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MÉMOIRES ORIGINAUX

Studies in *Borreliae*

Part I. A variant of *Borrelia parkeri* Davis 1942 isolated in California and its tick vector (1)

ETUDE SUR LES *Borrelia*. I. UNE VARIÉTÉ DE *B. parkeri* ISOLÉE
A PARTIR DE SON VECTEUR EN CALIFORNIE

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A new variety of *Ornithodoros parkeri* (*O. parkeri hastingsi*) from Monterey County, California and its associated *Borrelia* has been studied by Rafyi, Stiller and Maghami at Hooper Foundation, San Francisco, California (3).

The *Borrelia* strain isolated from the ticks which were collected in California was compared with *B. parkeri* Davis 1942 from the type collection which has been maintained in *O. parkeri* Cooley 1936.

The California strain was very scarce in the blood of infected mice but more individual organisms were seen in young, 20 to 24 days old rats. Their number, however, never exceeded 10^6 per ml rat blood. The infection did not kill the animals. Only in 9 to 11 % of the infected rats were relapse observed. The incubation time

(1) Part of these studies were carried out at the George William Hooper Foundation, University of California, San Francisco, California, during the senior author's sojourn, as a Post-Doctoral Research Fellowship Grant, N.I.H. FF-419.

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(3) A. Rafyi, D. Stiller and G. Maghami. — Preliminary observations on *Ornithodoros parkeri*, var. *hastingsi* and its associated *Borrelia*, from Monterey county (now in print).

between tick bite and borreliemia was usually 6 or 8 days while transfer of the blood from one rat to another by the syringe has a shorter average incubation time, i.e., 5 to 6 days in our strain of white rats. Typical *B. parkeri* had the same incubation time but relapses were observed in 27 to 30 % of the infected rats and up to 10^7 organisms were observed per ml blood.

Pathologic Changes

The rats did not succumb to this infection. Rats sacrificed for histologic studies did not show pathologic changes. With the aid of fluorescent microscopy, granula of *Borreliae* could be detected in the spleen but not in the central nervous system of 8 (13 %) of the 60 examined animals infected with the Californian strain, while degenerating forms and granula were revealed by the fluorescent antibody method in 20 (32 %) in the spleen and in 5 (9 %) in the central nervous system of 60 rats infected with the typical *B. parkeri* strain after the first attack.

Rabbits did not show borreliemia after infection with either variety of *B. parkeri* past the first few hours after intravenous injection. While borreliolytic antibodies developed to a titer of 1 : 80 or 1 : 160, there were no organisms demonstrable in the organs with the aid of the fluorescent antibody method and no morphologic organ changes were observed.

Forty young adult guinea pigs of mixed sex were infected with *B. parkeri* California strain. Twenty served as control.

For the consecutive 3 months a total of 39 of these were sacrificed, three every two weeks, under chloroform. Their weights increased from 220 gms to 650 during the period under study.

The examination of the central nervous system included coronal sections anterior to the optic tract, the hypothalamus, the mesencephalon, pons and cerebellum at the superior olive and through the medulla at the middle of the inferior olive. Two levels of the cord-thoracic and cervical were also examined. In general the sections were cut at 8-12 microns in paraffin and surveyed with hematoxylin and eosin or Cresyl Echt Violet. Bodian's silver and other special stain such as Giemsa were used whenever it was necessary for complete evaluation.

The other organs were examined after staining the sections with hematoxylin-eosin and by Bodian's method.

Out of the 40 guinea pigs 3 came down with flaccid paralysis of the hind quarters 3 days after having been infected. Two other animals demonstrated a bizarre behavior after 2 to 3 weeks, which was manifested by twitching, at times almost choreiform movements, hostility and biting of the cages which is unusual in the strain of guinea pigs used in this Institute. The 3 paralysed animals (average weight 240 gm) had purulent myelo-meningitis of the lower thoracic cord. No organisms were cultured. One animal which was most severely afflicted had some lymphocytic infiltration in the anterior white of the cervical cord but no neuron damages.

None of the other guinea pigs had central nervous lesions.

No spirochetal forms were seen and there was no evidence of any tissue reaction. In one animal several neurones in the frontal cortex had coccoid forms in the cytoplasm but there was no adjoining tissue response and no confirmation as to the nature of these coccoid forms could be obtained with special strains.

We conclude, therefore, that the *Borrelia* infection in these animals failed to involve the central nervous system or the meninges to an extent demonstrable by histologic methods.

The paralysis in three guinea pigs perhaps was unrelated or due to tick paralysis, the analogue of which has been reported in man. Attempts to isolate a viral agent by tissue culture failed.

Immunologic Studies

The *Borreliae* isolated from the blood of the rats were suspended in physiological saline solution and exposed to ultrasonic vibration in the Sonifer apparatus (Branson Instrument Co) under cooling. This crude antigen was used for further studies.

Rabbits were immunized with these sonicates. The first two injections were given with Freund's incomplete adjuvant, subcutaneously. They consisted of 0.5 and 1.0 of the antigenic material, respectively. Then intravenous injection of the sonicate, 0.1 to 1.0 ml, in 3 to 4 days intervals, were given. The last dose was repeated 6 times. The rabbits were bled under anesthesia and the sera preserved at -40° C.

Sera from healthy and from infected rats 5 to 7 days after the attack were collected, centrifuged at 1 500 X G at 0° C and stored at -40° C.

When the antigens from *B. parkeri* Californian strain and those from *B. parkeri* typical strain were permitted to act against homologous and heterologous antisera in the Ouchterlony agar gel diffusion technic, reactions were observed. At least 2 lines were identical and common to both strains but at least 2 lines were characteristic for each individual strain. This was demonstrated also by the agar gel electrophoresis of the antigens in a gel containing 1 % special Agar (Difco) in 0.025 M barbiturate buffer at pH 8.6 and 4 V/cm for 45 minutes. The results are illustrated in Fig. 1 which shows a diagram of an experiment in which the crude antigens from typical *B. parkeri* (P) and from the Californian strain of *B. parkeri* (P_c) were subjected to electrophoresis on agar slides after which the trough between them was filled with anti-P serum and the results read after 1, 7 and 14 days. Agar gel electrophoresis of the same antigens and subsequent testing against anti- P_c serum gave a similar pattern, with the P_c antigen showing the antigen-antibody reaction pattern demonstrated by the P antigen and anti-P serum.

Paper chromatography using the Buchler Standard Model, Whatman No. 3 MM paper, 0.025 M barbiturate buffer at pH 8.6 and 5 V/cm 16 to 18 hours revealed 3 main bands which had the same mobility both in P and P_c antigens, i.e., 0.9, and 2.5×10^{-5} /cm²/V, respectively.

The dry antigens of both P and P_c contained 32 ± 1.4 % N, 12.2 ± 0.5 % P and 2.3 ± 0.2 % lipopolysaccharide material. The lipopolysaccharide yielded

46.1 \pm 0.8 % reducing sugars, of which glucose was identified. Thus the gross chemical constitution of both antigens was the same.

Precipitation tests were set up with varying amounts of the antigens and standard amounts of rabbit antisera. The equivalence zones were established. When the proportions of the precipitable N were calculated, antigen P_c precipitated 31.2 \pm 4.1 % of the N which was precipitable from anti-P serum by antigen P, and antigen P precipitated 28.4 \pm 3.9 % of the N precipitable by antigen P_c from the anti-P_c serum. It was concluded, therefore, that the precipitating activities of P and P_c were related but not identical.

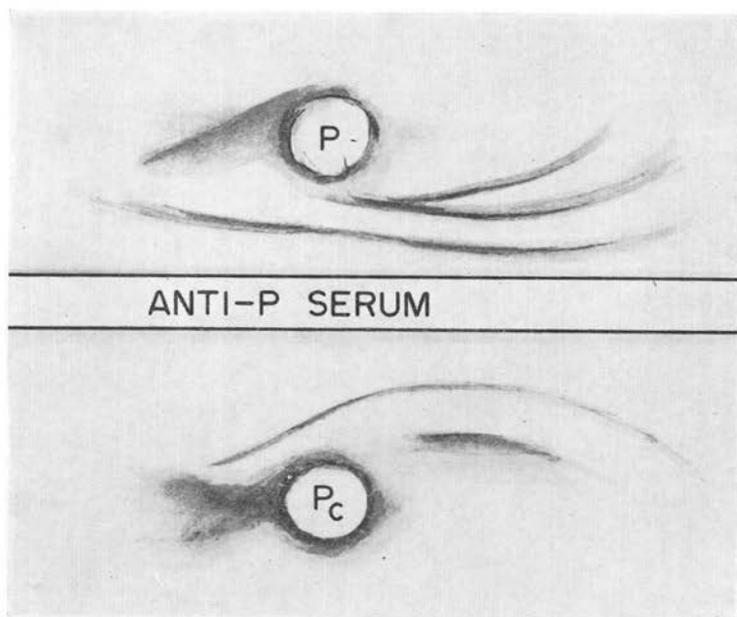


FIGURE 1. — Agar gel Electrophoresis of Crude Antigens from *B. parkeri* Standard Strain (P) and *B. parkeri* Californian Strain (P_c) Against *B. parkeri* Standard Strain Antiserum (P).

Borreliolysin tests were set up using 5 000 \pm 450 *Borreliae* per ml physiological saline and fresh convalescent rat sera. Two-fold dilutions of the sera, from 1 : 2 to 1 : 640 in physiological saline were employed. Two-tenths of a ml of the respective *Borrelia* suspensions were mixed with 0.8 ml of the antiserum dilutions, the mixtures incubated at 37° C for 2 hours, centrifuged for 10 minutes at 1 500 X G and the organisms observed under the microscope. The maximal effect was achieved with 1 : 2 diluted sera. In tubes containing 0.8 ml physiological saline instead of serum, 7 \pm 3 % of the *Borreliae* were lysed. Serum from uninfected rats lysed 10 \pm 4 %

of the *Borreliae*. Serum of rats after infection with P lysed $82 \pm 7\%$ of the *Borreliae* P but only 14 ± 4 of the P_c organisms. The serum of rats collected after borreliemia with strain P_c lysed $91 \pm 7\%$ of the P_c and $15 \pm 3\%$ of the P organisms (Table 1). Thus, the study of the lytic activity of the sera proved a significant difference between the two strains. In passive protection tests rabbit antiserum, 0.1 ml per 100 Gm body weight was given to 20 to 24 day old rats 5 to 10 minutes before the subcutaneous injection of 50 to 100 *Borreliae*. Eighteen of the 20 control animals (90%) which did not receive serum but only P and 15 out of 20 rats (75%) which were given only P_c organisms developed borreliemia. Of 60 rats protected with anti-P serum and infected with P, 3 (5%) developed borreliemia. Of 60 rats which were given anti-P serum and P_c organisms, 32 (53%) had *Borreliae* in their circulation. Of 60 rats receiving anti- P_c organisms, one (2%) developed infection. Of 60 rats which were protected with anti- P_c serum and which were given P organisms, 25 (42%) had this *Borrelia* strain in their blood when checked after, 5, 10, 12 and 15 days (Table 2). It was concluded, therefore, that the rat protecting against strains P and P_c were related to a certain degree but were not identical.

Discussion and Summary

A variety of *O. parkeri*, was found at the Hastings Reservation in Mountery County, California, its associated *Borrelia* has been studied in a comparative way with a typical *B. parkeri*.

TABLE 1

Borreliolytic Activity of B. parkeri Stock Strain and B. parkeri Californian Strain
(*Activité borréliolytique de B. parkeri*
(*souche entretenue au laboratoire et souche californienne*))

Serum	% <i>Borreliae</i> lysed	
	P*	P_c °
None	$7 \pm 3^*$	5 ± 3
Not Infected Rats	10 ± 4	8 ± 2
Infected Rats After:		
P	82 ± 7	14 ± 4
P_c	15 ± 3	91 ± 7

* = *B. parkeri* stock strain (*B. parkeri* de la souche du laboratoire).

° = *B. parkeri* Californian Strain (souche californienne).

* = Geometric means with standard deviation of 10 experiments. (Moyennes géométriques avec déviation standard dans 10 expériences).

While the chemical and immunological examination of the two strains of *Borrelia parkeri* had to be restricted because of the scarcity of antigenic material due to the paucity of the *Borreliae* in the blood stream, the results of this study indicate that the « typical » *B. parkeri* and its Californian strain are distinct. The gross chemical analysis, the general electrophoretic pattern of the antigens, the considerable cross-precipitating activity and the cross-protection in animal experiments but the sharing of only 2 of the 4 or 5 antigenic fractions in gel diffusion tests and the lack of a common bacteriolysin indicate that the two strains are related but not identical.

Borreliae are known to vary during the course of the disease. While the gross chemical constitution, the electrophoretic pattern, the antigenic components in gel diffusion technics and the quantitative precipitation reaction have not been used to our knowledge to compare so-called « attack » and « relapse » *Borreliae*, we feel that the difference observed in his study reach further than one may expect in mutations induced by the sojourn of *Borreliae* in infected animals (for proof, see Part 2 of this series of communications on « attack » and « relapse » *B. turicatae*, now in print). Considering also the difference between the typical *Ornithodoros parkeri* and its Californian variant, and the modern tendency of naming *Borreliae* according to the species or subspecies of the tick from which they were isolated, this *Borrelia* is a variant of the typical *B. parkeri* and the suggestion to label it *B. parkeri* var. *hastingsi* is tendered herewith with the reservation that further studies might prove that it is a subspecies of *B. parkeri* Davis 1942.

TABLE 2

Passive Protection Tests with *B. parkeri* Standard Strain and Californian Strain
(Tests de protection passive avec les souches standard et californienne de *B. parkeri*)

Serum	Rats infected with Strain	
	P*	P ^o
None	18/20 + *	15/20
Not Immunized Rabbit	17/20	16/20
Rabbit Immunized with :		
P	3/60	32/60
P _c	25/60	1/60

* = *B. parkeri* standard strain (souche standard).

^o = *B. parkeri* Californian strain (souche californienne).

* = No. of animals developing borreliemia (souche d'animaux présentant une borreliose).

+ = No. of animals injected with *Borreliae* (souche d'animaux inoculés avec des *Borreliae*).

Résumé

Une variété d'*Ornithodoros parkeri* a été trouvée dans la Réserve d'Hastings (Mountery County, Californie), et une étude comparative de la *Borrelia* qui lui est associée avec la souche typique de *B. parkeri* a été effectuée.

Bien que l'examen chimique et immunologique des deux souches ait été limité, en raison de la rareté des *Borrelia* dans le sang, donc du matériel antigénique, les résultats de cette étude permettent néanmoins d'affirmer que les deux souches sont distinctes.

L'analyse chimique, les caractéristiques électrophorétiques générales des antigènes, les réactions croisées de précipitation et d'immunité dans les expériences sur l'animal d'une part, la communauté de deux seulement sur les quatre ou cinq fractions antigéniques de l'électrophorégramme en gélose et l'absence d'une bactériolysine commune d'autre part, indiquent que les deux souches sont apparentées mais non identiques.

Considérant par ailleurs les différences existant entre la souche type d'*Ornithodoros parkeri* et sa variété californienne, et la tendance moderne des spécialistes à désigner les *Borrelia* d'après l'espèce ou la sous-espèce d'où elles ont été isolées, la *Borrelia* en provenance de la Réserve d'Hastings peut ainsi apparaître comme une variété de l'espèce type *B. parkeri*. Les auteurs suggèrent de la nommer *B. parkeri* var. *hastingsi*, sous réserve d'études ultérieures permettant de prouver qu'il s'agit bien d'une sous-espèce de *B. parkeri* Davis 1942.

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