A., quoted in Jeanselme, *Traité de la Syphilis*, Paris, 1931, ii, p. 124.—(16) Kolmer, A., and Kast, C. C., *Amer. Journ. Syph.*, 1929, xiii, p. 419.—(17) Kolmer, A., and Rule, A. M., *Arch. Derm. Syph.*, 1933, xxvii, p. 660.—(18) Krantz, W., *Centralbl. f. Haut. u. Geschlechtskr.*, 1926, xix, p. 718.—(19) Levaditi, C., *Ann. Inst. Pasteur*, 1905, xix, p. 41.—(20) *Idem* and Stoel, *C.R. Soc. Biol.*, 1931, cvii, p. 1528.—(21) Mulzer, P., *Handbuch der Haut- u. Geschlechtskr. ed.*, 1928, v. Jadassohn, xv, 1.—(22) Nyka, W., *C.R. Soc. Biol.*, 1933, cxiv, pp. 1060, 1148.—(23) Plaut, F., *Zeitschr. f. Immun.*, 1933/34, lxxxi, p. 479.—(24) Schamberg, J. F., and Rule, *Arch. Derm. Syph.*, 1926, xiv, p. 243.—(25) Scharnke and Ruete, *Zeitschr. f. d. ges. Neurol. u. Psychiatr.*, 1921, lxiv, p. 343.—(26) Sträussler, E., and Koskinas, G., *Wien. med. Wochenschr.*, 1923, lxxiii, p. 783; *Zeitschr. f. d. ges. Neurol. u. Psychiatr.*, 1925, xcvii, p. 176.— (27) Truffi, G., *Centenaire d'Alfred Fournier*, Paris, J. Peyronnet, 1932, p. 99.—(28) Weichbrodt, R., and Jahnel, F., *Deutsch. med. Wochenschr.*, 1919, xlv, p. 483.—(29) Zabolotny, D., and Maslakowetz, *Centralbl. f. Bakteriol.*, 1907, I. Orig., xliv, p. 533.

https://doi.org/10.1192/bjp.82.338.254 Published online by Cambridge University Press