

SUPPRESSION OF THE FIRST ATTACK WITH SUBSEQUENT
RELAPSE: AN IMMUNE PHENOMENON IN EXPERI-
MENTAL RELAPSING FEVER.

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(Received for publication, August 6, 1928.)

A series of experiments has already been reported (1) in which it was found that Chinese squirrels and chipmunks, when inoculated intraperitoneally with *Spirocheta recurrentis*, would show many spirochetes in the blood for a period of 1 to 5 days, and would recover without a relapse; but if the spleen were removed one or two relapses would occur. In a subsequent paper (2), the development of six distinct strains of spirochetes as the result of relapses was reported, and it was shown that strains which appeared in alternate attacks were closely related to one another, while strains appearing in consecutive attacks were immunologically quite different from each other.

During the course of the latter experiments a phenomenon was encountered, of which I have seen no report in the literature on relapsing fever. This phenomenon was the suppression of the first attack in animals which were reinoculated with a strain of spirochetes which had recently been present in the blood, and the subsequent occurrence of a relapse after the interval which usually occurred between attacks. That this delayed attack was a relapse and not a true first attack with a long incubation period, was proven by the fact that the spirochetes causing it were shown to belong to a different strain than the strain inoculated. The phenomenon was met with five times during the course of the experiments, as shown by the following protocols.

PROTOCOLS.

Observation 1.—A squirrel whose spleen had been removed 7 days previously, was inoculated with Strain I spirochetes. The tail blood was positive for the 4 succeed-

ing days, but no relapse occurred. After this attack the blood serum showed the presence of agglutinins only for Strain I. 32 days later the squirrel was reinoculated with Strain I. For 6 days thereafter no spirochetes appeared in the tail blood; but on the 7th day they appeared, and continued to be present in greatly increasing numbers for 4 days. After this attack the blood serum con-

TABLE I.
Course of Infection in Squirrel of Observation 1.

Date	Strain inoculated	Attacks			Negative intervals, duration	Titer of serum during interval for strain					Strain causing attack
		No.	Day	Tail blood		I	II	III	IV	V	
IV/1/27	I	1	1	+	32	1280	0	0	0	-	I
			2	++							
			3	+++							
			4	++++							
V/7/27	I	2	1	+	7	100+	100+	0	0	0	II
			2	++							
			3	++++							
			4	++++							
		3	1	+	6	100+	100+	100+	0	0	III
			2	+							
			3	+++							
			4	+++							
			5	+++							
		4	1	+	20						V*
			2	++							
			3	++							
			4	+							

* Origin of Strain V. Proved by agglutination tests not to belong to Strain I, II, III or IV.

tained agglutinins for both Strain I and Strain II, indicating that the spirochetes causing the delayed attack were not the strain inoculated but belonged to Strain II, the strain which usually followed Strain I in the first relapse. After another interval of 7 days another relapse occurred, which caused the development of additional agglutinins for Strain III. Another interval of 6 days then occurred, followed by another relapse. The spirochetes of this relapse were tested against

monovalent immune sera and were found to belong to Strain V (see Meloney (2) for details of strains). This squirrel, therefore, theoretically had four attacks following its reinoculation, but the first attack was suppressed because the animal was already immune to the strain of spirochetes inoculated. The record of this squirrel is summarized in Table I.

Observation 2.—A chipmunk was inoculated with Strain I spirochetes without previous splenectomy. Spirochetes were present in the tail blood on the 3 succeeding days. No relapse occurred. The blood serum after this attack contained agglutinins for Strain I but not for Strain II. Other strains were not tested. 12 days after the close of the attack the spleen was removed and 5 days after that the animal was reinoculated with Strain I spirochetes. The tail blood was negative for the next 5 days, after which an attack occurred which lasted 4 days. After this attack the animal's blood serum contained agglutinins for both Strain I and Strain II, indicating that the attack had been due not to the Strain I spirochetes with which it had been reinoculated, but to Strain II.

Observation 3.—A second chipmunk was inoculated with Strain I spirochetes without previous splenectomy. The tail blood was positive for spirochetes on the 3 succeeding days. No relapse occurred. The spleen was removed 16 days later, and 14 days after splenectomy the animal was reinoculated with Strain I spirochetes. The tail blood remained negative for 5 days, after which an attack ensued with spirochetes in the blood for 5 days. After this the animal's serum was found to contain agglutinins for Strain II, indicating that the attack had been due to that strain rather than to Strain I, with which the animal had been reinoculated. After another interval of 8 days a second relapse occurred lasting 2 days.

Observation 4.—A squirrel was inoculated on the day following splenectomy with Strain II spirochetes. After an incubation period of 3 days spirochetes appeared in the blood for 2 days. The attack was followed by a negative interval of 7 days and a relapse lasting 3 days. There was no second relapse. The animal's serum after the relapse contained agglutinins for Strain II, Strain I and Strain III.¹ After waiting 20 days from the close of the relapse, this squirrel was reinoculated with Strain III spirochetes. A negative interval of 6 days ensued, and then spirochetes appeared in the blood for 3 days. After this attack the animal's serum contained additional agglutinins for Strain V, indicating that the attack had been due to that strain rather than to Strain III, with which it had been reinoculated. After another interval of 4 days a relapse occurred in which the spirochetes were proven by agglutination tests to belong to Strain VI.

Observation 5.—This squirrel was inoculated three different times. The first inoculation was made with Strain I on the day following splenectomy. A single attack followed which lasted 5 days. After this attack the animal's serum contained agglutinins only for Strain I. After waiting 23 days it was reinoculated with Strain I and the tail blood contained spirochetes for the 3 following days.

¹ It was frequently found that, after a first relapse in an animal inoculated with Strain II, agglutinins for both Strain I and Strain III were present.

Thus the first reinoculation was not followed by suppression of the first attack as in the other four observations. After an interval of 7 days a relapse occurred lasting 3 days, and after another interval of 9 days a second relapse occurred lasting 3 days. The spirochetes of this second relapse were proven by agglutination tests with monovalent sera to belong to Strain III, but after this relapse the animal's serum contained agglutinins not only for Strains I, II and III, but also for Strain IV. 16 days later it was reinoculated with Strain IV spirochetes. The tail blood was negative for 8 days, after which spirochetes appeared for 3 days, and were found by agglutination tests to belong to Strain V.

COMMENT.

It is apparent from the above protocols that the reason that the "first attack" failed to appear following the reinoculation in these animals, was that they were immune to the strain of spirochetes with which they were reinoculated. The spirochetes which were injected into the immune animal found themselves in an unfavorable environment. Most of them were destroyed, but a few survived because they were able to adjust themselves to the environment. In other words, the course of the disease in these animals from the time of the reinoculation was the same as that in animals which have just reached the crisis of their first attack, when the blood still contains spirochetes and the immune bodies against those spirochetes have already begun to appear. In such animals the spirochetes *in the blood stream* are rapidly destroyed, causing the first attack to stop abruptly. In the reinoculated animals the first attack is entirely suppressed by the destruction of the spirochetes *in the peritoneal cavity*. In both types of animals a few spirochetes survive and undergo a biological change into a new strain. About 6 days is required for the spirochetes of the new strain to become numerous enough to be found in the peripheral blood. This is the negative interval, and it is followed by the relapse due to the new strain of spirochetes.

This phenomenon is an additional demonstration of the immunity to a single strain of *S. recurrentis* which is conferred by an attack due to that strain. It also emphasizes the limitation of such immunity to the strain or strains which have been present in preceding attacks or to closely related strains.

In some papers on experimental relapsing fever (3), long incubation periods are described in mice which have been reinoculated with the

same strain as that used originally. It may be that these cases are examples of the phenomenon here described. (See Kudicke and Feldt, Tables 2, 5 and 6.) The present observations emphasize the importance of determining the identity of the strain of relapsing fever spirochetes which appears in any attack other than one immediately following inoculation, if reliable conclusions are to be drawn as to the immunological reactions which take place between this parasite and its host. The strain of each relapse is a strain which was not present in the animal's blood in a previous attack. In order to distinguish between a prolonged incubation period and a suppressed first attack followed by a relapse, it is necessary either to test the spirochetes causing the attack, against monovalent immune sera, or to determine, after the close of the attack, what additional agglutinins have been added to the animal's serum as a result of the attack.

SUMMARY.

In five splenectomized squirrels and chipmunks which were reinoculated with a strain of *Spirochæta recurrentis* which had previously been present in their blood, the first attack was entirely suppressed because the animals were immune to the strain of spirochetes inoculated; but after the interval which usually occurred between attacks, a relapse ensued, in which the strain of spirochetes present in the blood was different from the strain inoculated.

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