EXPERIMENTS ON THE ORIGIN OF THE WASSERMANN REACTION IN THE CEREBRO-SPINAL FLUID.

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Whilst it is an undisputed fact that under certain clinical and experimental conditions various antibodies (antitoxins, agglutinins, bacterio- and hæmolysins, complement-fixing antibodies) can be demonstrated in the cerebro-spinal fluid, there is a divergency of opinion about their origin. Whereas some authors (Dujardin and Dumont, Ramon, Descombey and Bilal, Neufeld and Szyle, Nélis) ascribe their presence in the cerebro-spinal fluid to their passage from the blood through a damaged blood-cerebro-spinal fluid barrier, other investigators (Mutermilch, Illert, Grabow and Plaut, Friedemann and Elkeles) believe that the central nervous system or its membranes are able to produce antibodies on their own upon contact with an antigen. In the case of the Wassermann antibody in the cerebro-spinal fluid the question of its origin is of particular interest, because of the occasional occurrence of cases which show a positive Wassermann reaction in the cerebro-spinal fluid and a negative or weaker reaction in the blood. This divergence between blood and cerebro-spinal fluid is often quoted as an example of the independence of the cerebro-spinal fluid antibody.

In order to elucidate this problem, I have tried to produce experimentally a Wassermann reaction in the cerebro-spinal fluid of rabbits, and to obtain evidence of its nature by a close comparison of the blood and cerebro-spinal fluid reactions over a long period. Whilst most authors (Steiner, Plaut and Mulzer, Plaut, Mulzer and Neubürger) only report negative results with the Wassermann reaction in the cerebro-spinal fluid of syphilitic rabbits, Tani and his collaborators produced a positive Wassermann reaction in the cerebro-spinal fluid of rabbits by repeated intracerebral or intracisternal infections. Their procedure was followed in the present experiments.

Experimental Work.

For these investigations 16 rabbits were used, 11 of which were infected at weekly intervals by three to five intracisternal injections and the remaining 5 by repeated (three) intracerebral injections of syphilis spirochætes. Eight rabbits had been infected 5 to 12 months previously with syphilis by the usual testicular

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route; the other 8 rabbits were normal animals. The syphilitic animals were chosen because, according to Levaditi and his collaborators, the persistence of intracerebrally injected spirochætes seems to be enhanced by a previous testicular syphilitic infection. Only 11 rabbits lived to the end of the experiment, the 5 others died prematurely. Blood and cerebro-spinal fluid were taken at weekly intervals. The shortest period of observation was 7 weeks, the longest 12 weeks. The following tests were carried out: the Wassermann reaction of the blood and the Wassermann reaction, cell-count, Pandy and Nonne-Apelt reactions of the cerebro-spinal fluid. The reactions in the cerebro-spinal fluid were made by the micro-methods described by Plaut.

The present experiments were restricted to the study of the serological changes brought about by the spirochætes and—except in a few cases—no examinations were made as to the fate of the injected spirochætes in the rabbit's brain. Numerous previous investigators (for literature see Truffi and Levaditi) have shown that it is impossible to produce a permanent invasion of the rabbit's brain with spirochætes by intracerebral or intracisternal injection. I have also tried other methods described in the literature for obtaining such an invasion. Schlossberger reported that syphilis spirochætes might acquire the power of invading the rabbit's brain by a previous passage through a mouse's brain. This has been confirmed to a certain extent by Plaut and Raiziss, but Tani and Ogiuti had negative results. This experiment was repeated on 3 rabbits which had developed chancres after a previous infection with the brains of 3 syphilitic mice (Truffi strain). None of the brains of these 3 rabbits gave rise to a chancre when inoculated in the testes of 6 normal rabbits. In view of the suggestive histological findings of Hauptmann and Gärtner in the brain of so-called "Nuller" (zero) rabbits (rabbits with an asymptomatic syphilitic infection), the brain of two such rabbits was inoculated into the testes of fresh animals, also with negative results. The following experiments will show that the syphilis spirochæte, in spite of its inability to invade permanently the rabbit's brain, is able to produce definite serological changes in the cerebro-spinal fluid when brought into contact with the brain.

Technique of infection.—Broth emulsions were prepared from rabbits' testicular chancres (Truffi and Nichols strains), which had been excised under sterile conditions. The emulsions were freed from coarse particles by short centrifugation at low speed and used immediately afterwards for the infection. A sample was kept for the later counting of the spirochætes. Their number in the emulsion varied from 1 to 15 per field (magnification, × 950).

For the intracerebral infection the animal was anæsthetized by intravenous injection of 0·75 to 1·0 c.c. of somnifaine. Through a small trephine hole, made with a hand drill, a short and thin needle of the type used for intradermal injections was introduced about 2 to 3 mm. deep into the brain and 0·3 to 0·5 c.c. of the spirochætal emulsion was injected. The site of the injection was about 3 mm. to the right or left of the middle line, and at the level of a line joining the two posterior orbital angles.

The intracisternal puncture was made following Plaut's technique. Before

injecting the spirochætal emulsion the cerebro-spinal fluid was allowed to drain off. Thus it was possible to inject up to 1·0 c.c. of the emulsion.

As a preliminary to these experiments it was thought useful to gather some data about the cerebro-spinal fluid of the normal stock rabbits, and of rabbits infected with syphilis by the usual testicular route of infection.

Table I gives the results of these examinations :

TABLE I.*—*Giving the Results of the Examination of the Cerebro-spinal Fluid of Normal Rabbits and of Rabbits Infected Subscrotally with Syphilis.*

Origin of C.S.F.	Number of C.S.F.'s tested.	W.R. in C.S.F.		Cell-count (average in brackets).	Pandy reaction.	Nonne Apelt reaction.
		Positive.	Negative.			
Normal rabbits	20	...	20	0–21/3 (5/3)	All negative.	
Syphilitic rabbits	38	1	37	0–53/3 (6/3)		

It will be seen that in none of the 20 normal rabbits and in only 1 out of 38 syphilitic rabbits was a positive Wassermann reaction found in the cerebro-spinal fluid, although many rabbits of the latter group had a positive Wassermann reaction in the blood. The one positive reaction which was found was only a weak one. The corresponding cell-count in the cerebro-spinal fluid was 18/3, the Pandy and Nonne-Apelt reactions were both negative, the blood Wassermann reaction weakly positive. The rabbit in question showed extensive metastatic lesions of skin, bones, eyes and nose.

If 9/3 be the maximal cell-count for the cerebro-spinal fluid of a normal rabbit, only one of the normal rabbits and two of the syphilitic ones (one being the above-mentioned animal) showed a cell-count above normal (21/3 and 53/3 ; 18/3.) When these cases are excluded the average cell-count in normal and syphilitic rabbits is lowered to 4/3 and 3/3 respectively. Thus, with a few exceptions, the cerebro-spinal fluid of rabbits infected with syphilis by the testicular route and punctured once only was found to be normal.

TABLE II.—*Giving the Summarized Results of the Weekly Examinations of the Cerebro-spinal Fluid and Blood of Rabbits Infected Intracisternally or Intracerebrally with Syphilis.*

Way of infection.	Number of animals.	W.R. in C.S.F. positive.	Incubation time (in weeks) of W.R. in C.S.F. (average in brackets).	Highest cell-count (average in brackets).	Pandy positive.	Nonne-Apelt positive.	W.R. in blood positive.	Incubation time (in weeks) of W.R. in blood (average in brackets).
Intracisternal	7	3	2–4 (2·6)	86/3–1912/3 (1131/3)	5	5	5	2–6 (4)
Intracerebral	4	1	5	88/3–357/3 (286/3)	3	3	4	

Quite different results were found in the rabbits infected with syphilis by the intracisternal or intracerebral route. The results obtained with these rabbits are summarized in Table II. From this table it will be noted that 4 out

* In these as well as in the following examinations only cerebro-spinal fluids which were free of blood were counted.

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of 11 rabbits developed a positive Wassermann reaction in the cerebro-spinal fluid. One of them had been infected 5 months previously by subscrotal inoculation; the three others had no previous syphilitic infection. Further details concerning these rabbits will be seen from the charts below. Nine out of 11 rabbits, after an average incubation period of 4 weeks, also developed a positive Wassermann reaction in their blood, thus indicating the generalization of the injected virus.* One rabbit belonging to the group of animals with a previous testicular infection had a positive Wassermann reaction in the blood before the intracerebral infection was made. There were no other manifest symptoms (metastatic lesions) of generalization, such lesions being very rarely observed with the two strains at my disposal. All the rabbits showed a pronounced increase of the cell count in the cerebro-spinal fluid, and most of them also developed a positive Pandy and Nonne-Apelt reaction. The cell-count was usually highest following the injections and then steadily decreased. In the cases which developed a positive Wassermann reaction in the cerebro-spinal fluid it had—like the Pandy and Nonne-Apelt reactions—less tendency to diminish. The changes in the cell count and the globulin reactions are most probably only the response to the non-specific meningeal irritation set up by the injection itself and the injected tissue material, although the participation of a specific spirochætal factor cannot be ruled out.

The results of the weekly examinations of the blood and cerebro-spinal fluid of rabbits with a positive Wassermannn reaction in the cerebro-spinal fluid are shown in Charts 1 to 4. It will be seen that the highest titre of the Wassermann reaction reached in the cerebro-spinal fluid was 1 : 8 (Charts 1 and 4). In 2 cases the Wassermann reaction in the cerebro-spinal fluid persisted to the death of the animal (duration, 4 and 5 weeks respectively), and might even have persisted longer if the animals had been kept alive† (Charts 1 and 3). In the third case the duration of the Wassermann reaction was 2 weeks only and the titre did not exceed 1 : 1 (Chart 2). In the fourth case the Wassermann reaction in the cerebro-spinal fluid reached a titre of 1 : 8 and persisted for 6 weeks.

Whilst the 3 first-mentioned rabbits showed a close relation between the Wassermann reaction in the cerebro-spinal fluid and in the blood—the reaction in the cerebro-spinal fluid being always accompanied by a positive reaction in the blood of at least the same intensity—the fourth rabbit (B 443) showed a somewhat different behaviour. In this case a positive Wassermann reaction appeared in the cerebro-spinal fluid after the fourth intracisternal injection and

* In view of the divergency of opinions about the accuracy of the Wassermann reaction in the blood of rabbits, I should like to add that on examination of 215 rabbits' sera I observed a positive reaction in 6·8% and a doubtfully positive reaction in 3·4% of normal rabbits. The corresponding figures for syphilitic animals with clinical manifestations were 73·3% and 10%, and those for syphilitic rabbits without clinical manifestations 24·7% and 6·2% respectively.

† The animals were killed for the transfer of their brains and cerebro-spinal fluids into the testes of normal rabbits (see below).

persisted for 2 weeks whilst the reaction in the blood remained negative. Only after 3 weeks (6 weeks after the beginning of the injections) did the reaction in

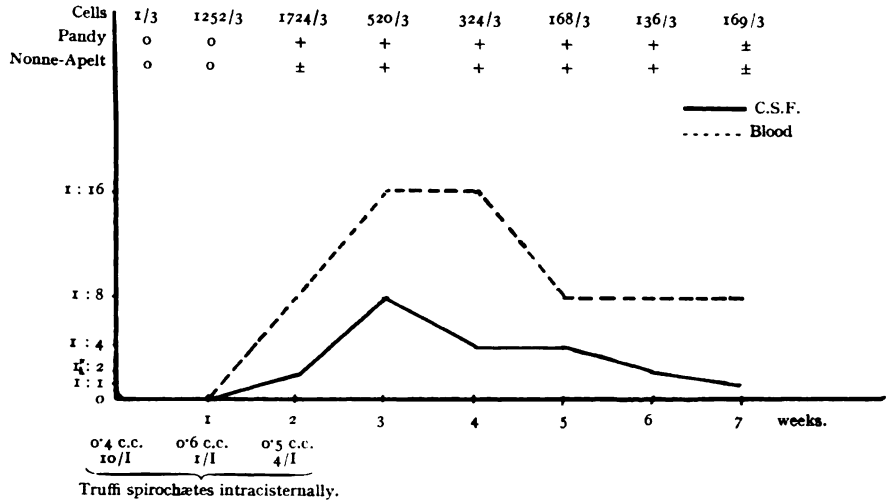

CHART 1.—Wassermann reaction in blood and cerebro-spinal fluid of rabbit B 243 (normal animal injected three times intracisternally with Truffi spirochætes.

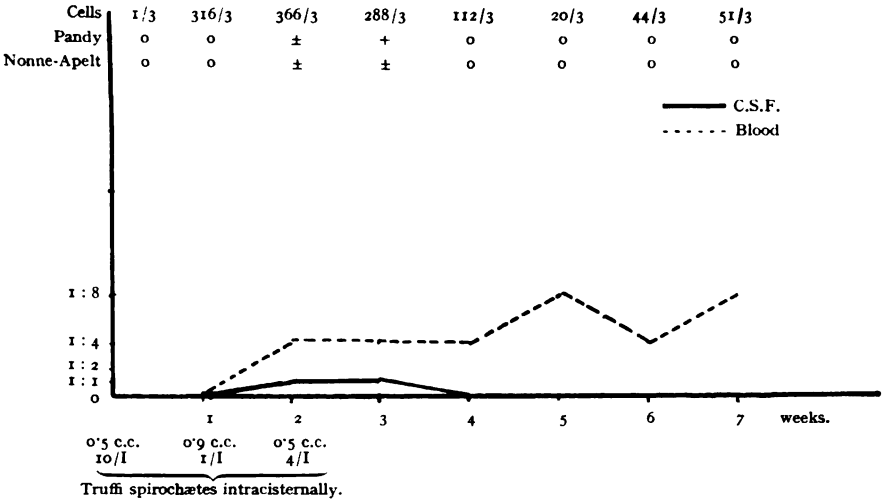

CHART 2.—Wassermann reaction in blood and cerebro-spinal fluid of rabbit B 170 (subscrotal Truffi infection 5 months previously; now injected three times intracisternally with Truffi spirochætes).

the blood become positive, its titre being at first lower than the titre in the cerebro-spinal fluid. But after 4 weeks the titre of the blood reaction exceeded the titre of the reaction in the cerebro-spinal fluid, which by now had weakened

and which finally disappeared 2 weeks before the blood became negative. In order to make sure that these isolated positive reactions in the cerebro-spinal fluid were not due to the fact that the serum alone had been inactivated (thereby

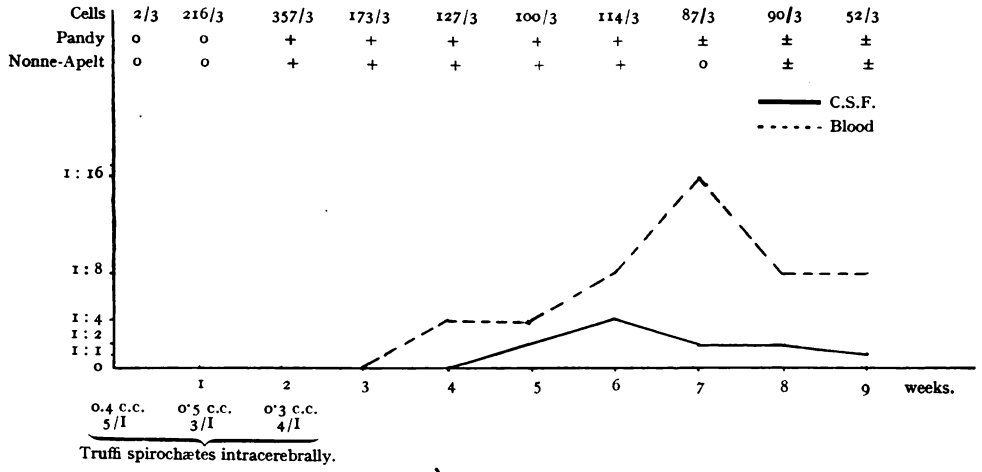

CHART 3.—Wassermann reaction in blood and cerebro-spinal fluid of rabbit B 3 (normal animal injected three times intracerebrally with Truffi spirochætes).

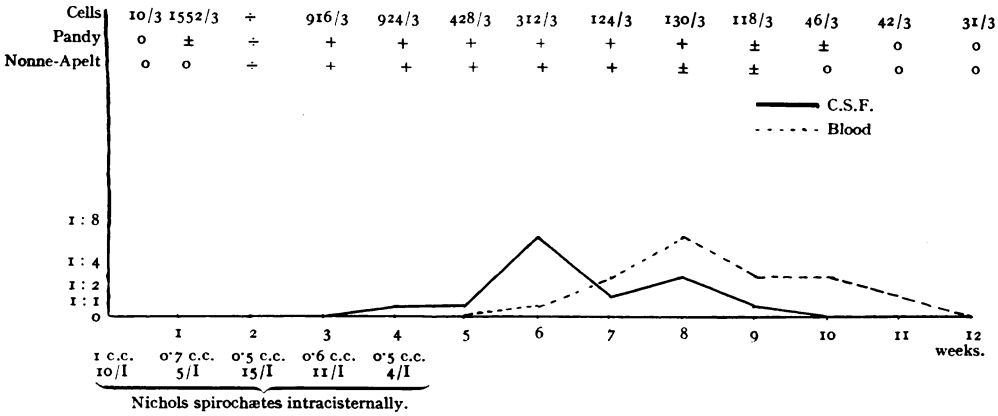

CHART 4.—Wassermann reaction in blood and cerebro-spinal fluid of rabbit B 443 (normal animal injected five times intracisternally with Nichols spirochætes).

destroying a possible weak antibody), the test was repeated with cerebro-spinal fluid which had also been inactivated. Although the intensity of the Wassermann reaction was thereby reduced, the second positive cerebro-spinal fluid specimen of rabbit B 443 (obtained 5 weeks after the beginning of the injections) still showed a positive Wassermann reaction in the absence of a positive reaction

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in the blood, and the Wassermann reaction titre of the third specimen was still distinctly higher than the corresponding blood titre. This goes to show that the difference found between the cerebro-spinal cord and the blood during the first three weeks was a genuine one.

In order to verify whether the Wassermann reaction in the cerebro-spinal fluid was dependent upon the persistence of spirochætes in the central nervous system, the brain and cerebro-spinal fluid of 3 rabbits with a positive Wassermann reaction in the cerebro-spinal fluid were inoculated, 7 and 9 weeks respectively after their first injection, into the testes of normal rabbits, in all 3 cases with negative result. It is noteworthy that 2 of these rabbits still had a positive Wassermann reaction in the cerebro-spinal fluid at the time of their death. Thus it appears that the Wassermann reaction, once it is established in the cerebro-spinal fluid, might persist there in the absence of spirochætes. These experiments at the same time illustrate the well-known difficulty of producing a persistent syphilitic infection of the central nervous system of rabbits.

Discussion.

At first sight the close relationship between the reactions in the blood and cerebro-spinal fluid which was found in the first three cases appeared more in favour of the hæmatogenous origin of the Wassermann antibodies in the cerebro-spinal fluid than of their independent formation—although it would not contradict the latter possibility. On the other hand, the independent behaviour of the cerebro-spinal fluid reaction found in the fourth case cannot be explained otherwise than by a local formation of antibodies in the central nervous system. Furthermore the following objection opposes itself to the assumption of the passage from the blood as the sole source of the Wassermann antibody in the cerebro-spinal fluid. If the damage to the blood-cerebro-spinal fluid barrier be alone responsible for the appearance of antibodies in the cerebro-spinal fluid, it is difficult to understand why no Wassermann reaction was found in the cerebro-spinal fluid of other rabbits with an equally high titre of Wassermann antibodies in the blood and with a meningeal irritation which, according to the cell-counts and the globulin reactions in the cerebro-spinal fluid, was of equal or even greater intensity than that found in the above-mentioned four rabbits. Thus it would appear that there is strong reason to assume the possibility of an independent rise of Wassermann antibodies in the cerebro-spinal fluid. On the other hand, the results obtained do not exclude the possibility that such locally formed antibodies might be increased by the passage of antibodies from the blood.

Summary.

1. In 20 normal and 38 syphilitic rabbits infected by the testicular route, a positive Wassermann reaction was found in the cerebro-spinal fluid only once, and that in a rabbit with severe generalized lesions.

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2. In 4 out of 11 rabbits a positive Wassermann reaction was obtained in the cerebro-spinal fluid by repeated intracisternal or intracerebral infections with syphilis spirochætes. Nine out of 11 of these rabbits also developed a positive Wassermann reaction in the blood.

3. In one of these 4 cases the Wassermann reaction in the cerebro-spinal fluid was found to rise and persist independently from the reaction in the blood. In the other 3 cases the cerebro-spinal fluid reaction followed closely the blood reaction.

4. The Wassermann reaction in the cerebro-spinal fluid may still persist when spirochætes are no longer demonstrable in the central nervous system by inoculation experiment.

5. The results described make it probable that at least a part of the Wassermann antibodies found in the cerebro-spinal fluid is formed locally in the central nervous system.

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