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ETIOLOGY OF OROYA FEVER.

X. COMPARATIVE STUDIES OF DIFFERENT STRAINS OF *BARTONELLA* *BACILLIFORMIS*, WITH SPECIAL REFERENCE TO THE RELATIONSHIP BETWEEN THE CLINICAL TYPES OF CARRION'S DISEASE AND THE VIRULENCE OF THE INFECTING ORGANISM.

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PLATES 7 TO 10.

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Dr. Sebastian Lorente, Director of the National Department of Public Health of Peru, during a visit to New York in 1926, expressed the wish that experimental studies of Carrion's disease might be further extended and agreed to forward specimens of patients' blood to The Rockefeller Institute for the purpose. Six samples of blood were received from Dr. Lorente on December 29, 1926, and six more on April 1, 1927. Comparative studies of the strains of *Bartonella bacilliformis* isolated from these specimens form the subject matter of the present report. I wish at the outset to express to Dr. Lorente my appreciation of the cooperation which made the work possible.

Isolation of Bartonella bacilliformis from Specimens of Patients' Blood.

The blood was collected in sterile vacuum bulbs containing dry sodium citrate sufficient to prevent coagulation (0.1 gm. per 10 cc. of blood). The containers were hermetically sealed and shipped in the steamer's refrigerator from Lima to New York, the time required for transportation being about 14 days. Three of the first six samples were unsuitable for cultural study, having become contaminated with extraneous bacteria by leakage of the container during transportation. The second shipment (Cases 7 to 12) arrived in perfect condition. *Bartonella bacilliformis* was obtained in pure culture from all six

specimens of the second lot and from the three uncontaminated samples of the first lot, nine strains in all being isolated.

The blood was inoculated into leptospira medium by the titration method, that is, several dilutions were made of each specimen (1:10, 1:100, 1:1,000, 1:10,000, 1:100,000), and a tube of culture medium was inoculated for each dilution and one for the undiluted citrated blood, a total of six tubes of medium for each sample of blood. The amount of inoculum was 0.2 cc.

Dark-field examination revealed no motile organisms. In stained smears forms resembling *Bartonella* were found, but they were not sufficiently numerous or characteristic to be identified with certainty.

Case 1, F. A., 18 years old, resident of Canta, Department of Lima. Had been ill 3 months. Verruga suspected because of patient's residence and his symptoms. Erythrocytes 2,900,000. Leucocytes 7,000; neutrophils 72 per cent, eosinophils 1 per cent, monocytes 7 per cent, lymphocytes 20 per cent; no basophils. *Plasmodium falciparum* was present in the blood. Temperature 37.8°C. at time blood was taken.

Case 2, J. M., 45 years old, resident of Oroya, Department of Junin. Had been ill 2 months. Generalized miliary verrucous eruption as well as subcutaneous nodules. There had been fever of remittent type, but patient was afebrile at the time of taking blood. Erythrocytes 2,500,000. Leucocytes, 13,600; neutrophils 65 per cent, eosinophils 2 per cent, basophils 1 per cent, monocytes 10 per cent, lymphocytes 22 per cent.

Case 3, G. H., 20 years old, resident of Matucana, Department of Lima. Had been ill 15 days. Verrucous eruption of miliary type, scattered. Fever of remittent type still present, highest temperature 40°C. Erythrocytes 3,400,000. Leucocytes, 9,000; neutrophils 69 per cent, eosinophils 1 per cent, monocytes 8 per cent, lymphocytes 22 per cent; no basophils. Barton's bodies present, bacilliform type. Wassermann reaction positive.

Case 4, L. W., 37 years old, resident of Oroya, Department of Lima. Had been ill 1 month. Verrucous eruption of subcutaneous miliary form, scattered, localized chiefly in lower limbs. Patient had no fever at time blood was taken.

Case 5, A. F., 31 years old, resident of Chosica, Department of Lima. Had been ill 4 months. Miliary eruption and subcutaneous nodules were present on both arms and on thorax. Fever of remittent type still present; highest temperature 39.5°C., temperature at time blood was taken 37.8°C. Erythrocytes 1,920,000. Leucocytes 4,600; neutrophils 40 per cent, eosinophils 3 per cent, basophils 1 per cent, monocytes 9 per cent, lymphocytes 47 per cent. Wassermann reaction positive.

Case 6, F. T., 28 years old, resident of Matucana, Department of Lima. Had been ill 2 months. No eruption at time blood was taken, but for 1 month patient

had subcutaneous miliary lesions. Erythrocytes 2,460,000. Leucocytes, 6,400; neutrophils 73 per cent, eosinophils 2 per cent, monocytes 2 per cent, lymphocytes 23 per cent; no basophils.

Case 7, S. N., 22 years old, resident of Puruhuay (Chosica), Department of Lima. No fever. Generalized verrucous eruption of miliary and nodular types. Erythrocytes 3,480,000. Leucocytes 4,400; neutrophils 72 per cent, eosinophils 1 per cent, basophils 1 per cent, monocytes 4 per cent, lymphocytes 22 per cent.

Case 8, J. A., 33 years old, resident of Canta, Department of Lima. Had been ill 6 months. No fever. Generalized miliary eruption. Erythrocytes 3,100,000. Leucocytes, 4,000; neutrophils 65 per cent, eosinophils 1 per cent, monocytes 8 per cent, lymphocytes 26 per cent; no basophils.

Case 9, J. M., 19 years old, resident of Chosica, Department of Lima. Had been ill 15 days. Continuous fever, 39°C. No eruption. (Malignant verruga?) Erythrocytes 1,250,000; normoblasts 4 per cent, erythroblasts 2 per cent. Anisocytosis, anisochromia, polychromatophilia. Leucocytes 8,400; neutrophils 73 per cent, eosinophils 2 per cent, monocytes 3 per cent, lymphocytes 22 per cent; no basophils. Barton's bodies present, bacilliform type predominating. Jolly bodies also present.

Case 10, E. C., 39 years old, resident of Chosica, Department of Lima. Had been ill 1 month. No fever. Verrucous eruption, miliary and nodular, scattered. Erythrocytes, 5,080,000. Leucocytes 5,200; neutrophils 44 per cent, eosinophils 7 per cent, basophils 1 per cent, monocytes 7 per cent, lymphocytes 41 per cent. Barton's bodies of coccoid type present.

Case 11, M. C., 27 years old, resident of Chosica, Department of Lima. Had been ill 12 days. No fever, and no eruption at time blood was taken. Erythrocytes 1,080,000; normoblasts 9 per cent, erythroblasts 2 per cent, neutrophilic myelocytes 3 per cent. Megalocytes. Leucocytes 22,200; neutrophils 88 per cent, monocytes 3 per cent, lymphocytes 9 per cent. No eosinophils or basophils. Barton's bodies, coccoid form, present.

Case 12, M. L., 28 years old, resident of Matucana, Department of Lima. Had been ill 1 month. No fever. Eruption of miliary type, discrete. Erythrocytes 4,800,000. Leucocytes 4,900; neutrophils 54 per cent, eosinophils 5 per cent, monocytes 10 per cent, lymphocytes 31 per cent; no basophils. Barton's bodies present, coccoid form predominating.

Comparative Studies of the Strains Isolated.

Appearance of Growth (30°C.).

On leptospira medium there is a light grayish, usually homogeneous, but sometimes finely granular, translucent layer of growth at the top of the column of medium. Single isolated colonies form denser central masses and are surrounded by a hazy zone of outgrowth from the periphery. All the strains pre-

sented a similar appearance except Nos. 9 and 12, which grew faster and more densely than the others. Horse blood agar plates or slants yield minute grayish, raised, shiny, firm miliary colonies which appear transparent but are grayish when covered with a layer of saline solution. They adhere to the medium and are difficult to scrape from the surface. Strains 9 and 12 form somewhat larger colonies, which reach a diameter of 2 mm. within 4 or 5 days.

Motility (Dark-Field Observations).

Cultures on leptospira medium or horse blood agar slants or plates contain very actively motile forms for the first 4 to 6 days, especially in the condensation water of the blood slants, but the organisms invariably become motionless sooner or later. The flagella apparently become abnormally large under the unfavorable conditions existing in a semisolid medium and are thrown off; for detached flagella, so large as to be visible by dark-field examination, appear in cultures about a week old (Figs. 32 and 33).

I have been unable to detect any differences in movement among the strains studied; they all exhibit rotation and progression, singly or in groups of two or three, or in masses. Occasionally there is no motility at all even in young cultures, owing undoubtedly to unfavorable conditions of cultivation.

Staining Properties and Dimensions.

All the strains are frankly Gram-negative and take basic fuchsin rather poorly. Giemsa's solution gives satisfactory results and brings out the fairly sharp contours of the organisms in cultures which have been grown 4 to 6 days on the surface of blood agar slants or plates. This definition of the form is quickly lost as the cultures grow older. Organisms grown on leptospira medium or in the condensation water of a blood agar slant stain only poorly, and it is often impossible to recognize individuals; nevertheless they thrive well and remain viable under these conditions much longer than on the surface of slants or plates. There is a tendency for most of the strains to take up the stain more intensely in the interior portion of the body than on the outer layer. Often the chromatophilic material is seen to be heaped up toward either end, giving the appearance of a diploid coccobacillus. Sometimes the accumulation is in nodular masses at irregular intervals throughout the entire length, the effect simulating a chain of ill stained cocci, while in shorter forms there may be only a single deeply stained dot. The organisms often assume a kind of triangular or elongated wedge shape.

In fresh preparations of young, actively motile cultures, the organisms all appear to be of the same size and shape, but certain variations in size and form are recognizable in stained smears (Plates 7 and 8). Special care has been taken to exclude various external factors which might influence the comparison. For example, cultures have been grown on the same medium (horse blood agar plates prepared on the same day with the same material) for 6 days at 30°C.

under identical conditions, and film preparations have been made in the same way (the culture put into a drop of distilled water, the mixture spread on the slide, and dried in the air for a given period), fixed in methyl alcohol for 5 minutes, and then stained with Giemsa's solution in the same jar for 30 minutes. Another set of slides has been treated by Gram's method and counterstained with dilute carbol fuchsin, washed, blotted, and dried in the air. The measurements given in Table VI, therefore, should be fairly reliable. Strain 7 (Figs. 5 and 6) is distinctly coarser and Strain 11 (Figs. 13 and 14) decidedly finer than the rest. There is, however, a gradual transition, some approaching the coarser type and others the finer, hence striking differences are brought out only by comparing the two extremes. Some strains are short, others more rod-shaped.

Flagella (Zettnow-Fontana Combination Stain).

Determination of the type of flagella in newly isolated pathogenic microorganisms is not of morphological interest merely; it may prove to be of immunological importance. Orcutt¹ has demonstrated that the serological specificity of certain organisms resides in the flagella, and Bauer² recently found in this laboratory that serological types of tetanus bacilli possess dissimilar flagella; for example, Type III, having a single unipolar heavy spiral flagellum, and Type IV, having peritrichal, rather coarse regularly wavy flagella, of moderate length, are quite distinct from the other types. All strains of *Bartonella bacilliformis* were therefore stained for flagella by a modified procedure, in which Zettnow's mordant is followed by reduction with Fontana's ammoniac silver nitrate. The cultures used were those utilized for the measurements of size, that is, the surface growth on horse blood agar plates after 6 days at 30°C.

Certain strains proved to have comparatively shorter, smoother, and more delicate flagella than others (Plate 9). Strains 3, 9, 10, 11, and 12 (Figs. 22, 26 to 29) are of this type, while Strains 2, 7, 8 (Figs. 21, 24, and 25), and the two old strains, S.A.³ and P.5⁴ (Fig. 30) have distinctly more wavy or spiral and coarse appendages. The number of flagella seems to vary among different strains, some showing a single flagellum, others as many as four. In all instances, however, the flagella are unipolar. Short flagella scarcely exceed 3 μ , long ones may measure 10 μ or more.

Pathogenicity.

Macacus rhesus monkeys were used to determine the virulence of the original samples of blood, as well as of the newly isolated cultures,

¹ Orcutt, M. L., *J. Exp. Med.*, 1924, xl, 43, 627.

² Bauer, J. H., not yet published.

³ Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, xliii, 851.

⁴ Noguchi, H., *J. Exp. Med.*, 1927, xlv, 175.

the tests being made by three different methods. (1) The effects of local inoculation of the original samples of blood were simultaneously compared by intradermal inoculation of each specimen into separate sites on the abdominal skin of the same monkey (the experiment was done in duplicate), previous experience having shown⁵ that an experimental local lesion in *Macacus rhesus* remains confined to the site of inoculation, and that several intradermal inoculations with different materials may be made simultaneously on the same animal. (2) The effect of local inoculation of each of the cultures isolated from the blood

TABLE I.
Tests of Infectivity of Blood, Cases 1 to 6. Multiple Intradermal Inoculations, Dec. 29, 1926.

Case No.	<i>M. rhesus</i> 1A	<i>M. rhesus</i> 2A
1	—	—
2	—	—
3	—	—
4	—	—
5	—	—
6	—	—
Blood cultures, Jan. 6, 1927.		
	—	—
Blood cultures, Jan. 24, 1927.		
	+ (1:100)	+ (1:100)

was compared in the same animal with that of the inoculation of passage strains of known virulence. (3) Each strain was individually tested on a monkey by both intradermal and intravenous inoculation.

The first two types of test have an advantage over the third in that the variations in the susceptibility of individual monkeys is not involved. The third method is the more usual one, but in testing a large number of strains it is necessary to economize in monkeys, hence the factor of individual variation may enter into consideration. The results in this series of animals, however, were determined not only by local reactions but also by blood cultures at appropriate intervals.

⁵ Noguchi, H., *J. Exp. Med.*, 1927, xlv, 455.

For virulence tests, cultures grown for 8 days on leptospira medium at 30°C. and those grown for 4 to 6 days on horse blood agar slants and plates at the same temperature were pooled, the mixture comprising first, second, and third generation cultures of a given strain. The original samples of blood were tested again simultaneously with the cultures, a procedure which yielded interesting results, inasmuch as the blood, in contrast to the cultures, produced no cutaneous lesions. The results of the various tests are outlined in the tables.

Table I summarizes the results of the direct pathogenicity tests of the blood of Cases 1 to 6 inclusive. No local lesions were induced,

TABLE II.
Parallel Infectivity Tests of Blood of Cases 2, 3, and 5, and of Cultures Obtained from the Blood. Multiple Intradermal Inoculations, Feb. 7, 1927.

Case No.	<i>M. rhesus</i> 3A
2 { Blood.....	—
{ Culture.....	++
3 { Blood.....	—
{ Culture.....	++++
5 { Blood.....	—
{ Culture.....	++++

though invasion of the blood by *Bartonella bacilliformis* occurred, as shown by the results of blood culture in both animals 26 days after inoculation. The cultures from Cases 1, 4, and 6 were contaminated, those from Cases 2, 3, and 5 yielded marked local lesions (Table II; Fig. 31). Similar results were obtained with the specimens from Cases 7 to 12, the original blood being non-virulent for the skin of the monkey, while the cultures in three instances (Cases 9, 10, 11) gave rise to typical verruga lesions. Parallel tests of the blood and the cultures in Cases 7 to 12 are recorded in Tables III and IV. Strains 7 to 12 were tested separately on different monkeys (Table V) with results which agree with those of the multiple tests.

The experiments show that of nine strains tested, six possessed definite specific pathogenicity for *Macacus rhesus*; the other three

were non-pathogenic, as shown by three separate tests. The three non-virulent strains (7, 8, and 12) came from cases of benign miliary or nodular verruga, in two of which the microscopic examination of the blood had been negative for *Bartonella*; in Case 12 *Bartonella* was present in the blood, though the red count was practically normal. The six virulent strains, with the exception of Strain 10, came from patients whose blood showed either microscopically detectable num-

TABLE III.

Parallel Infectivity Tests of Blood of Cases 7 to 12 and of Cultures Obtained from the Blood. Multiple Intradermal Inoculations, May 3, 1927.

Case No.		<i>M. rhesus</i> 4A	<i>M. rhesus</i> 5A
7	Blood.....	—	—
	Culture.....	—	—
8	Blood.....	—	—
	Culture.....	—	—
9	Blood.....	—	—
	Culture.....	+++	+++
10	Blood.....	—	—
	Culture.....	+++	+++
11	Blood.....	—	—
	Culture.....	++++	++++
12	Blood.....	—	—
	Culture.....	—	—
Control (P. 5 strain, from verruga nodule)		++++	++++

bers of *Bartonella bacilliformis* or marked anemia, or both. In three of these cases there were no skin lesions, and the red counts were very low (1,080,000 to 1,250,000). These represent the pure septicemic form of Carrion's disease, without cutaneous involvement.

The fact that the virulent strains came from the severe types of Carrion's disease, and the non-virulent ones from benign verruga seems to indicate that the highly fatal disease is caused by strains possessing a greater virulence. The first strain of *Bartonella bacilli-*

formis isolated,³ which was obtained from the blood of a fatal case of Carrion's disease, was found to produce in monkeys of average susceptibility the clinical picture of a human case of verruga, but in unusually susceptible animals, which are rather rare, it induced a fatal infection similar to that of human Oroya fever. The strains from the severe

TABLE IV.

Parallel Infectivity Tests of the Original Blood, of Cultures on Leptospira Medium, and of Blood Broth Mixtures, Cases 7 to 12. Multiple Intradermal Inoculations, May 18, 1927.

Case No.	<i>Macacus rhesus</i> 6A			
	Original blood	Cultures		
		Leptospira medium	Blood broth	
			30°	37°
7	—	—	—	—
8	—	—	—	—
9	—	+++	—	—
10	—	++	—	—
11	—	+	—	—
12	—	+	—	—

TABLE V.

Individual Tests of Strains 7 to 12. Intradermal and Intravenous (2 cc.) Inoculations, July 1, 1927.

Strain No.	Monkey No.	Skin lesions	Blood cultures
7	7A	—	— (Aug. 8, 1927)
8	8A	—	—
9	9A	+	—
10	10A	+++	—
11	11A	++++	—
12	12A	—	—

cases of the present series have not so far, in the small number of monkeys inoculated, reproduced the fatal Oroya fever, but they have invariably induced cutaneous lesions similar to those occurring in benign human cases, while the strains isolated from the blood of benign verruga have not proven sufficiently virulent to set up any infection in the same monkeys.

TABLE VI.
Summary of Data.

<i>B. bacilliformis</i> strain No.	Clinical data				Observations on cultures							Pathogenicity for <i>Macacus rhesus</i>		
	Fever	Erythrocyte count	<i>Barionella</i> in blood	Eruption	Duration of illness	Cultural titer of blood	Growth on leptospira medium 30°C.	Growth on blood agar 30°C.	Measurements				Flagella	
									Predominant forms	Extremes			Character	Length
									Width	Length				
2	2,500,000			General military	2 mos.	1:10,000	Light grayish haze	Minute, almost transparent, raised, shining, firm, round colonies	0.3 × 0.8 μ	0.25-0.35 μ	0.5 -1 μ	Unipolar, 2 or more, smooth	3-5 μ	+
3	3,400,000	+		Few eruptions	15 days	1:1,000	"	"	0.3 × 1 μ	0.25-0.4 μ	0.4 -1.2 μ	Similar, but spiral	3-5 μ	+
5	1,920,000			Miliary and nodular	4 mos.	1:10,000	"	"						+
7	3,480,000			General military and nodular		1:10	"	"	0.4 × 1.4 μ	0.3 -0.4 μ	0.8 -1.6 μ	Unipolar, 1 or 2; spiral	5-10 μ	-
8	3,100,000			General military and nodular	6 mos.	1:10	"	"	0.3 × 1.2 μ	0.25-0.3 μ	0.5 -1.4 μ	Same as 7	5-10 μ	-
9	39° 1,250,000	+		-	15 days	1:10,000	" (denser)	" (coarser)	0.3 × 1 μ	0.25-0.3 μ	0.4 -1.4 μ	Unipolar, 2 or 3; smooth or wavy	3-6 μ	+

10	-	5,080,000	+	Few mili-ary nodular	1:1,000	Light gray- ish haze	Minute, almost transparent, raised, shin- ing, firm, round colonies	0.35 × 1 μ	0.3 -0.4 μ	0.3 -1.2 μ	Unipolar, 1 to 3; smooth or slightly wavy	3-8 μ	+
11	-	1,080,000	+	-	12 days	"	"	0.2 × 1 μ	0.2 -0.25 μ	0.3 -1.2 μ	Same as 10	3-8 μ	+
12	-	4,800,000	+	Few mili-ary	1:100	"	"	0.3 × 0.7 μ	0.3 -0.4 μ	0.5 -1.2 μ	"	3-5 μ	-
S.A.						(denser) Light gray- ish haze	Minute, almost transparent, raised, shin- ing, firm, round colonies	0.25 × 1 μ	0.25-0.35 μ	0.45-1 μ	Unipolar, 2 to 4; spiral	5-10 μ	+
P. 5 (nod- ule)						"	"	0.25 × 1 μ	0.2 -0.3 μ	0.3 -1.2 μ	Same as S.A.	5-10 μ	+

As has previously been suggested, the variety of types of human *Bartonella* infection may be accounted for by variations in the susceptibility of different individuals or different tissues of the same individual, or by variations in the virulence of the microorganism, or by both. The present experiments appear to emphasize more particularly the factor of variation in virulence of the parasite. According to clinical observations, the skin lesions more usually follow the acute febrile anemic stage (*fiebre grave de Carrion*, or Oroya fever); the lesions in these instances are evidently caused by a strain virulent enough to have invaded the blood. Yet there are cases in which the infection is chiefly confined to the cutaneous tissues, the anemia being very mild, and strains isolated from such cases would presumably be less virulent, as was found to be true in the present experiments. In instances in which the severe bartonellemia supervenes in the course of an apparently benign verruga, we may conclude that the strain belonged to the virulent type, but that the individual was of the resistant type, and that the defenses of the resistant organism were finally overcome by continued constitutional disturbances, due perhaps to the presence of the parasite in question, perhaps to some other cause. Phenomena of this sort are not uncommon in infections with other organisms which may give rise to septicemia following local infection (streptococcus, staphylococcus, the tubercle bacillus).

Serologic Studies.

Suspensions of *Bartonella bacilliformis* which would be suitable for agglutination tests are difficult to obtain, owing to the fact that the masses of organisms are well nigh impossible to disperse. Complement fixation tests proved easier to interpret.

Two samples of immune sera, obtained by inoculating rabbits intravenously on several occasions at appropriate intervals with live cultures of Strain S. A.³ were employed against a non-fixing dose of saline suspensions of plate cultures (horse blood agar) of each strain of *Bartonella bacilliformis*. The suspensions were heated at 60°C. to kill the microorganisms and the immune sera inactivated at 56°C.

All the strains gave complete fixation with the antiserum with the exception of Strain 12, which gave partial fixation (30 per cent). The

results indicate that on the whole the strains belong to the same serologic group.

DISCUSSION.

Carrion's disease offered a singularly complex problem, because of its two clinically dissimilar aspects, the grave fever of Carrion (Oroya fever), and benign verruga. The presence of the endoglobular bodies discovered by Barton⁶ practically settled the etiology of the severe cases, but the relation of Barton's bodies to the cutaneous lesions of verruga remained obscure, since it had not been proven that the same bodies were present in the blood or skin lesions of patients with the mild cutaneous disease. Moreover, while the cutaneous tissues affected were definitely infective, inducing similar skin lesions in monkeys, the parasites present in such large numbers in the blood of patients with malignant anemia, with or without skin lesions, appeared to be non-infective for monkeys, inducing neither skin lesions nor systemic infection so far as could be determined. It has recently been shown, however, that the apparently negative findings were not due to the absence of *Bartonella bacilliformis*, but to the fact that, except in severe systemic infection, the parasite cannot be detected by microscopic examination, its presence being revealed only by a suitable culture method.⁷ The unsolved portion of the problem of the etiology of Carrion's disease has been cleared up by the cultural procedure, which permits the ready isolation of *Bartonella bacilliformis* and its detection even when it is present in extremely small numbers.

The importance of the selection of the culture method cannot be over-emphasized, since on the special features of the medium used depended the success of the entire investigation. Had blood agar been used for the isolation experiments—and it is by far the best of the many other media tried—many strains would undoubtedly have been missed, some because growth of the microorganism is not uniformly successful on this medium, some because of loss of virulence due to the frequent subculturing required with blood agar cultures. Once the parasite had been obtained in culture on the semisolid leptospira medium, and its special properties studied, it became possible to discover the causes

⁶ Barton, A. L., *Crón. med.*, Lima, 1909, xxvi, 7.

⁷ Noguchi, H., *J. Exp. Med.*, 1926, xlv, 697.

for the apparently conflicting clinical and experimental evidence. Moreover, by special histological technique⁸ it was shown that *Bartonella bacilliformis* is microscopically detectable in large numbers in human and experimental lesions, where it had previously escaped detection.

The reason for the difference in pathogenicity between the endoglobular parasites, which are present in such large numbers in verruga maligna, and those existing in the skin lesions of verruga benigna has not been brought to light. The fact that in the first mentioned condition the microorganism is incapable of inducing in monkeys anything more than a slight transitory blood invasion, detectable only by blood culture, explains the negative results obtained by the Harvard Commission⁹ with blood from severe Oroya fever, results which seemed unmistakably to indicate a totally different etiology for Oroya fever and verruga peruana.

SUMMARY.

Through the cooperation of Dr. Sebastian Lorente, Director of the National Department of Public Health of Peru, nine strains of *Bartonella bacilliformis* have been isolated, by means of the semisolid leptospira medium, from nine of twelve specimens of blood withdrawn from cases of verruga and forwarded from Peru under conditions of refrigeration. The cultural titer of the blood specimens immediately after their arrival (2 weeks after withdrawal) varied from 1:10 to 1:100,000. Blood from the severe anemic type of the disease, in which there was no eruption, had the highest titer. Blood agar slants yielded irregular results, but some strains grew well on these media.

⁸ The technique is that used by Nicholson (*J. Exp. Med.*, 1923, xxxvii, 221) for the detection of *Rickettsia* in tissues. Fixation in Regaud's fluid (*Arch. Anat. Micr.*, 1910, xi, 296), which consists of 4 parts of 3 per cent potassium bichromate and 1 part of commercial formalin, is followed by staining with Giemsa's solution, according to the method recommended by Wolbach (*J. Med. Research*, 1919-20, xli, 1). 1.25 cc. of Giemsa's solution (Grübler's, for bacteria) is diluted with 1.5 cc. of methyl alcohol and 50 cc. of distilled water to which has been added 3 drops of 1 per cent sodium bicarbonate.

⁹ Strong, R. P., Tyzzer, E. E., Sellards, A. W., Brues, C. T., and Gastiaboru, J. C., Report of first expedition to South America, 1913, Harvard School of Tropical Medicine, Cambridge, 1915.

Morphologically the strains differed very little in fresh preparations examined by dark-ground illumination. In stained preparations some strains appeared coarser, others finer than the average. Special staining indicated that the flagella were characteristically unipolar and varied in number from one to four, some strains showing distinctly more wavy and heavier flagella than others. Young cultures grown on the surface of horse blood agar for 3 to 6 days show individuals with fairly sharp contours, short rods, often varying in thickness toward one or both ends, being intermingled with smaller oval or coccoid elements. Some strains show a predominance of bacillary, some of coccobacillary forms. It is not known whether these features are inherent or are due to conditions of growth, which, though identical, may react differently upon different strains. Definiteness in outline disappears with the age of the culture.

More striking variations are found in the virulence of the different strains for the monkey (*Macacus rhesus*). Three of the nine strains isolated proved to be non-pathogenic for the monkeys. All three of these were derived from cases of benign verruga. The remaining six strains all gave rise to local lesions when intradermally inoculated and were recovered in culture from the blood of the animals. So far, severe anemia has not developed in any of the monkeys.

It is significant that most of the severe cases yielded virulent strains, while some of the strains from benign verruga were non-pathogenic. It appears highly probable that the severe form of Carrion's disease is, in general, caused by a virulent strain, while the benign forms are due to a strain of low virulence. On the other hand, a virulent strain may cause benign verruga in unusually resistant persons and a weak strain may give rise to severe blood infection in unduly susceptible individuals. The form of Carrion's disease is probably determined primarily by the inherent virulence of the strain of *Bartonella bacilliformis* and is modified secondarily by individual predisposition in a given case.

An interesting phenomenon brought out by the present investigation was the failure of the nine human blood specimens to induce local verruga in the same monkeys in which the corresponding cultures, inoculated simultaneously at separate sites, gave rise to typical lesions. Yet the original blood samples were shown by cultivation to have

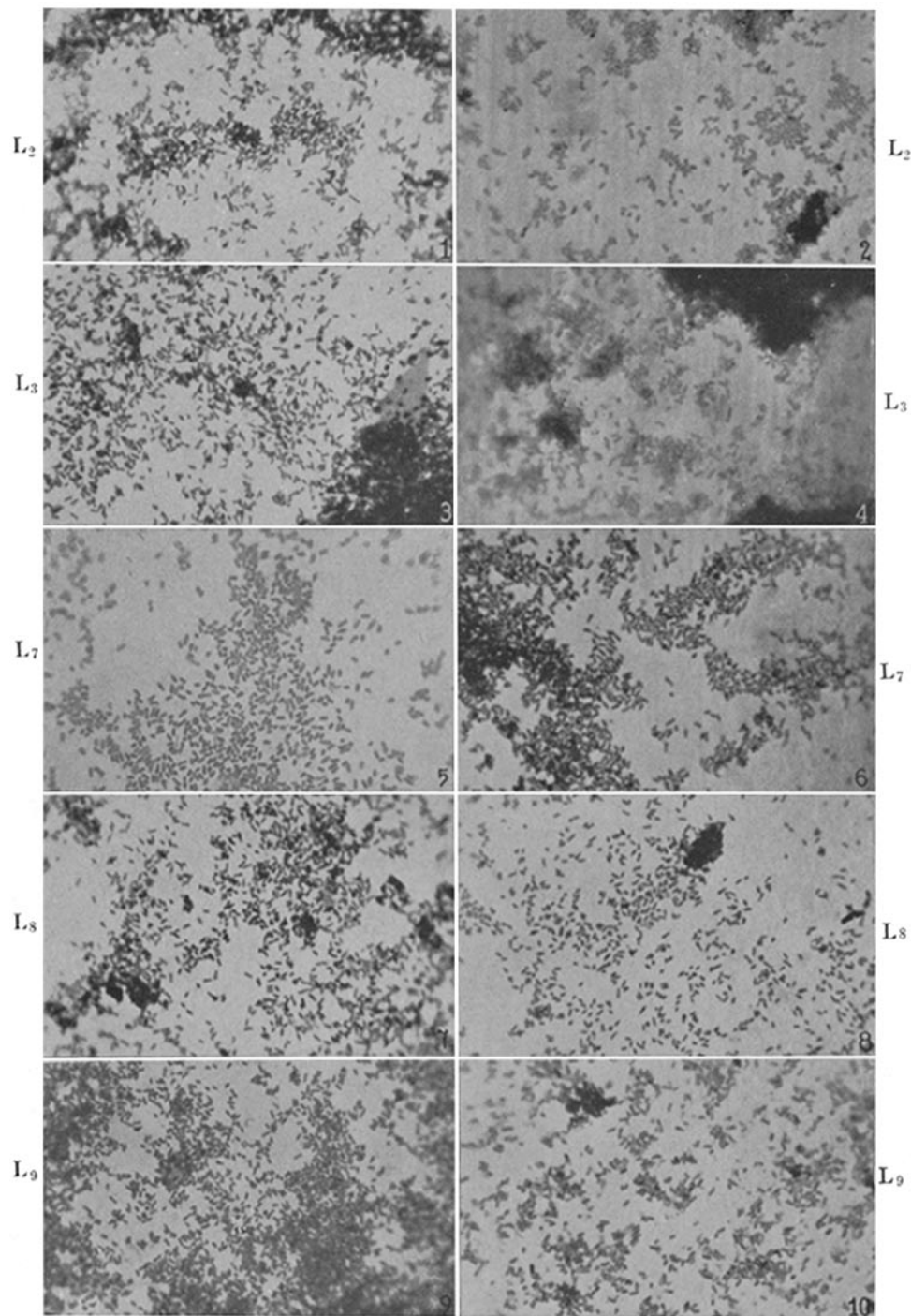
contained live bartonellas at the time they were inoculated, and blood culture revealed the presence of the microorganisms in the blood of monkeys which showed no other signs of infection after inoculation with the human blood. Whether this striking difference is merely a quantitative one or is due to some factor still unknown—such as, for example, a biological phase of the microorganism—has not been determined. The uniformly negative results of transmission experiments with blood by previous investigators is explained by an actual inability of the blood to induce skin lesions and the lack, until now, of a reliable method of detecting *Bartonella bacilliformis* in the monkeys' blood.

The strains isolated showed similar serologic properties, as tested by complement fixation.

Comparison of different strains of *Bartonella bacilliformis*. $\times 1000$.

Giemsa's stain.

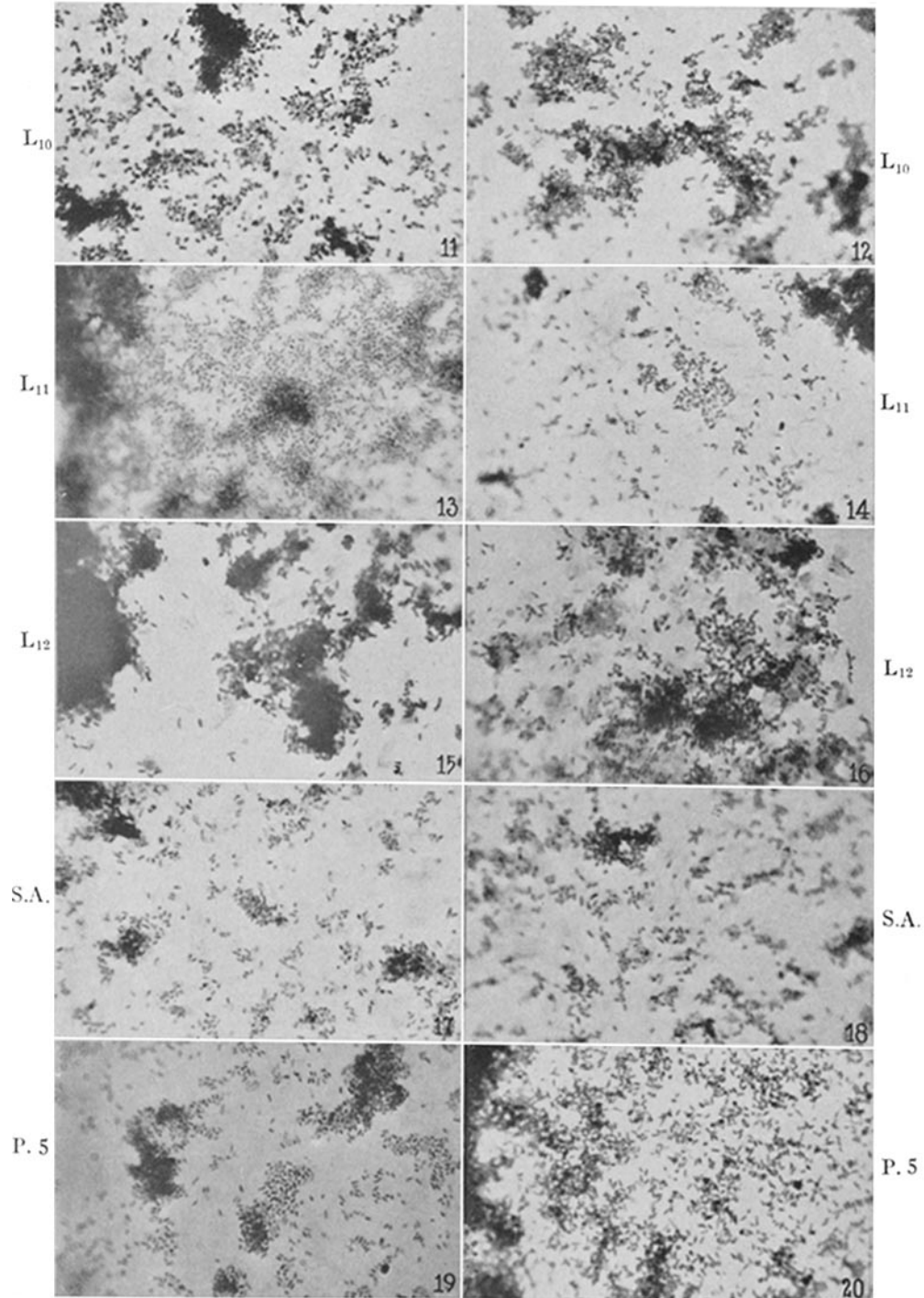
Gram's stain, counterstained.



Comparison of different strains of *Bartonella bacilliformis*. $\times 1000$.

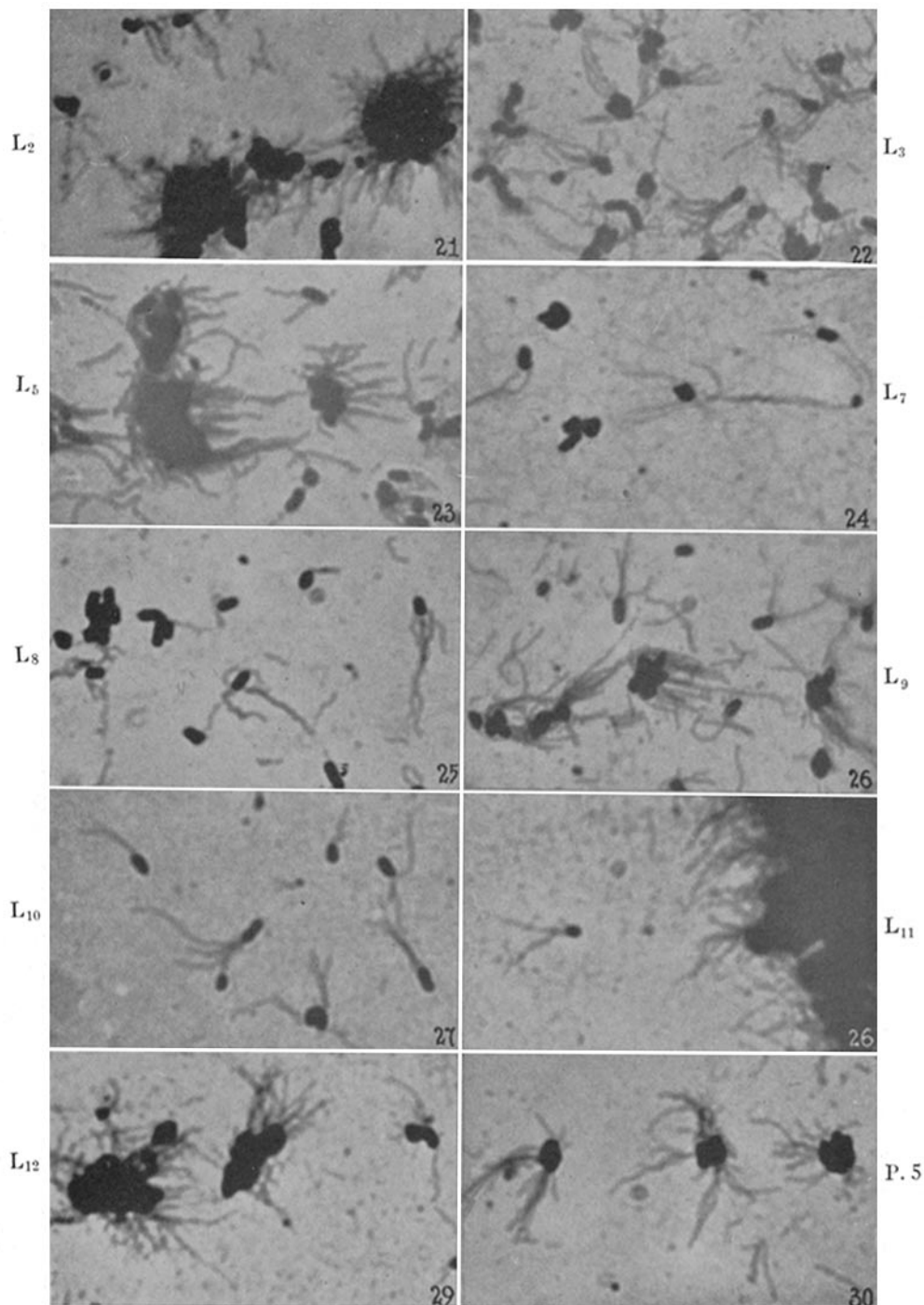
Giemsa's stain.

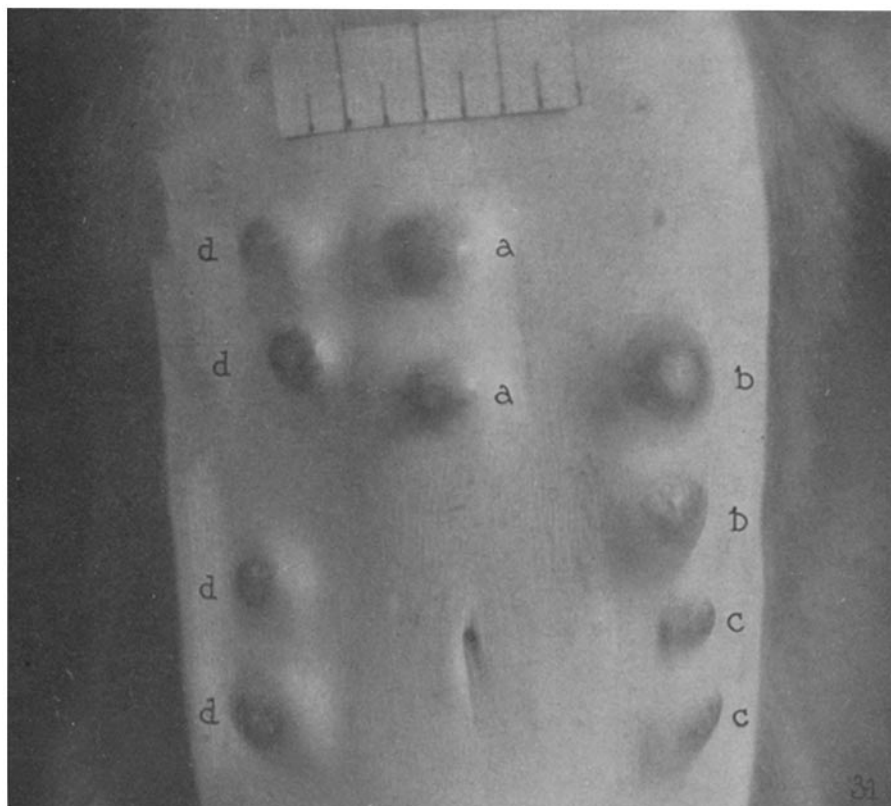
Gram's stain, counterstained.



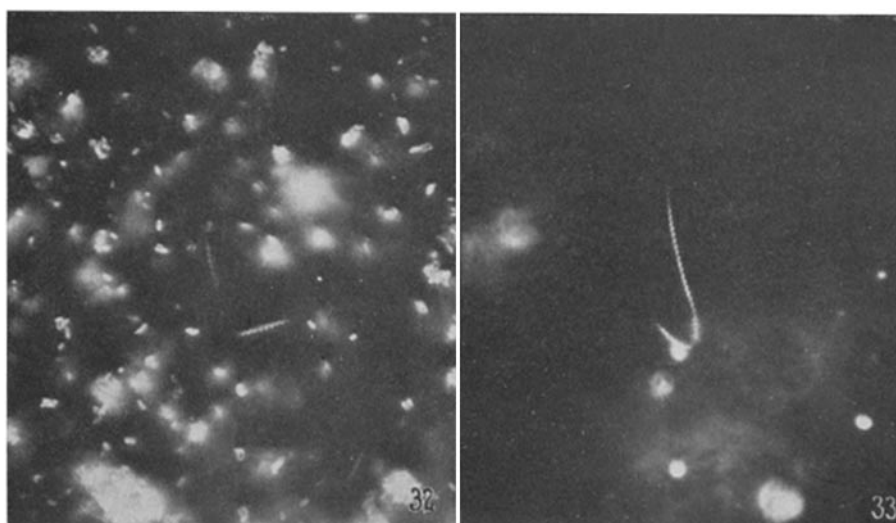
Comparison of different strains of *Bartonella bacilliformis*. $\times 2000$.

Zettnow-Fontana stain.





Lesions produced by cultures of (a) Strain 2, (b) Strain 3, (c) Strain 5, and (d) Strain P. 5. 30 days after inoculation. *M. rhesus* 3A. Natural size.



Abnormal detached flagella. Dark field. $\times 1000$.

(Noguchi: Etiology of Oroya fever. X.)