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SPOTTED FEVER IN MEXICO

Immunological relationship between the virus of the rickettsiosis observed in Sonora and Sinaloa, Mexico, and other Spotted Fever viruses

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In 1929 an infection identical with Rocky Mountain Spotted Fever was recorded in Brazil, first in the State of São Paulo (1, 2), then in Minas Gerais (3), and lately in the State of Rio de Janeiro (4). Cross immunity tests, made by several investigators (5-9), proved the identity of both infections, and gave evidence that Rocky Mountain Spotted Fever, transmitted to man chiefly by *Ixodidae* of the genus *Dermacentor* and possibly by *Amblyomma americanum* (20), is immunologically identical with São Paulo and Minas Gerais Spotted Fever, transmitted by ticks of the genus *Amblyomma*.

More recently (10,11), under the name of "Fiebre petequial de Tobia", an infection has been described in Colombia the clinical characteristics of which resemble those of the Rocky Mountain Spotted Fever group. *Amblyomma cajennense,* found spontaneously infected was indicated as one of the *Ixodidae* responsible for the transmission of the disease (10). Preliminary protection tests against Rocky Mountain Spotted Fever, made with serum of "Fiebre petequial de Tobia" cases, showed that both infections are identical.

Hence, presently, Spotted Fever has been recorded and thoroughly studied in America in several regions of only three countries, namely in the United States, Brazil (São Paulo, Minas Gerais and Rio de Janeiro) and Colombia (Tobia).

In other paper (12) we studied several properties of a virus strain of a new rickettsiosis, isolated from a suspected human case of Spotted Fever and clinically identified with several other cases lately recorded in the States of Sonora and Sinaloa (Mexico).

The strain studied by us showed, in laboratory animals, an experimental behaviour similar to that of the most virulent strains isolated from severe Spotted Fever cases, either in the United States, or in Brazil, with a possible

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greater tendency to scrotal reactions in guinea-pigs. These findings confirm the preliminary papers of Bustamante and Varela (13, 14), who conclude that owing to the clinical characteristics of the infection in man, the epidemiological data, the mortality and clinical aspect of the infection in the guinea-pig, Sonora and Sinaloa cases resemble Rocky Mountain Spotted Fever.

In the mentioned paper (12) we describe certain cultural characteristics of the rickettsia and show that the *Ixodidae* of the genus *Amblyomma* (*Amblyomma striatum*) are able to infect themselves on guinea-pigs during the period of virus' circulation and transmit the disease by biting other animals.

All these experimental results would suggest that the exanthematic infection, known as "Fiebre pinta" or "Fiebre de Choix", recognized among the populations of rural zones of the States of Sonora and Sinaloa, is identical with Rocky Mountain Spotted Fever.

The study of the immunological identity of the virus recently isolated by Bustamante and Varela in Mexico is therefore of high importance, since it might confirm the suspicion held by several investigators that the geographic distribution of the infection is much wider than pointed out until presently.

In this paper we report the results of experiments made with the purpose of studying the immunological properties of this new Mexican rickettsiosis virus by means of cross immunity and neutralization tests against strains of Spotted Fever virus isolated in certain regions of the State of São Paulo. We shall also report experiments on the protective value of vaccines prepared with the viruses of São Paulo and Rocky Mountain Spotted Fever against the "Fiebre de Choix".

EXPERIMENTAL

Material and methods

A. *Virus strain*

1. *BV strain.* Received from Mexico, where it was isolated from a human case of "Fiebre pinta" by Bustamante and Varela and sent to us in a female of *Ornithodoros* sp. fed on infected animal. Received in our laboratory on December 11th, 1944 and maintained by passages in guinea-pigs.

2. *S strain.* Isolated from B. S., who died from Spotted Fever in June, 1941, in Araras, State of São Paulo. At the beginning of this study it counted 184 serial passages in guinea-pigs.

3. *L. strain.* Isolated from spontaneously infected *Amblyomma cajennense* nymph removed from a hare in Araras, State of São Paulo, in September, 1942. Number of passages at the beginning of this study: 86.

4. *F strain.* Derived from a batch of adult *Amblyomma cajennense* specimens, found spontaneously infected and removed from a horse in Loreto, near the city of Araras, in August, 1942. 101 passages in guinea-pigs.

All the three virus strains isolated in São Paulo are highly virulent and proved antigenically identical in experiments carried out in our laboratory.

The number of passages in guinea-pigs of each of the viruses used in the tests will be marked in parenthesis immediately behind the letter of the strain.

B. *Virus blood*

Obtained by cardiac puncture from guinea-pigs experimentally inoculated with infectious material on the second or third day of thermic reaction. The blood was received in sodium citrate solution to prevent coagulation or shaken with glass beads immediately after bleeding. When larger amounts of inoculum were required, blood samples of several guinea-pigs were pooled and allowed to stand overnight in the ice-box, while the sterility tests were performed.

Occasionally, mixtures of two antigenically identical virus strains were made: these cases are especially indicated in the text.

C. *Immune-protective serum*

The animals, rabbits or guinea-pigs, which survived infection and were employed for production of immune serum containing protective antibodies, were bled through the heart a few days after the last day of temperature over 39.6°C. The blood was allowed to clot at room temperature for one hour and then placed in the ice-box (+ 4°C.) where it was left overnight. After separation, the serum was stored at 4°C.

The amounts of serum used in the neutralization tests were deliberately high, since we had only the intention to prove the immunological identity of the various strains, disregarding the quantitative estimation of homologous antibodies.

D. *Guinea-pigs*

All animals inoculated with BV virus strains were kept under observation in well isolated cages, far from any contact with animals infected with other Spotted Fever virus strains under study in the laboratory.

In the comparative tests for the antigenic properties of different virus strains we have employed guinea-pigs weighing from 350 to 370 gr. In groupe tests we have always tried to use animals from the same breeding. The animals were checked daily, and the rectal temperature taken once or twice a day. The curves show the temperatures taken between noon and 1:00 p.m. 39°C. was considered the maximum normal temperature limit for guinea-pigs. Temperatures exceeding this maximum limit are black printed in the curves.

I. CROSS IMMUNITY BETWEEN THE VIRUS OF THE NEW MEXICAN RICKETTSIOSIS AND THE SPOTTED FEVER VIRUS

a) *Protection against the Mexican virus shown by guinea-pigs recovering from Spotted Fever* — In early experiments on the therapeutic action of penicillin (sodium salt), a large number of guinea-pigs were inoculated with 0.5 cc. of a mixture of São Paulo Spotted Fever virus-blood. From those

animals, 23 survived typical, but comparatively benign infection, including in this total number several animals used as controls for virus activity. In this test the action of penicillin was nihil, no influence being detected upon infection's course or mortality rate in relation to untreated controls.

We took advantage of the rare opportunity of having at our disposal such a large number of animals surviving an infection produced under uniform conditions, in order to test the protection against the homologous virus, as well as against the Mexican virus.

Twenty days after the infective inoculation, and 4 to 7 days after the temperature fall, the animals were bled from the heart and the sera (1.5 to 2 cc. from each guinea-pig) pooled and stored at +4°C.

The animals remained under observation for another 5 days after bleeding, in order to exclude those dying from heart puncture.

The survived guinea-pigs were thereupon reinoculated intraperitoneally as follows:

12 with 0.5 cc. of BV virus-blood (Mexico)

11 with 0.5 cc. of F+S virus-blood (São Paulo, Brazil)

10 normal guinea-pigs served as controls of the virus activity

5 inoculated with 0.5 cc. of BV virus and

5 with 0.5 cc. of a virus-blood mixture prepared with the blood of guinea-pigs infected with F or S strains.

All inoculated animal remained under observation for a fortnight when the survivors were killed for examination of the internal lesions.

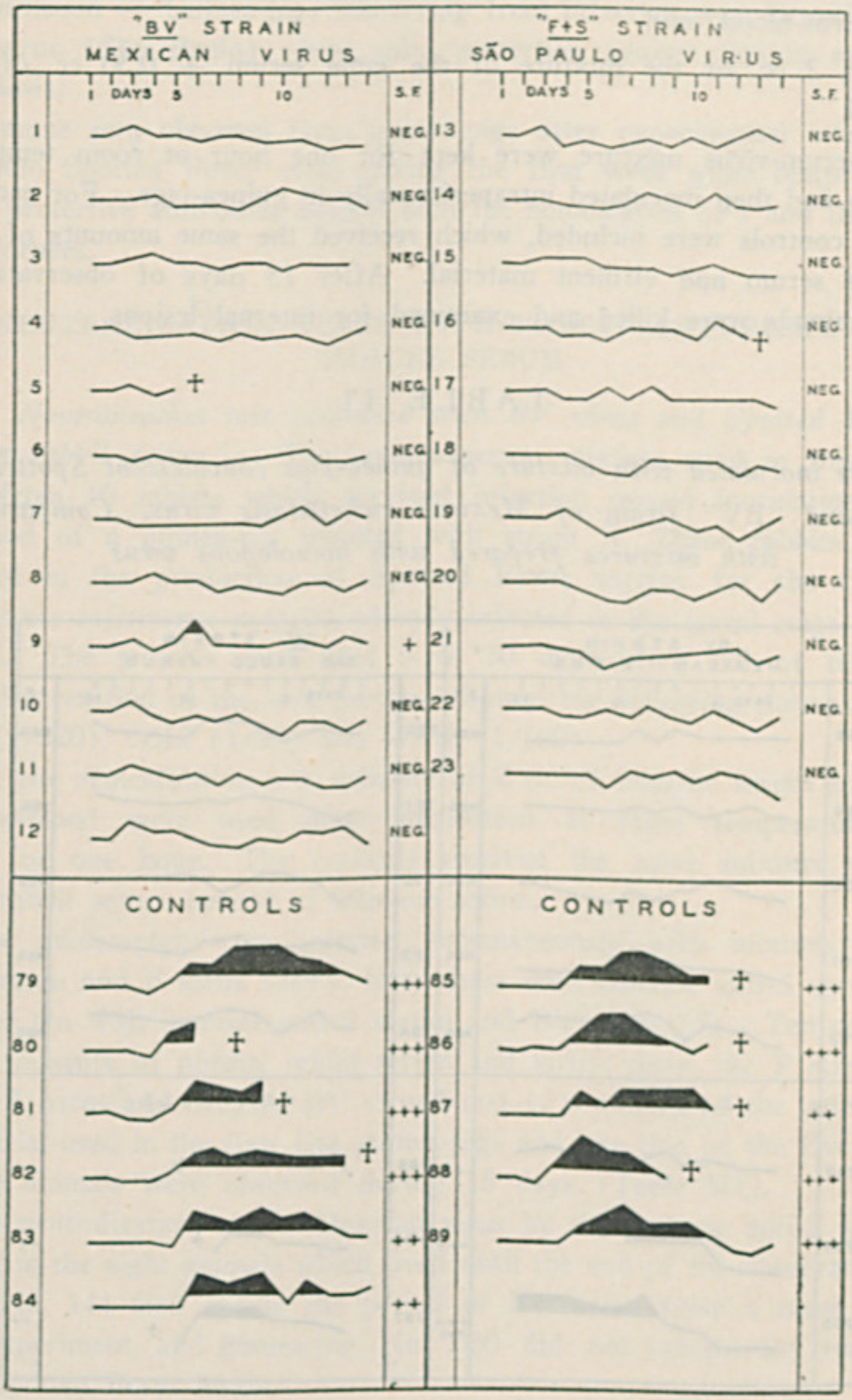
The behaviour of the controls was satisfactory, since all the animals reacted typically and showed at the necropsy lesions common to the most virulent Spotted Fever strains. The guinea-pigs inoculated either with homologous virus or with BV virus, displayed complete protection against infection and death. Animals No. 5 and No. 16 apparently did not die from Spotted Fever, since no post-mortem alterations were found to support this diagnosis; in one of them we found a small abcess in the rectum, probably caused by the introduction of the thermometer as the temperature was taken. In another survivor, guinea-pig No. 9, there was at necropsy a slightly large, congested spleen, but two hepatic abcesses were also detected.

All test animals, in contrast with the controls, failed to show any lesion suggestive of Spotted Fever. No conspicuous spleen enlargement was observed, although discrete congestion of the organ has been recorded in some cases and among the male animals not a single scrotal reaction was found.

The significance of these findings is especially pointed out, since such a complete protection is very seldom observed.

TABLE I

Cross immunity. Comparative study of the behaviour of spotted fever survived guinea-pigs, reinoculated with homologous virus and BV strain of Mexican rickettsiosis virus



b) *Presence of protective antibodies against BV virus in sera of guinea-pigs recovering from São Paulo Spotted Fever* — In order to confirm the preceding results another test was set up with a mixture of sera collected from guinea-pigs used in the former experiment. Ten guinea-pigs were inoculated:

5 with 2 cc. of the mixture of guinea-pigs convalescent sera + 0,30 cc. of "F" virus-blood. and

5 with 2 cc. of the mixture of the same serum + 0,30 cc. of "BV" virus-blood.

The serum-virus mixture were kept for one hour at room temperature (18-20°C.) and then inoculated intraperitoneally in guinea-pigs. For each virus type three controls were included, which received the same amounts of normal guinea-pigs serum and virulent material. After 15 days of observation, the survived animals were killed and examined for internal lesions.

TABLE II

Guinea-pigs inoculated with mixture of guinea-pigs convalescent Spotted Fever sera and "BV" strain of Mexican rickettsiosis virus. Comparison with mixtures prepared with homologous virus

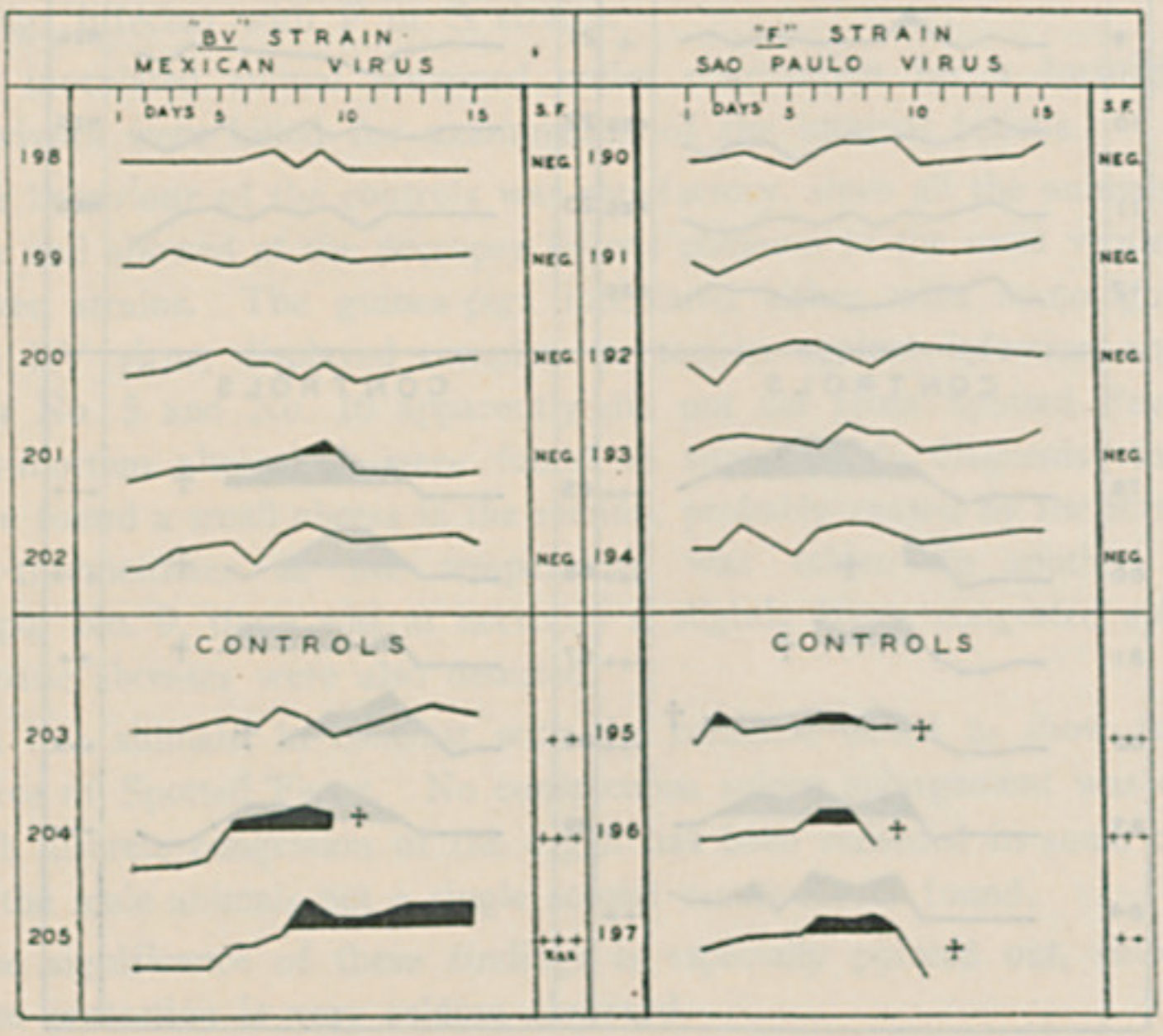


Table II gives the temperature curves of the guinea-pigs, as well as the intensity of the lesions of the internal organs. The results obtained show a satisfactory protection against the homologous virus, as well as against BV strains, although the controls have not exhibited a very intense thermic reaction, due, perhaps, to a spoiling effect of the room temperature upon the small dose of virulent material used.

Conclusion — Guinea-pigs recovering from infection with Brazilian Spotted Fever virus (São Paulo), show solid protection against Sonora and Sinaloa rickettsiosis.

Immune sera obtained from guinea-pigs atter experimental infection with São Paulo Spotted Fever virus during the first week after temperature fall contain protective antibodies against both the homologous (F) and the Mexican (BV) viruses.

2. NEUTRALIZATION TESTS WITH RABBIT ANTI-RICKETTSIAL IMMUNE SERUM

a) *Neutralization test perfomed with BV virus and Spotted Fever convalescent rabbit serum* — The immune-serum mixture used in this test was pooled from 10 rabbits which survived infection caused inoculation of virulent blood of a guinea-pig infected with strain S. These rabbits had been employed in the production of Spotted Fever vaccine for the feeding of *Amblyomma cajennense* nymphs, already infected in the larval stage, also with virus S. The animals were bled 6 to 10 days after fall of temperature. Weil-Felix reaction of the sera mixture showed the following titers: *B. proteus* OX19 (1/320), OX2 (1/80) and OXK (1/160).

For the neutralization test, mixture of 2 cc. of immune serum and 0.30 cc. of virus-blood were used after incubation at room temperature (about 20ºC.) for one hour. The controls received the same mixture containing normal rabbit serum instead of immune serum.

Five guinea-pigs were injected subcutaneously with mixture of imune anti-S serum and F virus (*101*), five others with immune anti-S serum and L virus and ten with immune anti-S serum and BV virus (*5*). Ten controls received a mixture of normal rabbit serum and virus: three, the F strain (*101*), two, the L virus and five, the BV virus-blood (*5*). Three of the latter received the material used in the first five guinea-pigs and two that of the five last ones.

The animals were observed during 15 days (Table III).

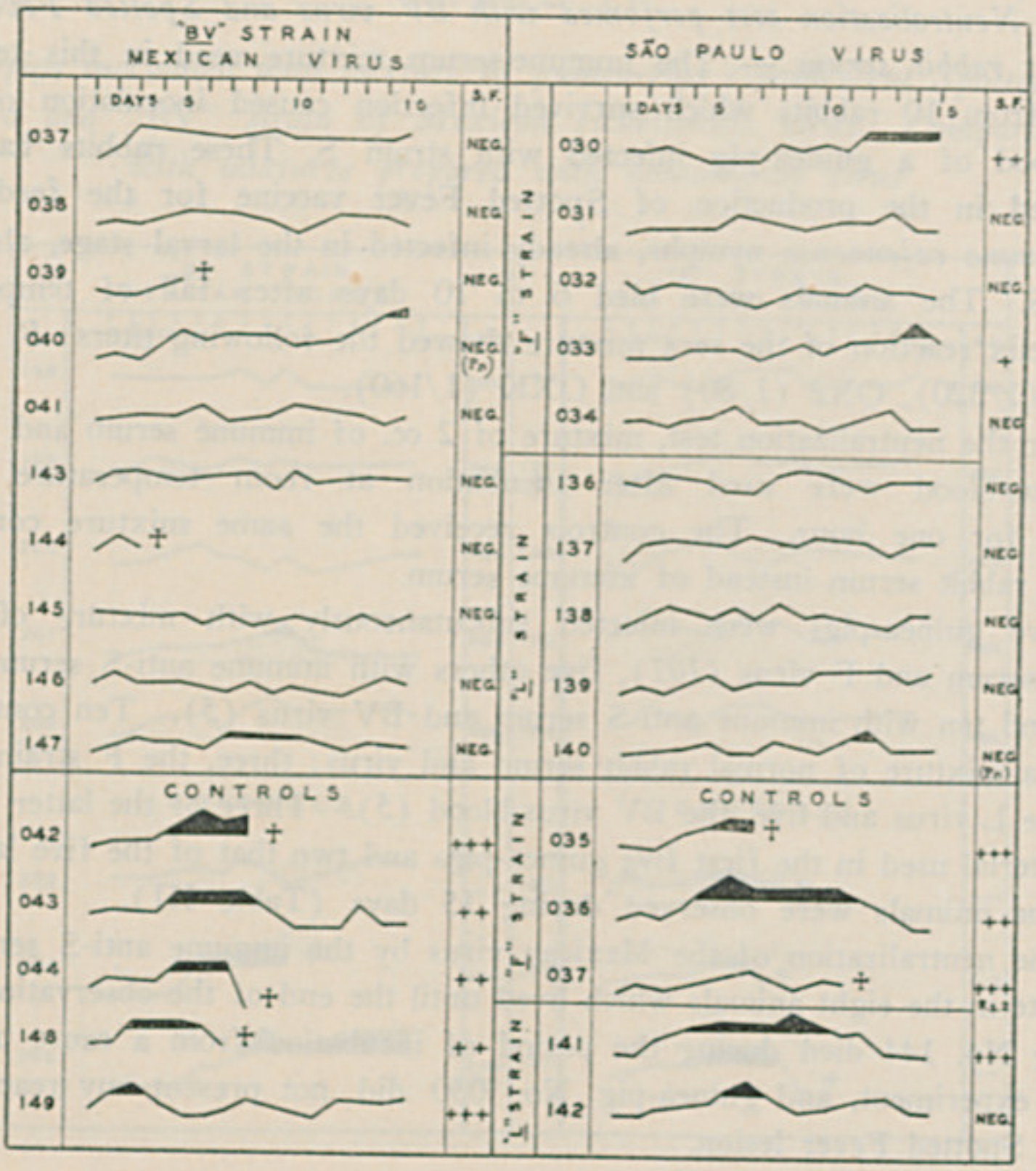
The neutralization of the Mexican virus by the immune anti-S serum was complete in the eight animals which lived until the end of the observation. Guinea-pig No. 144 died during the period of incubation from a cause unrelated to the experiment, and guinea-pig No. 030 did not present any reaction nor typical Spotted Fever lesion.

The results of the protection gained by the immune anti-S serum against the F and L strains were also satisfactory. The neutralization was complete for the L strain; as to the F virus, only guinea-pig No. 030 suffered delayed thermic reaction, with temperature over 40°C., beginning on the 12th day of inoculation and lasting only until the 15th. Post-mortem, there were alterations of moderate intensity, with a more or less enlarged spleen.

b) *Neutralization test with immune anti-BV serum S São Paulo rickettsiosis virus* — The serum of rabbits infected with BV virus *(6)* and bled a few days after temperature fall was used for the protection test against strain S of São Paulo Spotted Fever virus. Since only three rabbits were available and only a small serum volume could be obtained, we were obliged to reduce the number of guinea-pigs in the test.

TABLE III

Neutralization test. Behaviour of guinea-pigs inoculated with mixture of anti-S São Paulo Spotted Fever convalescent rabbit serum and virus BV (*México*), F and L (*São Paulo*)

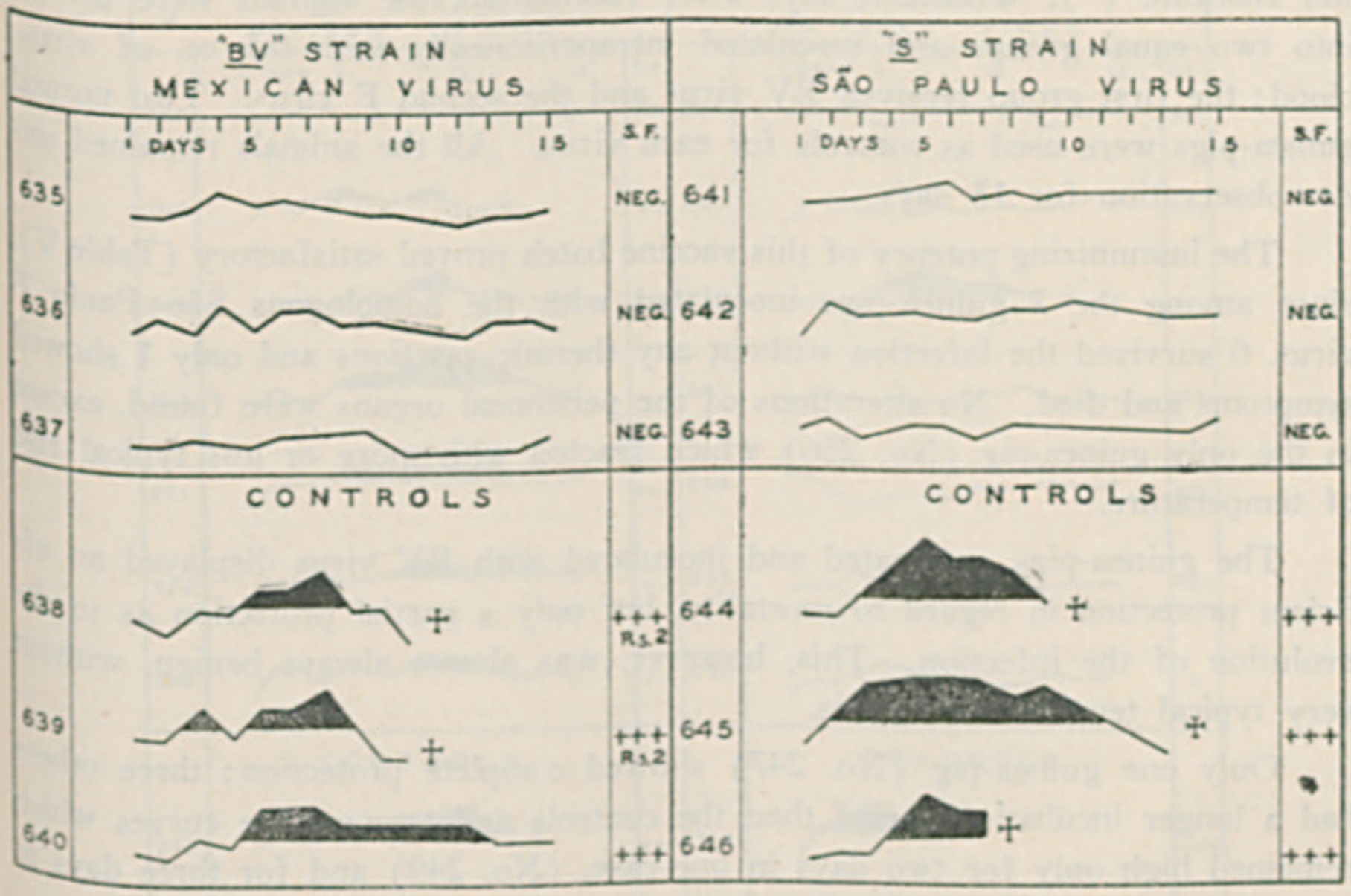


Six male guinea-pigs were injected subcutaneously with 2.5 cc. of a mixture of 2 cc. rabbit serum and 0.5 cc. virus-blood, prepared at the moment of injection. The test animals received immune serum and the controls normal rabbit serum. Of the test guinea-pigs, three were injected with mixture of serum and homologous virus and the other three with serum and S virus-blood (*98*).

The protection obtained by anti-BV serum (table IV) was complete against S virus *(98)*, as well as against the homologous virus. Notwithstanding the small number of animals included in the test, the results obtained are highly significant, because of the high virulence of the virus-blood strains used. as shown by the reactions, mortality and lesions in the control group. Of the three

TABLE IV

Neutralization test. Results of inoculations of guinea-pigs with mixture of anti-BV convalescent serum and S São Paulo spotted fever virus as compared with mixtures of homologous virus



controls injected with BV virus and normal rabbit serum, two suffered scrotal reaction of moderate intensivity, whereas none of the three animals injected with immune serum presented edema of the scrotum skin or, at necropsy, inflammation of the vaginals or any other alteration of the type of those which occur in cases of scrotal reactions.

Conclusion — The results of the virus neutralization tests prove that the immune anti-S serum, obtained from rabbits recovering from experimental Spotted Fever by S virus strain, isolated in the State of São Paulo from a human case, contains antibodies capable not only of neutralizing other strains of viruses (F and L), isolated in the State of São Paulo from spontaneously infected *Ixodidae,* but also the BV virus of the new Mexican rickettsiosis.

In the same way, anti-BV serum of rabbits convalescing from the Mexican rickettsiosis under study, gave a fair protection against S virus of São Paulo Spotted Fever.

3. STUDY ON THE PROTECTION SHOWN BY SPOTTED FEVER VACCINATED GUINEA-PIGS AGAINST THE VIRUS OF THE NEW MEXICAN RICKETTSIOSIS

a) *Spencer-Parker vaccine (Butantan)* — Fourteen guinea-pigs were vaccinated subcutaneously with a single dose of 1 cc. of vaccine, batch No. 257, prepared with *Amblyomma cajennense* by the Spencer-Parker method at Butantan Institute (*). Fourteen days after vaccination, the animals were divided into two equal groups and inoculated intraperitoneally with 0.5 cc. of virus blood: the first group received BV virus and the second F virus. Two normal guinea-pigs were used as controls for each virus. All the animals remained under observation for 15 days.

The immunizing potency of this vaccine batch proved satisfactory (Table V), since among the 7 guinea-pigs inoculated with the homologous São Paulo F virus, 6 survived the infection without any thermic reactions and only 1 showed symptoms and died. No alterations of the peritoneal organs were found, except in the only guinea-pig (No. 256) which reacted with more or less typical rise of temperature.

The guinea-pigs vaccinated and inoculated with BV virus displayed an efficient protection in regard to mortality, but only a partial protection as to the evolution of the infection. This, however, was almost always benign, without very typical temperature curves.

Only one guinea-pig (No. 247) showed complete protection; three others had a longer incubation period than the controls and temperature curves which remained high only for two days in one case, (No. 249) and for three days in another case (No. 253). Two animals reacted typically.

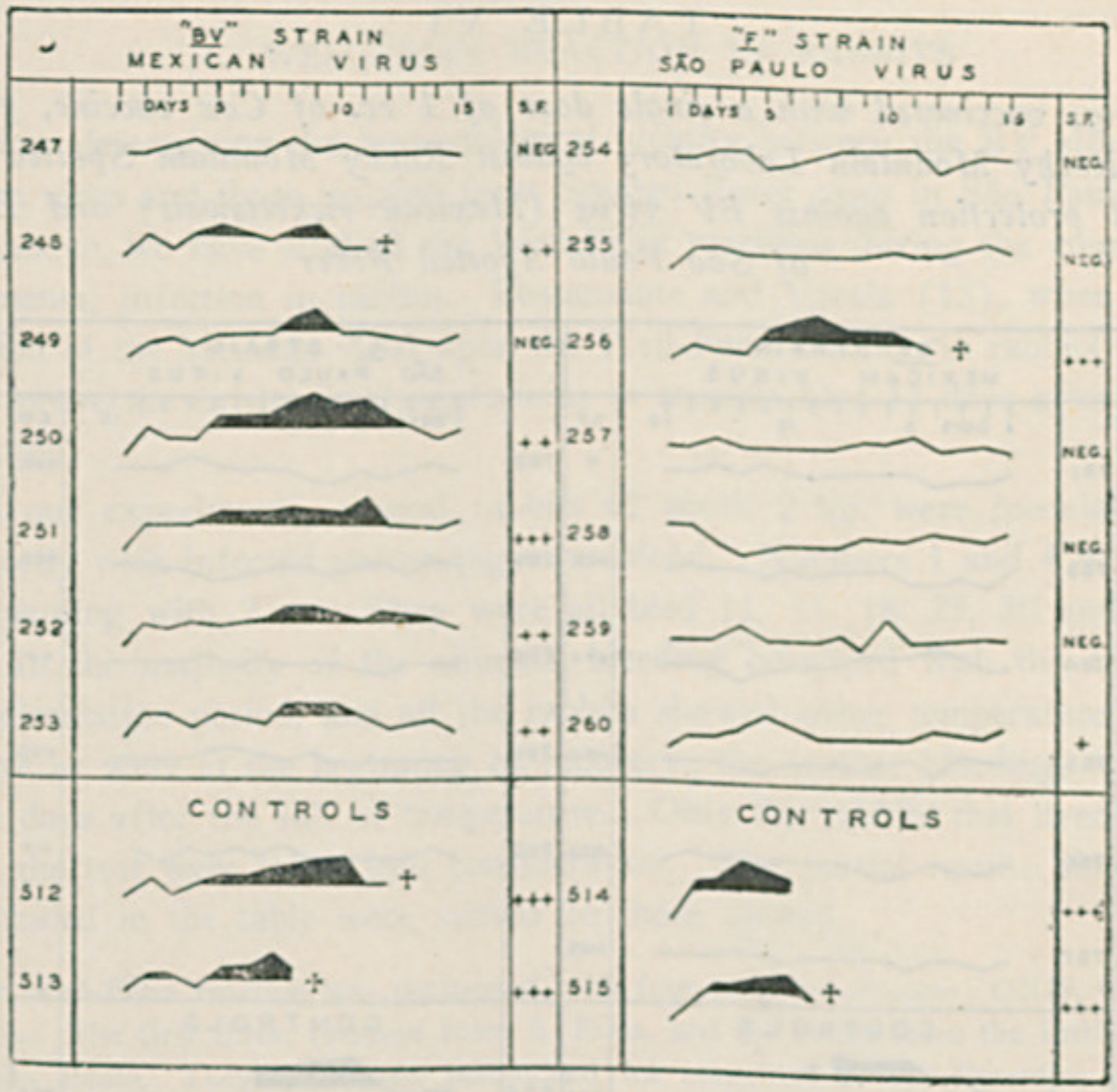
(*) For details concerning the technique of producing this vaccine see: Travassos, J. & Vallejo-Freire, A. Novos dados sobre a biologia de *Ixodidae.* Criação artificial do *Amblyomma cajennense* para o preparo da vacina contra a Febre Maculosa. *Mem. Inst. Butantan,* **18**:145-236, 1944/45.

Thus the Spencer-Parker vaccine prepared with São Paulo Spotted Fever rickettsiae is able to produce a fairly significant immunity in guinea-pigs against experimental infection with Mexican rickettsiosis virus.

A careful consideration of the quantitative variations of the virulence of the strains used in the protection tests might possibly explain whether this apparent variation is related to the presence of different antigenic fractions in both strains. Experiments with two doses of vaccine and the use of a titrated and more homogenous inoculum obtained from yolk sac membrane are hence suggested. (*)

TABLE V

Behaviour of guinea-pigs vaccined with a single dose of 1 cc. of São Paulo Spotted Fever vaccine, prepared in the Instituto Butantan with Amblyomma cajennense by the Spencer-Parker method, and thereupon inoculated with 0.5 cc. of virus-blood BV *(México) and* F *strain of São Paulo Spotted Fever*



(*) Guinea-pig virus-blood may contain from 50 to 1.000 infective doses per cc. In average it varies from 200 to 400.

b) *Cox vaccine* (*Rocky Mountain Laboratory, Hamilton, Montana*) — Twelve male guinea-pigs were vaccinated subcutaneously with 1 cc. of Cox vaccine against Rocky Mountain Spotted Fever prepared at the Rocky Mountain Laboratory, in Montana. One animal died two days after vaccination from a cause unrelated to the experiment. After 14 days, 6 guinea-pigs were inoculated intraperitoneally with 0.5 cc. of BV virus-blood (7) and five other with 0.5 cc. of L virus-blood *(104)*.

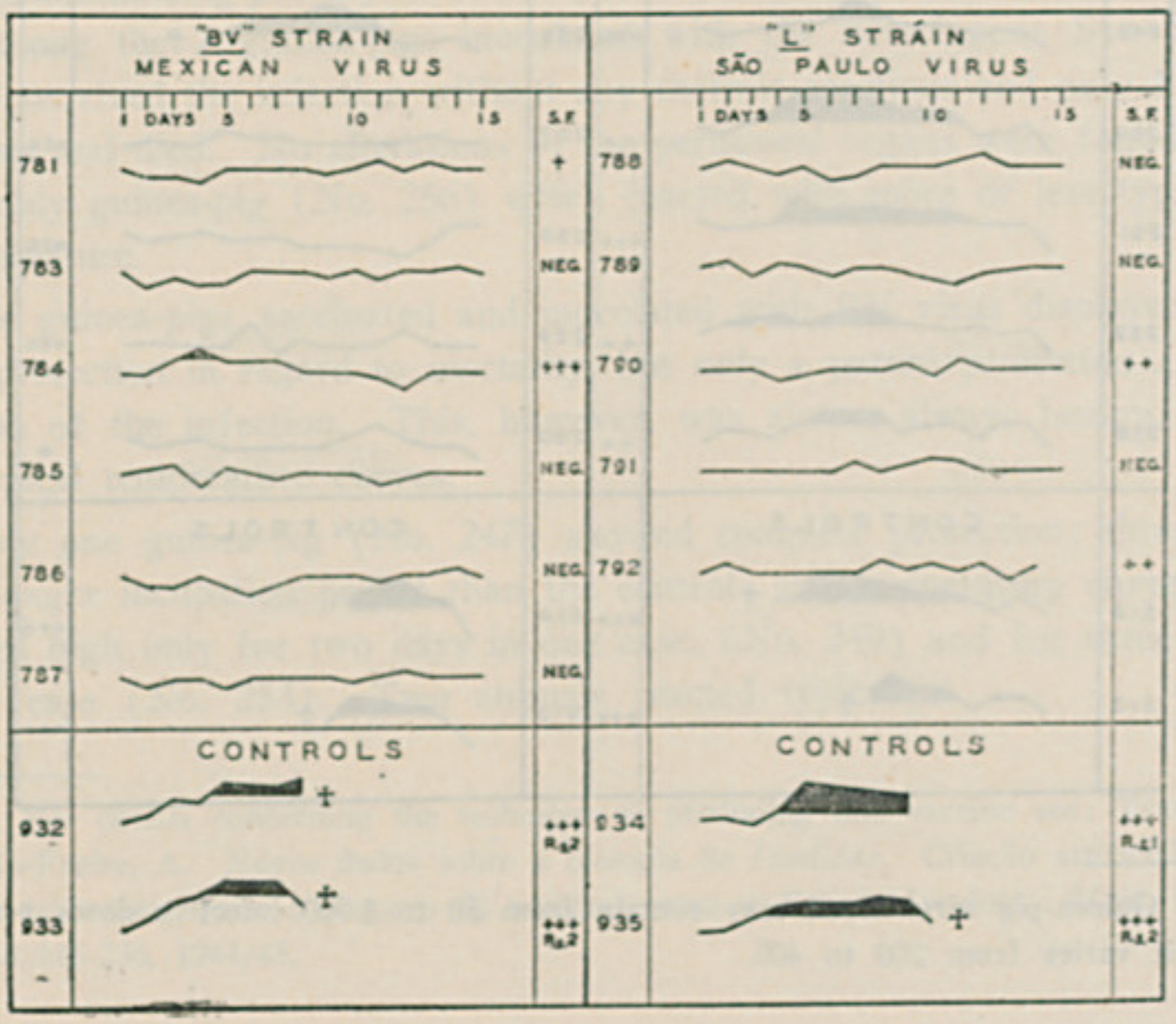
For each virus 2 male controls were inoculated with the same volume of inoculum, also through the peritoneal route.

As shown in table VI, the protection obtained was excellent regarding both BV (Mexican) and L (São Paulo) strains.

Only one of the guinea-pigs inoculated with Mexican virus and two of the L virus showed, at necropsy, marked spleen enlargement, giving evidence of inapparent infection. All the controls exhibited thermic and scrotal reactions and died; post-mortem there were the characteristic internal lesions.

TABLE VI

Guinea-pigs vaccinated with a single dose of 1 cc. of Cox vaccine, prepared by the Rocky Mountain Laboratory against Rocky Mountain Spotted Fever. Obtained protection against BV virus (Mexican rickettsiosis) and L strain of São Paulo Spotted Fever



In this test only male animals were used and a 100% scrotal reactions obtained with BV strain, thus enabling us to determine the influence of immunity induced by Cox vaccine in preventing the action of the rickettsiae upon the *tunica vaginalis* and the scrotum of the inoculated animals.

Among the vaccinated guinea-pigs not a single case of scrotal reaction was observed, not even the initial edema of the scrotal sac skin. Both the BV virus controls showed scrotal reactions of moderate intensity, with marked swelling, hemorrhages and necrosis of the scrotum skin. At necropsy, adherence of the tunica caused by inflammation or necrosis, desintegration of the polar fat of the testis and sometimes very discrete periorchitis were observed.

The tests here reported show the high protective value of the Cox vaccine, prepared with antigens from Rocky Mountain Spotted Fever cases, against the Mexican virus those isolated from Spotted Fever cases in São Paulo, as described against the São Paulo virus.

The neutralization tests leave, hence, little doubt concerning the identity of the viruses isolated in the United States, Brazil and Mexico.

4. WEIL-FELIX REACTION IN RABBITS

After determining the immunological identity between the BV strain of the Mexican virus and those isolated from Spotted Fever cases in São Paulo, as described above, we have studied the Weil-Felix reactions during the course of the experimental infection in rabbits. Bustamante and Varela (13), when studying the action of the Mexican virus upon the Weil-Felix reaction in rabbits, were unable to prove the existence of agglutinins in animals bled 11 days after infective inoculation.

In our experiments, several rabbits of about 2 kg. were inoculated intraperitoneally with infected guinea-pig virus-blood. Numbers 1 and 4 with 0.5 cc. the remaining with 2 cc. They were all bled 11, 15, 18, 25, 30 and 35 days later. In the majority of the animals, bleeding coincided with the end of the virus' circulation period, and all the rabbits showed either temperature of 40°C. or above or were at the beginning of recovery; the further bleedings were made several days after the fall of temperature. Only the rabbits that lived until the end of the test were taken into consideration. The partial results obtained and not included in the table were similar to those quoted.

The Weil-Felix reaction was performed with four races of *Proteus*: OX19, OX2, OXK and OXL. The first three, received from A. Felix, and the latter, from the Instituto Adolfo Lutz, São Paulo. They have been maintained for sometime at the Butantan Institute in plain agar, without condensation water. Before the preparation of the agglutinable suspensions, plate cultures were made, and S cultures used to inoculate ordinary agar, which was allowed to stand at 37°C. for 48 hours.

Suspensions of alive germs were used, at a concentration corresponding to MacFarland's tube 3, the suspensions being tested for roughness, according to White's test.

The tests were read macroscopically after 4 hours' inoculation in a water bath at 37°C., and again after 18 hours in the ice-box at 0°C.

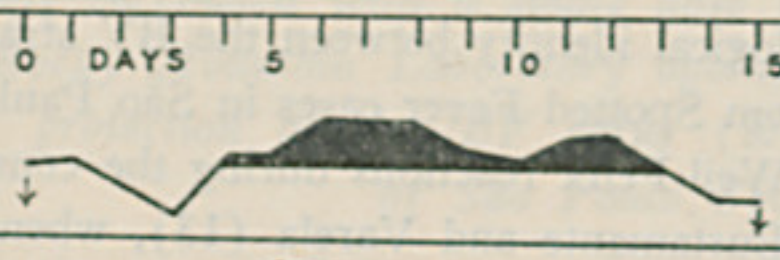
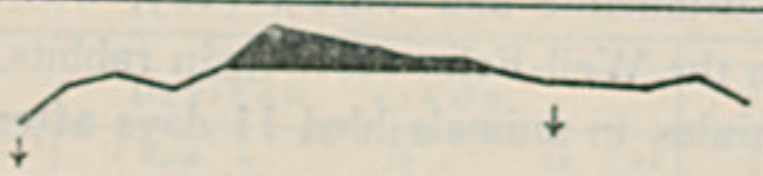
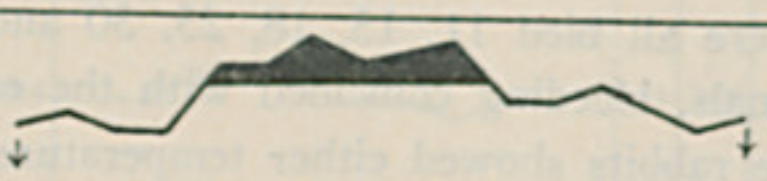
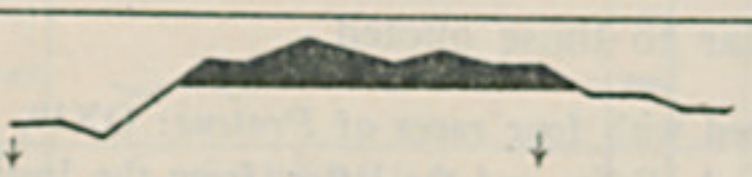
Table VII gives the results of the Weil-Felix reaction in three rabbits inoculated with São Paulo Spotted Fever virus, and in four others inoculated with Mexican BV virus.

Agglutinins for *Proteus* OX19, OX2 and OXL were practically absent in the pooled sera from rabbits before the infective inoculation.

Only in one case a titer above 1:20 was recorded with the OX2 strain in the serum of rabbit No. 6 (1:80).

TABLE VII

Weil-Felix reaction in rabbits inoculated with Mexican and São Paulo virus

VIRUS	RABBIT	TEMPERATURES INOCULATED RABBITS	PROTEUS	AGGLUTINATION TITER 0 DAYS	11	15	18	25	30	35
SÃO PAULO STRAIN	1		OX19	0		320	320	160	80	
			OX2	10		160	160	80	40	
			OXK	10		20	10	10	10	
			OXL	0		320	320	80	80	
	2		OX19	0	80		80	80	10	10
			OX2	20	320		320	160	10	0
			OXK	80	40		40	40	20	10
			OXL	0	160		160	80	10	0
	3		OX19	0	40		40	80	40	40
			OX2	10	640		320	320	80	80
			OXK	320	640		640	160	40	20
			OXL	10	40		160	160	40	40
MEXICAN STRAIN	4		OX19	0		160	80	80	40	
			OX2	0		1280	640	640	320	
			OXK	10		10	10	10	20	
			OXL	0		80	80	40	40	
	5		OX19	10	10		10	10	10	10
			OX2	0	80		80	80	20	0
			OXK	10	10		10	10	0	0
			OXL	0	10		10	10	10	10
	6		OX19	0	80		80	80	20	40
			OX2	80	160		320	320	320	80
			OXK	640	320		320	80	40	40
			OXL	20	160		320	80	80	80
	7		OX19	0	0		160	160	40	20
			OX2	10	10		160	40	10	10
			OXK	10	10		80	80	20	20
			OXL	10	10		320	160	20	20

The Weil-Felix reaction shows sometimes considerably high titers in normal rabbits with *Proteus* OXK; positive reactions are frequently found at the dilution of 1:80 or above. In three of the seven rabbits employed in this study, positive reactions were recorded at considerably high dilutions (1:80, 1:320 and 1:64). In connection with this observation, it must be emphasized that special care has been given to the technique of the reaction by using only emulsions free from H antigen and submitted to White's test, in order to exclude non-specific agglutination. Besides, all the reactions have been made separately on the day immediately following bleeding and repeated in a group test with the same bacterial suspension.

Felix (16) has also recorded sometimes high titers for OX2 and OXK, in normal rabbits, never exceeding, however, 1:50. This investigator does not consider these rabbits as "normal", suggesting that these high titers would be accounted for by infections provoked in rabbits by *Proteus vulgaris,* which possesses the same group antigens as the strains of *Proteus* X. Therefore, he advises, in studies on the Weil-Felix reaction, only the use of rabbit sera the titers of which do not exceed 1:20.

In the present paper we are including as additional evidence the results of the Weil-Felix reaction performed in rabbits, which have presented high titers for *Proteus* OXK before inoculation, in order to verify once more the non-influence of the action of São Paulo Spotted Fever virus and also of the virus of the new Mexican rickettsiosis upon *Proteus* OXK.

The Weil-Felix reaction was practically identical in the rabbits inoculated with BV virus-blood, and in those inoculated with São Paulo Spotted Fever virus. All the animals, with only one exception (No. 5), showed significant increase in agglutination titer for X19, X2 and XL strains. On the contrary, no significant increase in agglutinins for *Proteus* XK was verified except in the last rabbit, the original titer (1:10) of which increased to 1:80 eighteen days after inoculation; in the other rabbits no increase at all was recorded, but rather a decrease during the period of observation.

The highest titers for 0X19, 0X2 and 0XL were recorded on the 11th day after inoculation, corresponding to the final period of thermic reaction on the beginning of normalization of temperature. The agglutinin content remained stable for about 14 days and then decreased, reaching again the original titers between the 30th and 35th day after inoculation, or 20 to 25 days after the fall of temperature.

Usually, the titers for *Proteus* X2 strains were higher than for *Proteus* X19 in the rabbits inoculated with both virus strains. This fact has already been described for the São Paulo Spotted Fever virus by Travassos and Monteiro (17), and by Davis and Parker (19) for the Rocky Mountain Spotted Fever virus.

With *Proteus* OXL the differences were not frequent enough to be significant, and to a certain extent, a parallelism with the *Proteus* OX19 titers was observed.

Rabbit No. 7 did not present a conspicuous thermic reaction, showing temperature above 39.6°C. only on the 9th and 10th day. The Weil-Felix reaction on the 11th day was negative; on the second bleeding, however, 18 days after inoculation, in spite of the mildness of the reaction, agglutination titers increased for the four strains, but decreased much faster than in the other animals, which showed more serious symptoms and more typical temperature curve.

The possible meaning of these high OXK titers in our rabbits coming from the breeding place and their decrease during the stay in the laboratory after inoculation with São Paulo Spotted Fever virus is being submitted to further investigation.

DISCUSSION

The observations presented in this paper afford sufficient evidence that the virus of the new human rickettsiosis, isolated in Mexico, in the States of Sonora and Sinaloa by Bustamante and Varela, may be considered immunologically identical with the Spotted Fever viruses.

Cross immunity and neutralization tests carried out comparatively with São Paulo Spotted Fever virus and with the virus of the Mexican rickettsiosis, as well as the behaviour of the Weil-Felix reaction in experimentally inoculated rabbits, led to the conclusion that the rickettsiae responsible for both infections have a similar, if not identical antigenic structure.

This identity is evident in regard to virus strains obtained from human cases, as well as to strains of spontaneously infected *Amblyomma striatum* and *Amblyomma cajennense*.

The protection against the Mexican virus shown by guinea-pigs inoculated with a tick-vaccine against São Paulo Spotted Fever, and above all with an egg-vaccine against Rocky Mountain Spotted Fever, together with the proved immunological identity of the São Paulo and Rocky Mountain Spotted Fever viruses (7-11) and the known experimental transmission of the Mexican rickettsiosis by *Amblyomma striatum*, allows the inclusion of the etiological agent of the "Fiebre pinta" or "Fiebre de Choix" in the genus *Dermacentroxenus* Wolbach, 1919, in spite of the fact that up to now no spontaneously infected tick species has been found either in Sonora or Sinaloa.

If we adopt Philip's (18) classification of pathogenic rickettsiae, as a temporary nomenclature basis, the rickettsia of Mexican Spotted Fever would be included in the genus *Rickettsia* Rocha Lima, 1916, subgenus *Dermacentroxenus*

Wolbach, 1919, species *rickettsi* (Wolbach, 1919), i. e., the same found in Rocky Mountain Spotted Fever as well as in the Spotted Fevers occurring in Brazil and in Colombia.

According to Pinkerton and Hass (21), the differentiation between the rickettsia of the typhus group infections (*Rickettsia prowazeki* Rocha Lima, 1916) and those belonging to the genus *Dermacentroxenus* (name given originally by Wolbach (22) to the rickettsia responsible for Spotted Fever) could be based on the fact that the latter are visible within the nuclei of the infected tissues, whereas the former are only found in the cytoplasm.

According to Hass and Pinkerton (23), the rickettsia of "boutonneuse" fever should also be included in the genus *Dermacentroxenus*, since it is also found within the nuclei of the tissues of *Rhipicephalus sanguineus*, as well as in tissue cultures. This fact, together with the positive cross immunity tests between the "boutonneuse" and the Rocky Mountain Spotted Fever (24), enabled these authors to place the so called "boutonneuse" fever in the Spotted Fever group (*). It is also possible by means of cross immunity tests, to enroll South African tick-bite fever among infections of this group, which quite increases the list of Spotted Fevers outside the American continent.

For the Spotted Fevers occurring outside the United States, several names have been proposed, recalling either the geographic distribution of the disease ("Fiebre de Tobia", "Fiebre de Choix", "Kenya fever", etc.), or certain striking symptom, such as in "Fiebre pinta", "Fiebre petequial", "Fièvre boutonneuse". Other denominations such as "São Paulo and Minas Gerais exanthematic typhus", may be considered as misgiven.

In the last case, the name "Exanthematic typhus", brings practical inconveniences for the establishment of an adequate treatment and control of the disease. To those not acquainted enough with the characteristics of the rickettsiosis most frequently found in Brazil, that name reminds infections of the typhus group, rickettsioses caused by *Rickettsia prowazeki* and transmitted through the bite of human louse or flea. Laymen and even physicians of certain rural regions, when facing the Spotted Fever diagnosis made by official laboratories under the heading "Exanthematic typhus" have often taken wrong or unnecessary epidemiological measures.

The denomination "Spotted Fever" should be adopted for all exanthematic typhus infections transmitted by *Ixodidae* and immunologically related to the

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(*) Recently, Plotz, Reagan and Wertman (25) by means of complement fixation reaction with rickettsial antigen of tissue cultures, were able to distinguish the boutonneuse fever virus from that of the Rocky Mountain Spotted Fever.

rickettsioses formerly found in the Rocky Mountain region, in the United States. The name "Spotted Fever", although derived from symptoms common to several infections, will not bring confusion with other infectious diseases, not even with other rickettsiosis of the typhus group. Its use in the medical literature already reminds a well studied and individualized morbid entity.

Hence, for priority's reason, all infections that are or will be related to the so called Rocky Mountain Spotted Fever should be called "Spotted Fever" (**). If we use the name "Rocky Mountain Spotted Fever" for other rickettsiosis of this group, we will have to face the significant inconvenience of a geographic limitation, preventing its generalization.

For the same reason, we cannot adopt the denomination "American Spotted Fever", suggested by Bustamente and Varela, nor any other name which includes in its designation any specification concerning a city, region, country or continent, since as we have pointed out, there are or might be recorded tick transmitted rickettsioses in countries outside the American continent, the viruses of which present an immunological behaviour related to that of the Spotted Fever group.

There is "Spotted Fever" in the Rocky Mountains (United States), in São Paulo, Minas Gerais (Brazil), in Tobia (Colombia), in Sonora, Sinaloa (Mexico), etc., as well as there is Malaria, Yellow Fever, etc. in so many regions of the world.

It is probable that new foci of Spotted Fever will be detected mainly in those regions, where *Ixodidae* are abundant and can feed on man. Being an infection which essentially strikes rural zones, Spotted Fever is usually diagnosed only after serious cases are reported in sufficient number to attract attention, such as has been the case in Brazil, in Colombia and now in Mexico. In Brazil, the new foci that presented a considerable number of cases, could usually be traced back to a loss of balance in the normal tick biology, due to the introduction of means which favoured an exaggerated increase of its population.

On the other hand, as soon as the disease is better known clinically by a larger number of physicians and the means for laboratory diagnostic become available, as in the United States and now in Brazil, a much larger number of sporadic cases are reported, and cases in new regions identified. In the same way and for the same reason, it has been possible to observe benign cases of Spotted Fever and to isolated less virulent virus strains from individuals, in whom other wise the disease would not have been recognized.

(**) In Portuguese "Febre Maculosa", in Spanish "Fiebre manchada".

ABSTRACT

The paper deals with the study of the immunological relationship between a virus isolated by Bustamante and Varela from a new rickettsiosis recorded in Mexico, in the States of Sonora and Sinaloa, and other strains of Spotted Fever virus. Following results are reported:

1. Guinea-pigs surviving infection with São Paulo Spotted Fever virus displayed complete protection when reinoculated with Mexican virus. Furthermore, the pooled sera obtained from these guinea-pigs before reinoculation showed neutralizing antibodies against São Paulo Spotted Fever virus.

2. Convalescent serum from rabbits infected with São Paulo or Mexican virus exhibited protective antibodies against the homologous, as well as against the heterologous strain.

3. Guinea-pigs inoculated with a 1 cc. dosis of a Spencer-Parker vaccine prepared at Butantan Institute (São Paulo, Brazil), with local Spotted Fever virus strains, proved partially immune against the virus of the new Mexican rickettsiosis.

4. Guinea-pigs vaccinated with a 1 cc. dosis of a Cox vaccine prepared at the Rocky Mountain Laboratory (Hamilton, Montana, U. S. A.) against Rocky Mountain Spotted Fever. showed solid immunity to the Mexican virus.

5. The course of the Weil-Felix reaction is identical in rabbits experimentally infected with the Mexican virus or with the São Paulo Spotted Fever virus. In both cases there is a rise of the agglutination titer for the *Proteus* OX19, OX2 and OXL strains, reaching the maximum at end of the fever reaction or beginning of recovery, remaining stable for a few days and decreasing rapidly within 30 or 35 days after inoculation or within 20 or 25 days after recovery. No significant increase of agglutination for strain OXK was observed. In general the titer was higher for *Proteus* OX2, than for OX19 and OXL.

The experiments reported allow the conclusion that an immunological identity exists between the viruses of Mexican Sonora and Sinaloa rickettsiosis and São Paulo Spotted Fever.

RESUMO

Neste artigo são estudadas algumas propriedades imunológicas do virus isolado por Bustamante e Varela de doente de uma nova rickettsiose verificada no Mexico nos Estados de Sonora e Sinaloa, em comparação com amostras de virus da febre maculosa. São os seguintes os resultados obtidos:

1. Cobaios sobreviventes da infecção provocada com virus de febre maculosa, isolado no Estado de São Paulo, mostram sólida imunidade perante reinoculações feitas com virus mexicano. Mistura de soros obtidos destes mesmos cabaios no periodo da convalescença e antes da reinoculação apresentaram anticorpos neutralizantes ativos para amostras de virus de febre maculosa de São Paulo.

2. Soros de coelhos convalescentes de infecção com virus de São Paulo ou do México apresentaram anticorpos protetores, tanto para o virus homólogo, como para a amostra heteróloga.

3. Cobaios inoculados com uma dose de 1 cm³ de vacina tipo Spencer-Parker, preparada no Instituto Butantan com amostras de virus isoladas em São Paulo, mostram-se parcialmente protegidos contra o virus da nova rickettsiose mexicana.

4. Cobaios vacinados com uma dose de 1 cm³ de vacina tipo Cox, preparada no Rocky Mountain Laboratory, nos Estados Unidos, contra a febre maculosa das Montanhas Rochosas, apresentaram sólida proteção às inoculações feitas com virus mexicano.

5. A reação de Weil-Felix, estudada em coelhos experimentalmente infetados com virus mexicano, mostrou idêntico comportamento ao que se verifica com amostras de virus da febre maculosa de São Paulo, isto é, elevação do título aglutinante para as amostras de *Proteus* OX19, OX2 e OXL, atingindo o máximo no fim da reação febril ou início da convalescença, mantendo-se estável durante alguns dias e decaindo rapidamente dentro de 30 a 35 dias a contar da inoculação ou sejam entre 20 e 25 dias do início da convalescença. Não houve aumento significativo de aglutinação para a amostra OXK. Em geral o título foi mais elevado para *Proteus* OX2, do que para OX19 e OX2.

Estas experiencias permitem concluir pela identidade imunológica existente entre a amostra de virus isolada no Mexico e as amostras provenientes de casos de febre maculosa em São Paulo.

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