ETIOLOGY OF OROYA FEVER.

XIII. Chemotherapy in Experimental Bartonella bacilliformis Infection.

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Plates 15 and 16.

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In the course of our studies on *Bartonella bacilliformis* infection in monkeys, we submitted several *Macacus rhesus*, in which experimental verrucous lesions had been induced by means of cultures, to treatment with chemicals which had proved therapeutically useful in spirochetal and leishmania infections. Salvarsan had already been recommended by Arce[1] in the treatment of malignant verruga in man. In our experiments salvarsan, neosalvarsan, bismuth lactate, esters of chaulmoogra oil, sodium gynocardate, neutroflavine, and urotropin were tested. It was not deemed suitable to test tartar emetic, since it is a drug of slow therapeutic action, and the experimental verrucous lesions in the monkey lead in any case to spontaneous retrogression.

Chemical Action in Vitro.

Although it was not expected that a relationship would be shown to exist between the action of the chemical when tested on *Bartonella in vitro* and the verrucous lesions *in vivo*, it was considered of interest to determine the direct effects of the chemicals on the bacilli. The substances to be tested were added directly to the culture media, and the cultures were incubated at 25°C. for a period of 13 days. The results are shown in Table I. Neutroflavine inhibited growth in 1:10,000,000 dilution. Formalin was almost as effective, and neosalvarsan, novasurol, and mercuric chloride were effective up to

[1] Arce, J., *An. Facultad Med. Lima*, 1918, i, No. 3, 21–53, 130–161; No. 4, 24–52.

TABLE I.

Growth-Inhibiting Properties in Vitro.

	Final concentration of substance in culture medium					
	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	1:10,000,000
Bismuth albuminate	− (Turbid)	− (Turbid)	++++	++++	++++	++++
Tartar emetic	− (Turbid)	− (Clear)	−	+	++++	++++
Neosalvarsan	− (Clear, deep brown)	− (Brown)	− (Yellowish)	−	++	++++
Tryparsamide	± (Clear)	++++	++++	++++	++++	++++
Mercuric chloride	− (Turbid)	− (Clear)	−	−	++++	++++
Novasurol	− (Clear)	−	−	−	++++	++++
Mercurochrome	− (Deep red, clear)	− (Deep red)	− (Eosin red)	++++ (Lt. eosin)	++++ (Tr. pink)	++++
Neutroflavine	(Turbid, deep gold)	(Turbid, gold)	− (Greenish yellow, clear)	− (Greenish)	− (Lt. green)	− (Tr. green)
Optochin	(Turbid, white)	− (Opalescent)	− (Clear)	+++	+++	+++
Sodium taurocholate	(Turbid,brownish)	?	+ (Clear)	+	+++	+++
Phenol	− (Opalescent)	?	+++	+++	+++	+++
Formalin	− (Clear)	−	−	−	±	
Lugol's solution	− (Clear)	++++	++++	++++	++++	++++

− = complete inhibition of growth.

++++ = no inhibition of growth.

1:100,000. Mercurochrome and tartar emetic required at least 1:10,000 concentration to prevent growth.

The first experiments were made on monkeys in which the cherry-red verrucous lesions on the abdominal skin and eyebrows had reached maximal size and had persisted in this state for several days. Blood cultures taken shortly before or at the time of first injection of the chemical into the circulation proved subsequently to be negative, and bits of excised nodules taken at the same time[2] showed few bacilli or even none at all by culture or in section. These last findings could not be known at the time of treatment, since the bacilli require 10 to 14 days to become evident in culture.

However, distinction between the ordinary or spontaneous regression of the nodules and the regression taking place after the use of chemicals, is entirely possible. The mature nodules undergo spontaneous regression slowly,[3] while in the animals given chemicals there occurred rapid loss of cherry-red color, usually in 24 to 48 hours after the first injection, followed by a still more rapid reduction in size most pronounced in the nodules located in the abdominal skin. At the expiration of 5 to 6 days small pale fibrous areas alone remained to indicate the site of the nodules, and in 10 to 14 days all vestiges had disappeared, the lesions of the eyebrows persisting somewhat longer than those of the abdomen. The protocols of these experiments follow.

Protocols.

Macacus rhesus 1-T, injected intravenously on Oct. 14, 1926, with 0.5 cc. of a mixture of:

4 cc. defibrinated blood (culture +++) from *M. rhesus* 54 (P. 5 strain[4]),

5 cc. culture of *Bartonella bacilliformis* (P. 5 strain) grown on leptospira medium for 72 hours,

5 cc. culture of *Bartonella bacilliformis* (P. 5 strain) grown for 6 days on blood agar slants.

In addition, 4 intradermal injections of the mixture were made on the left abdominal wall and 2 on the left eyebrow. Also nodular tissue freshly excised from *M. rhesus* 54 was applied to scarified areas on the right abdominal wall and right eyebrow.

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[2] Ether anesthesia was used in all the operations.

[3] Noguchi, H., *J. Exp. Med.*, 1927, xlv, 455.

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

Experimental nodules appeared on the left eyebrow in 14 days. Within a month large nodules were present on the abdomen at the sites of intradermal inoculation, and the scarified areas showed the characteristic miliary lesions. On Nov. 23 (40 days after inoculation) the hemoglobin was 35 per cent (Sahli), and the red cells 2,502,000, and there had been no fever. 0.05 gm. of salvarsan was given intravenously. Within the following week the nodules grew small and became paler. On Dec. 3 a second injection of 0.05 cc. salvarsan was administered. Within the week the nodules had become very small and pale, the erythrocytes rose to 5,600,000 and the hemoglobin to 80 per cent. When the animal was sacrificed on Dec. 13, *Bartonella bacilliformis* could not be detected in the nodular tissue either microscopically or by culture, and blood, lymph nodes, and spleen also failed to yield cultures. Sections of the nodules showed fibrous tissue.

Macacus rhesus 2-T. Inoculated at the same time as No. 1-T, and in the same manner, except that the eyebrows were spared. On Oct. 19 the culture titer of the blood was 1:100,000. On Nov. 15, or 32 days after the inoculation, the animal showed large mature subcutaneous nodules on the left abdominal wall and numerous red miliary lesions on the scarified areas on the right side (Fig. 1). Blood cultures made on this day later proved to be negative. A mixture of 1 cc. of 1 per cent bismuth lactate, 1 cc. of 1 per cent neutroflavine, and 1 cc. of 1 per cent urotropin was injected intravenously. The temperature rose to 105.2°F. on the following day (Nov. 16), but the animal appeared well. On Nov. 23, a second injection of the same mixture was given. The nodules soon became bluish, smaller in size, and continued to diminish in volume after the second dose of the drugs (Fig. 2). On Dec. 3 a third injection was given. During the following 9 days the nodules became very small and pale (Fig. 3). Nodules and spleen removed on Dec. 16 did not yield *Bartonella bacilliformis* in culture.

Macacus rhesus 3-T, inoculated in same manner as *Macacus* 2-T. By Nov. 24 the abdomen showed large, mature subcutaneous nodules (Fig. 4), and large bluish red nodules on each leg where the injections had been made into the saphenous veins. A general miliary eruption was also present on the abdomen (Fig. 5). The hemoglobin was 45 per cent (Sahli), and the red blood cells 3,960,000. No fever. A suspension of one of the nodules yielded cultures of *Bartonella bacilliformis* in a dilution of 1:1,000, but the blood proved negative, although the titer had been 1:100,000 on Oct. 19 and Nov. 3. At this time (*i.e.*, on Nov. 24, 41 days after inoculation), 1 cc. of moogrol[5] was given intravenously. Within 48 hours the nodules had become bluish in color and somewhat smaller. Fig. 6 shows the appearance of the nodules 9 days after the first treatment. On Dec. 3 another injection of 1 cc. of moogrol was given. The temperature rose on the 2 days following to 104.4–104.6°F. By Dec. 13 the nodules had contracted considerably (Fig. 7). The spleen was negative for culture on Dec. 16, and all lesions had disappeared by Dec. 30. The blood culture was negative a week later.

[5] Burroughs Wellcome and Company's preparation of the esters of chaulmoogra oil.

Macacus rhesus 4-T was inoculated intravenously on Nov. 24, 1926, with 1 cc. of a mixture of cultures of *Bartonella bacilliformis*, besides which a suspension of an abdominal nodule of *M. rhesus* 3-T was given intravenously and introduced intradermally into both eyebrows and the abdominal skin. On Dec. 21 the blood cultures were positive for *Bartonella bacilliformis* in a 1:100,000 dilution. The nodules were fully developed by Dec. 28, 34 days after inoculation (Figs. 8 and 11), when the first intravenous injection of bismuth lactate, proflavine, and urotropin was given (a mixture of 1 cc. of a 1 per cent solution of each). Blood cultures were negative at this time. The temperature rose to 105°F. on the day following the treatment, and the nodules had already shrunk and become paler. A double dose was given on Dec. 30, and again on Jan. 3, 1927. No febrile reactions. Blood culture was negative on Jan. 5, 1927. The hemoglobin was 78 per cent (Sahli), and the erythrocytes 4,400,000. Cultures made from nodules and lymph nodes on Jan. 10, 1927, were negative. The lesions regressed rapidly (Figs. 9 and 12), and only fibrous traces remained on Jan. 28, 1927 (Figs. 10 and 13).

Macacus cynomolgus 5-T, inoculated intradermally with a suspension of nodular tissue from *M. rhesus* 1-S[6] into the right eyebrow and the abdominal skin on Dec. 7, 1926. This animal remained afebrile but developed large cherry-red nodules on eyebrows and abdomen by Dec. 27. The blood culture titer was 1:10 on Jan. 6, 1927. On Jan. 8 and 14, 1927, or 32 and 38 days after inoculation, an intravenous injection was made of 1 cc. of 5 per cent bismuth lactate, 1 cc. of 5 per cent urotropin, and 1 cc. of 1 per cent neutroflavine. Figs. 14 and 15 show the appearance of the lesions on Jan. 7. The nodules began to shrink in the following week and within 2 weeks had become small and fibrous. The appearance on Feb. 8 is shown in Figs. 16 and 17.

The next step was to test the action of the chemicals on the appearance and development of the nodules in instances in which the chemicals were administered *before* the lesions reached full development, *i.e.*, 2 to 3 weeks after the inoculation of the infective material into the skin, while the lesions were growing in size daily. Under these circumstances the drugs failed to influence the progress of the lesions, which in one instance attained unusually large proportions (Figs. 19, 20). The usual cherry-red color developed without hindrance, and the scarified areas became covered with the characteristic deep red miliary nodules. As in untreated animals, the skin of the lower half of the abdomen became edematous.

[6] Noguchi, H., *J. Exp. Med.*, 1928, xlvii, 821.

Protocols.

Macacus rhesus 6-T, inoculated intravenously (2 cc.) and intradermally on Dec. 28, 1926, with a mixture of cultures of *Bartonella bacilliformis* and a suspension of nodular tissue of *M. rhesus* 4-T. The nodules were well advanced on Jan. 24, 1927, 27 days after the inoculation, when intravenous injection of a mixture of bismuth, urotropin, and proflavine (1 cc. of 1 per cent proflavine, 1 cc. of 5 per cent urotropin, and 1 cc. of 5 per cent bismuth lactate) was begun. Blood taken just before the treatment yielded cultures in a dilution of 1:10. No change was observed in the nodules after the first injection. The second injection, given on Jan. 26, 1927, was followed by slight diminution in the size of the nodules on the eyebrows while the abdominal lesions continued to enlarge.

Macacus rhesus 7-T, inoculated by scarification and intradermal injection on Mar. 8, 1927, with a suspension of the nodule from *M. rhesus* 3-A, which had been infected by means of the L_5 strain of *Bartonella bacilliformis*.[7] Small nodules were present 20 days after inoculation (Fig. 18), when the animal was given the first intravenous injection of 0.1 gm. of neosalvarsan. The injection was repeated 2 days later. The nodules continued to grow gradually and within 2 weeks they attained unusually large dimensions (Figs. 19 and 20). Certainly no inhibitory action was apparent.

Macacus rhesus 8-T. This animal was inoculated in the same manner and on the same date as the foregoing monkey. Nodules had appeared by Mar. 28 (Fig. 21), when an intravenous injection of 2 cc. of 3 per cent sodium gynocardate and an intramuscular injection of 0.15 cc. of chaulmestrol[8] were given. This was followed 2 days later by an injection of 3 cc. of the sodium gynocardate and an injection of 0.2 cc. of chaulmestrol. No inhibitory effect upon the development of the cutaneous lesions was apparent and the large nodules are shown in Figs. 22 and 23.

SUMMARY.

The therapeutic effect of several antiparasitic chemicals on experimental verruga peruana is described. The drugs were administered by intravenous injection according as the nodules (1) were already developed to an approximate maximum, or (2) were still in the active period of growth.

The effect of the drugs was different under the two circumstances of

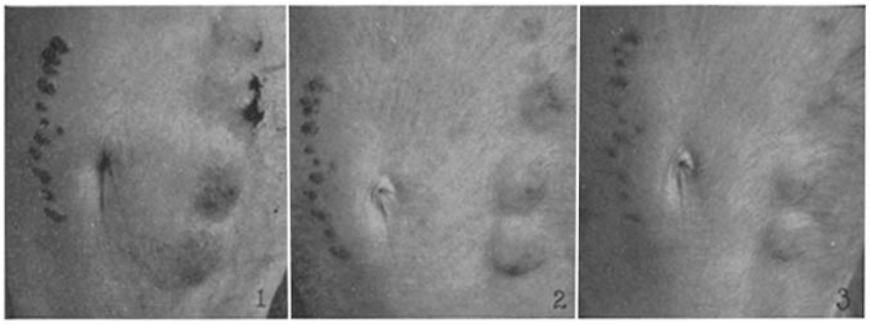
[7] Noguchi, H., *J. Exp. Med.*, 1928, xlvii, 219.

[8] The name given by the Winthrop Chemical Company to their preparation of the esters of chaulmoogra oil, of which the Company kindly furnished a sample.

their administration. When they were given after the maturity of the nodules they hastened the regressive process, but when given during active growth of the lesions no action whatever was detected.

Bartonella bacilliformis in culture is acted upon injuriously by a number of the chemicals employed in the therapeutic tests, the most active being formalin and neutroflavine.

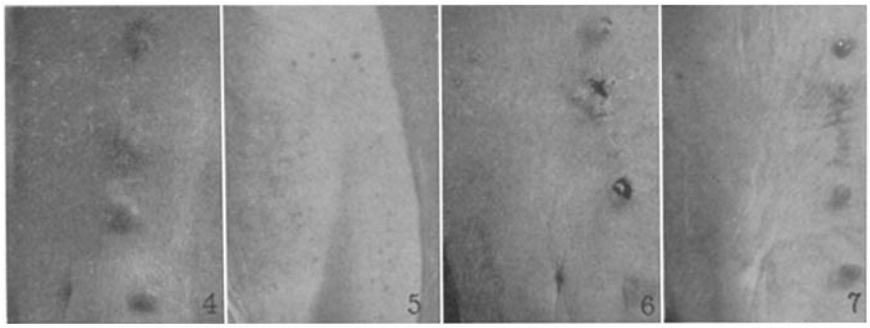
M. rhesus 2-T.



Before treatment (32 days after inoculation).

17 days after treatment. 25 days after treatment. (One nodule removed for study 32 days after inoculation.)

M. rhesus 3-T.



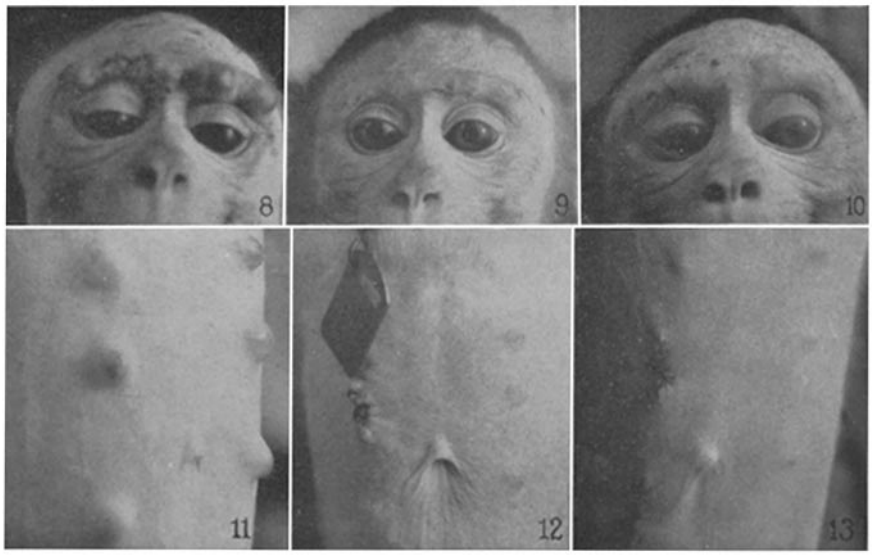
Before treatment. Nodular and miliary eruptions 41 days after inoculation.

9 days after treatment.

19 days after treatment.

(One nodule removed for study 41 days after inoculation.)

M. rhesus 4-T.

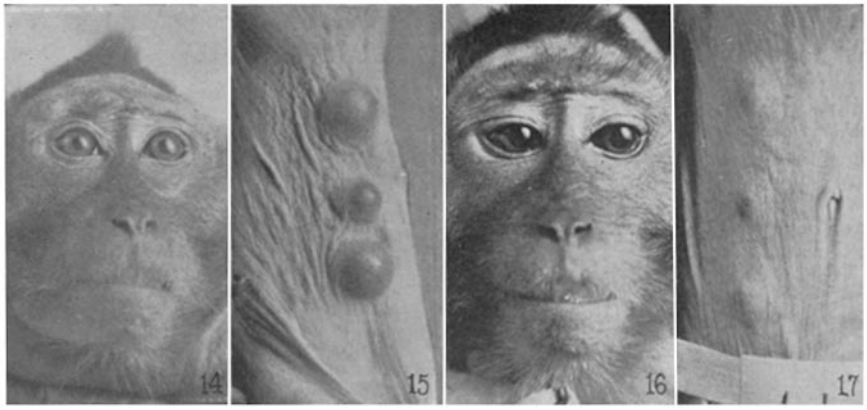


Before treatment. Nodules on eyebrows and abdomen 34 days after inoculation.

13 days after treatment. 38 days after treatment. (One nodule removed for study 34 days after inoculation.)

(Noguchi: Etiology of Oroya fever. XIII.)

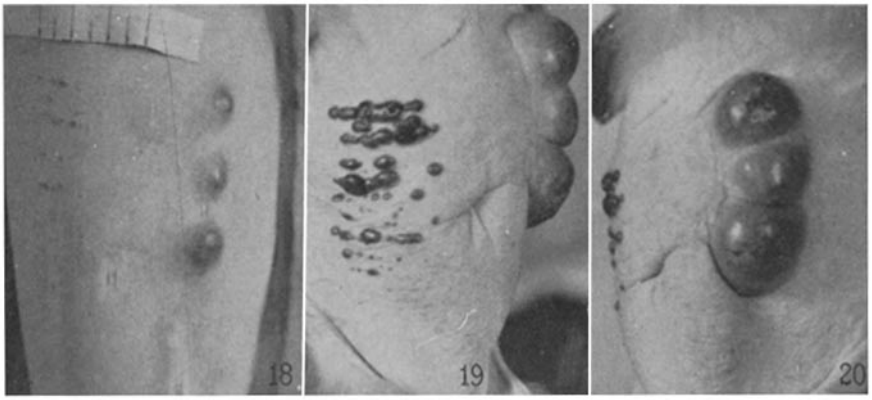
M. cynomolgus 5-T.



Before treatment (32 days after inoculation).

31 days after treatment was begun.

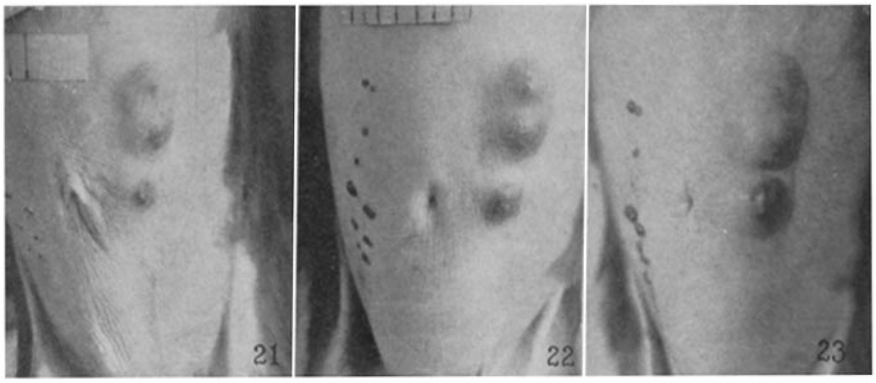
M. rhesus 7-T.



Before treatment (20 days after inoculation).

15 days after treatment was begun.

M. rhesus 8-T.



Before treatment (20 days after inoculation).

8 days after first treatment.

15 days after treatment was begun.

(Noguchi: Etiology of Oroya fever. XIII.)