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SPOTTED FEVER IN PANAMA; ISOLATION OF THE ETIOLOGIC AGENT FROM A FATAL CASE[1]

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Although spotted fever is known to be endemic in this hemisphere in Canada, the United States, Mexico, Colombia and Brazil, its occurrence on the Isthmus of Panama has not yet been demonstrated. It seems of importance, therefore, to report the isolation of a strain of *Rickettsia* belonging to the spotted fever group from the blood of a native Panamanian, permanently resident in the Republic, who died of a fulminant infection 12 hours after admission to the Santo Tomas Hospital located in the City of Panama. Due to the grave clinical course of the disease, the jaundice and vomiting, yellow fever was strongly suspected and the assistance of this laboratory solicited in an attempt to isolate the virus. For this purpose the chief pathologist, Dr. J. M. Herrera and the physician in charge of the ward Dr. J. Nicosia, submitted the following specimens: blood drawn on the day of admission, blood drawn from the heart post-mortem and sections of liver, spleen and cerebral tissue. Macroscopic and microscopic findings later proved negative for yellow fever and a tentative diagnosis of acute hemolytic malaria was made by the pathologist.

The following clinical details are available: The patient, A. S., a 26 year old negro farmer resident in the town of Chorrera in the Republic of Panama all of his life, entered the hospital on February 7, 1950. He had suffered from fever and bilious vomiting for eight days prior to admission. He had no history of vaccination against yellow fever. His condition on entry was grave with temperature of 39.0°C, pulse rate 140 per minute, respiration 40 per minute, conjunctival jaundice and generalized maculo-hemorrhagic efflorescences. The liver was palpated four fingers below the costal margin. The sensorium was clear. The urine showed two plus albumen and granular tubes. The red cell count was 5.1 million and the white count 13.750 with 74 per cent neutrophiles, 24 per cent lymphocytes and 2 per cent monocytes. Blood smears for malaria were negative. An admitting diagnosis of parenchymatous hepatitis (probably yellow fever) and purpura hemorrhagica was made. Death occurred 12 hours after admission on February 8.

In view of the suggested diagnosis of yellow fever, serum in various dilutions and suspensions of liver, spleen and cerebral tissue were inoculated intracerebrally into white mice. Also one cubic centimeter of serum from the blood specimen drawn on February 7th was inoculated subcutaneously into a young female *Macacus rhesus* No. 336 B and three cubic centimeters of a mixture of tissue suspensions and blood drawn postmortem on February 8th were inoculated into a second young female *Macacus rhesus* No. 333 B. All mice remained perfectly well throughout a two month observation period. However, Rhesus No. 333 B

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developed a rectal temperature of 104.8°F. on Feb. 13, 5 days after inoculation. The temperature ranged between 104.8 and 105.2°F. for the succeeding 4 days and then dropped gradually to 102° by February 20, after which the animal recovered completely and is alive and well at the present time. Rhesus No. 336 B developed a temperature of 104.8°F. on February 15, 7 days after inoculation. The temperature ranged between 103.8° and 104.8°F. during the succeeding 3 days. There was increasing inappetence and apathy and the animal was found dead on February 19th. The eyelids were edematous and the eyes filled with a serous exudate. The inguinal lymph nodes were moderately enlarged and reddened, the spleen enlarged and firm and the liver pale with patches of yellowish discoloration. Otherwise gross postmortem pathology was negative.

Daily samples of serum from these two monkeys during the febrile period were inoculated intracerebrally into white mice with negative results. However, serum drawn from Rhesus No. 336 B on the second day of fever was inocu.ated into the yolk sacs of 6 seven-day old chick embryos producing death in all in a period of 1 to 4 days. Successive passages were made in further 7-day embryos but *Rickettsiae* in sufficient numbers for definite identification were not observed until the 4th passage. To date 16 successive passages have been effected. Morphology is typical but growth is usually scanty and irregular perhaps due to the fact that only one incubator has been available for these studies, which we have found it convenient to maintain at 37.5°C. The *Rickettsiae* take on a pale purple coloration with Giemsa's stain, a blue color with Castañeda's. The majority show the form of a minute lanceolate diplococcus although spherical diplococcoid, coccoid, bacillary and filamentous forms of varying size may be present. They are found in the intranuclear, intracyloplasmic and extracellular positions. Intracytoplasmic forms are most abundant, intranuclear forms more rare. The extracellular forms could be accounted for by the extensive cellular rupture and destruction which is usually observed. The chick embryos die within 24 to 72 hours. There may be marked vascular congestion and hemorrhages in the embryo proper and the vitelline membrane, lesser hemorrhages into the chorioallantoic membrane. Embryos dying on the 3rd and 4th days show greater numbers of *Rickettsiae* than those dying earlier and have been preferred as passage material.

THE DISEASE IN GUINEA-PIGS

Two male guinea-pigs were inoculated intraperitoneally with a third passage yolk sac suspension before the appearance of typical *Rickettsiae*. Both developed fever on the third day after inoculation which continued for 5 and 6 days followed by death on the 8th and 9th days respectively. Sporadic passages have been made in additional guinea-pigs employing both yolk sac suspensions and homologous blood drawn on the second or third day of fever as the inoculum and the intraperitoneal or subcutaneous route of injection. The incubation period has varied from 2 to 5 days, being longer usually after inoculation with homologous blood than with yolk sac suspensions. There is continuous high fever commonly reaching a peak of 105 to 106°F. and lasting for from 2 to 7 days. The temperature may then fall rapidly or gradually to normal or subnormal ranges. Mortality after

injection with yolk sac suspensions has been 100 per cent. Of 20 animals so far injected with homologous blood two have survived, a mortality of 90 per cent. The animals show inappetence, weight loss, roughening of the coat and debility. Inflammation of the scrotum is regularly present. This organ shows a swollen dark red appearance and may later develop areas of necrosis. In some pigs there is a sero-purulent exudate over the conjunctivae. Congestion of the ears and foot-pads may also be observed, and rarely hemorrhage from the nose. Constant post-mortem findings include enlargement of the spleen which may be 2 to 4 times normal size and is frequently covered with a greyish fibrinous exudate, enlargement and hyperemia of the inguinal and to a lesser extent the axillary lymph nodes with congestion of the surrounding connective tissue, reddening and edema of the scrotal sac with adhesions between the visceral and parietal lamina of the tunica vaginalis, congestion and at times petechia of the superficial vessels of the testes and hemorrhages into the tunica, scrotum and polar fat of the testes. Small discrete areas of necrosis in the liver may be observed, especially after inoculation of yolk sac suspensions. Hemorrhages into the wall of the stomach and intestine are seen rarely. A sero-sanguinolent exudate may be present in the peritoneal cavity in some animals. *Rickettsiae* have been observed in relatively small numbers in scrapings of the tunica vaginalis and parietal peritoneum, and occasionally also in impression smears of the liver and spleen, in animals dying after inoculation with either yolk sac suspensions or homologous blood.

Extensive bacteriological cultures in a wide variety of media both by aerobic and anaerobic methods were carried out with the blood of the patient, blood and tissues of infected animals and yolk sac suspensions with negative results.

CROSS-IMMUNITY STUDIES

Several experiments to test possible cross-immune reactions between the AS strain of spotted fever and a strain of murine typhus (MM) and a strain of Q fever (JD) both isolated locally by Rodaniche and Rodaniche (1) were conducted. Pigs were challenged with spotted fever *Rickettsia* in yolk sac suspensions or homologous blood. No cross-immunity was demonstrated, the incubation period of the disease and the severity of infection being approximately the same in all groups. Details of one such experiment are presented in Table I. A yolk sac suspension of spotted fever *Rickettsiae* was administered intraperitoneally as the challenging inoculum. Reverse cross-immunity experiments have been limited by the difficulty in obtaining survivors of infection with the AS strain of *R. rickettsi*. In one experiment three guinea-pigs surviving in a protection test (see Table II) were utilized. Of these, two were inoculated with blood of a guinea-pig infected with Q fever and the third with blood of a guinea-pig infected with murine typhus. All showed incubation and febrile periods closely corresponding to those of the controls.

PROTECTION TESTS

The convalescent blood of Rhesus No. 333 B which survived inoculation with tissue suspensions and postmortem blood of the patient was employed in an in-

traperitoneal protection test in Swiss mice for yellow fever and an intraperitoneal protection test in guinea-pigs for spotted fever. The yellow fever protection test gave a negative result. The following technique was employed for the protection test in guinea-pigs for spotted fever. One cubic centimeter of serum drawn from Rhesus No. 333 B on March 9, 18 days after defervescence, was combined with 1 cc. respectively of a 5 per cent and 0.5 per cent yolk sac suspension of the AS strain of *Rickettsia*. The mixture was allowed to stand one hour at room tempera-

TABLE I

Cross-immunity test in guinea-pigs between spotted fever and Q and murine typhus fevers

NO. OF GUINEA-PIG	IMMUNE STATUS	INTERVAL BETWEEN INOCULATIONS	INCUBATION PERIOD	MORNING TEMPERATURES DEGREES FAHRENHEIT					HEMORRHAGIC TESTICULAR REACTION
				2nd day	3rd day	4th day	5th day	6th day	
		days	*days*						
754	Normal	—	2	106.3	105	103.2	D		Yes
755	Normal	—	2	105.4	105.4	104	105	D	Yes
687	Q fever immune	62	2	104.8	105.2	104.7	103.6	D	Female
694	Q fever immune	52	2	106.4	105.4	105.6	105.4	D	Female
681	Typhus immune	67	2	105	105.2	104.5	103.4	D	Yes
669	Typhus immune	84	2	105.2	104.6	104.8	104.6	D	Yes

D indicates death.

TABLE II

Protection test in guinea-pigs with immune serum and spotted fever rickettisa

GUINEA-PIG NO.	INOCULUM	INCUBATION PERIOD	NO. DAYS FEVER	DAY OF DEATH
		days		
715	5% yolk sac suspension + immune serum	12	3	12th
716	5% yolk sac suspension + immune serum	15	8	Survived
717	0.5% yolk sac suspension + immune serum	13	5	Survived
718	0.5% yolk sac suspension + immune serum	15	4	Survived
719	5% yolk sac suspension + normal serum	3	3	6th
720	5% yolk sac suspension + normal serum	3	3	6th
721	0.5% yolk sac suspension + normal serum	3	2	5th
722	0.5% yolk sac suspension + normal serum	3	5	9th

ture and then 1 cc. of each dilution was injected intraperitoneally into paired male guinea-pigs. As a control, serum from the same monkey drawn one month prior to inoculation was similarly prepared and inoculated. Results are presented in Table II. Positive protection is shown by the prolonged incubation period and higher survival rate of the test as compared to the control animals. This experiment demonstrates identical etiology for the disease in the two monkeys injected with blood or tissues of the patient AS.

IN VITRO SEROLOGICAL REACTIONS

Various in vitro serological reactions using the blood of the patient, blood of Rhesus No. 333 B drawn prior to inoculation and during the febrile period of the

disease and convalescence, and blood of two guinea-pigs convalescent from an injection of homologous blood were carried out. These included the Weil-Felix reaction using Proteus OX19, OX2 and OXK and the complement-fixation reaction for Q, typhus and spotted fevers. The following antigens were employed: Q fever (Italian Strain-Henzerling), typhus fever (Epidemic) and typhus fever (murine), all purchased from Lederle Laboratories Division of the American Cyanamid Company; and a rickettsialpox antigen kindly forwarded to us by Dr. R. J. Huebner of the National Institute of Health in response to our request for Rocky Mountain spotted fever antigen which was not then available, with the following comment "there is little or no serological difference between the two antigens (rickettsialpox and Rocky Mountain spotted fever) as they are or-

TABLE III

Weil-Felix and complement-fixation reactions of spotted fever serums

SOURCE OF SERUM	TIME OF BLEEDING	WEIL-FELIX REACTION			COMPLEMENT-FIXATION REACTIONS				
		OX19	OX2	OXK	Rickettsialpox	Typhus (murine)	Typhus (epidemic)	Q fever	Homologous antigen
Rhesus no. 333B	Prior to inoculation	0	0	0	0	0	0	0	NT
Rhesus no. 333B	2nd day of fever	0	0	0	0	0	0	0	NT
Rhesus no. 333B	2nd day of convalescence	160	0	0	256	0	0	0	NT
Rhesus no. 333B	19th day of convalescence	40	0	0	512	0	0	0	1024
G-pig no. 742	4th day of convalescence	NT	NT	NT	32	0	0	0	NT
G-pig no. 759	12th day of convalescence	NT	NT	NT	128	0	0	0	NT
A.S.	one day prior to death	0	0	0	0	0	0	0	NT
A.S.	postmortem	0	0	0	0	0	0	0	NT

NT indicates no test was made.

dinarily prepared . . . for purposes of screening diagnosis rickettsialpox antigen can be used in both diseases". One test was also conducted with the convalescent serum of Rhesus No. 333 B and an ether extracted soluble type antigen prepared from yolk sacs of chick embryos inoculated with the AS strain of spotted fever according to the method 2F of Topping and Shepard (2). This antigen gave a slightly higher endpoint than the rickettsialpox antigen as may be noted in Table III. Due to the sparse and irregular growth of the *Rickettsia* in chick embryos, it has not been possible as yet to prepare sufficient antigen to test other serums. Results of these various serological reactions are presented in Table III. The highly positive reactions with rickettsialpox antigen place this organism in the spotted fever group. The negative reactions with the blood of the patient were to be expected in view of the fulminant nature of the case. No cross serological reactions were noted with Q fever and typhus antigens. The high specificity of the

complement fixation reaction using rickettsial antigens has been adequately demonstrated by Plotz and Wertman (3) Cox (4) and others.

DISCUSSION

The serological reactions and behavior in experimental animals of the strain of *Rickettsia* here described place it beyond question in the spotted fever group. Unfortunately the highly specific washed rickettsial antigens recommended by Plotz, Reagan and Wertman (5) have not been available for serological differentiation within that group. However, the clinical picture in the patient and the high mortality in the guinea-pig serve to differentiate it from rickettsialpox, fievre boutonneuse and South African tick typhus (4, 6). It may further be differentiated from rickettsialpox as described by Huebner (7) by the production of a positive Weil-Felix reaction with Proteus OX19 in the rhesus monkey and the different experimental host range; *e.g.* insusceptibility of the mouse and susceptibility of the monkey.

In every respect the organism isolated by us corresponds most closely to *Rickettsia* (*Dermacentroxenus*) *rickettsi*, the etiological agent of Rocky Mountain spotted fever, as do also apparently the causative organisms of Sao Paulo typhus of Brazil as summarized by Dias and Martins (8) Tobia fever of Colombia identified by Patiño, Afanador and Paul (9) and Mexican spotted fever studied by Bustamante and Varela (10).

CONCLUSIONS

Isolation of a strain (AS) of *R. rickettsi* from a fatal case of spotted fever in the Republic of Panama is reported and the characteristics of the strain discussed. This is the first definite identification of spotted fever on the Isthmus of Panama.

Since the preparation of this paper, a second case of Rocky Mountain spotted fever, also from the vicinity of La Chorrera, R.P. has been indentified. This patient, V.R., entered the Santo Tomas Hospital on May 15, 1950 with classical symptoms. A strain of *R. rickettsi* similar in all respects to the one here described was isolated from acute-phase blood and the complement-fixation reaction with rickettsialpox antigen changed from negative during the acute phase to positive in a titer of 1 to 128 in early convalescence.

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