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ETIOLOGY OF OROYA FEVER.

XII. Influence of Malarial Infection (Plasmodium Inui?), Splenectomy, or Both, upon Experimental Carrion's Disease in Monkeys.

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Mixed infections with *Bartonella bacilliformis* and plasmodia are not uncommon in regions where both malaria and Carrion's disease (verruga peruana, Oroya fever) are endemic. Both parasites induce swelling of the spleen, and both invade the red blood cells. It is conceivable that the preexistence of malaria might have an unfavorable influence upon a subsequent infection with *Bartonella bacilliformis*, as a result of impairment of the defensive powers of spleen and blood by the plasmodia, or that the introduction of plasmodia during the course of a dormant or partially controlled verruga infection might result in renewed invasion of the blood and lymphatic system by *Bartonella bacilliformis*. The abrupt development of malignant Oroya fever in the course of apparently benign verruga would be explicable on this latter hypothesis.

The opportunity to obtain definite experimental data on the effect of malaria infection on the course of experimental verruga presented itself when malaria parasites were found in the blood of a monkey (*Macacus cynomolgus*) which had been splenectomized in connection with an experiment to determine what influence the operation would have upon the susceptibility of monkeys to *Bartonella bacilliformis*.

Protocol 1.—Macacus cynomolgus 1-M, splenectomized on Dec. 1, 1926, to serve as control for *M. cynomolgos 2-S*, which was splenectomized the same day and inoculated with *Bartonella bacilliformis*. Examination of the blood of Monkey 1-M was made daily in order that the appearance of any *Bartonella*-like intracor-

puscular elements (such as appear in splenectomized rats[1]) might be detected. These elements were not found, but the examinations revealed plasmodia[2] on Dec. 6, 9, 10, 11, and 16, 1926, and the parasites were still present at the time of death on Mar. 10, 1927. No fever was detected in this animal at any time. The monkey had shown no signs of illness during several months of captivity previous to the operation. It would appear that it was a chronic malarial parasite carrier, a condition already established for man. Death may have been due to aggravation of the malarial infection through splenectomy.

Preexisting or Concomitant Malarial Infection and Verruga.

A *Macacus rhesus* intravenously inoculated with the blood of *Macacus cynomolgus 1-M* on December 10 soon developed fever, and the malarial parasites were found in the blood. One month later verruga material was intradermally inoculated. In due course the cutaneous lesions appeared and attained moderate size. Blood taken on February 3, 1927, yielded cultures of *Bartonella bacilliformis*, but no severe form of blood invasion and anemia developed. The animal recovered from both infections within a few weeks.

Protocol 2.—Macacus rhesus 2-M, inoculated intravenously with 0.5 cc. of the blood of Monkey 1-M on the day when plasmodia were first noticed (Dec. 10, 1926). The febrile reaction began on Dec. 15, that is, 5 days after inoculation, and continued daily for 9 successive days, being high (105–106.2°F.) for 5 days and moderate (104.2–104.6°) for 4 days longer. The plasmodia were present on Dec. 16. The temperature was normal (about 102°) from Dec. 26 to Jan. 3 but rose to 104.6° on Jan. 4, 1927, and to 106° on Jan. 5. Plasmodia were found in the blood on Jan. 3. After Jan. 5 there was no more fever. On Jan. 10, 1927, cultures of *Bartonella bacilliformis* and a saline suspension of the nodule of *M. rhesus 73* were intradermally inoculated. The signs of verruga were already evident on Jan. 19, and by Feb. 3 the nodules were of moderate size, and the blood had a culture titer of 1:100. The animal had no further paroxysm of malaria and recovered from both infections within a few weeks.

Another monkey was inoculated simultaneously with malaria and verruga material.

Protocol 3.—Macacus rhesus 3-M was inoculated on the same day as *M. rhesus 2-M* with 0.5 cc. of blood containing plasmodia and in addition received, both

[1] Mayer, M., *Arch. Schiffs.- u. Tropenhyg.*, 1921, xxv, 150. Mayer, M., Borchardt, W., and Kikuth, W., *Klin. Woch.*, 1926, v, 559; *Arch. Schiffs.- u. Tropenhyg.*, 1927, xxxi, 295. Noguchi, H., *J. Exp. Med.*, 1928, xlvii, 235.

[2] The parasite would appear to be *Plasmodium inui* (Wenyon, C. M., Protozoology, New York, 1926, ii, 968–973).

intravenously and intradermally, cultures of *Bartonella bacilliformis* and a suspension of verruga tissue. The animal's blood showed plasmodia on Dec. 16, 1926, 3 days before the onset of fever. The first paroxysm lasted 3 days (temperature 104.6°, 106.2°, 106.2°). The second rise of temperature (to 105.4°) was first detected on Dec. 27, temperatures not being taken daily during the Christmas holidays, and fever was still present on Dec. 28 (104°). Plasmodia were present in the blood on Jan. 3, and there was fever on Jan. 4, 5, and 6 (104°, 105.8°, 104°). Verruga nodules arose at the sites of intravenous inoculation into the saphenous veins (where several unsuccessful punctures had been made in the course of intravenous injection), but none at the sites of intradermal injections on the abdomen. Blood cultures made on Dec. 21, 1926, failed to yield cultures of *Bartonella bacilliformis*. The temperature of 106.2°F., recorded on that day, may have been due either to malaria or to verruga. A second injection of virulent verruga material intradermally on Jan. 10, 1927, resulted in only slight induration at the sites of inoculation, but the nodules on the back of the legs reached moderate size, and marked edema of the scrotum developed. The animal recovered.

The experiments outlined gave no evidence that the malarial infection in *Macacus rhesus* influenced unfavorably the result of infection with *Bartonella bacilliformis* when the plasmodia were introduced a month previous to inoculation with verruga or when both parasites were simultaneously inoculated.

Malarial Infection during Convalescence from Verruga.

The lesions resulting from experimental infection with *Bartonella bacilliformis* in monkeys usually heal within a few months, the microorganisms persisting longest in the spleen and lymph glands, where they can be demonstrated late in convalescence. It was of interest to determine whether, at this stage, the infection would again become active as a result of introduction of plasmodia.

As the protocols show, the inoculation of malaria parasites during convalescence had no influence whatever upon the course of recovery. In one instance the animal was reinoculated with virulent verruga material a month after the introduction of the plasmodia, but reinfection was not induced. The malarial infection apparently had not interfered with the development of immunity.

Protocol 4.—Macacus rhesus 4-M had had an infection with *Bartonella bacilliformis* during the autumn, but the skin lesions had almost healed. It was inoculated with 0.5 cc. of the malarial blood intravenously on Dec. 10, 1926. There

was fever lasting for 6 days, Dec. 22 to 27, 1926 (temperatures varying from 104.4° to 105°), and the blood was positive for plasmodia on Jan. 3, 1927. Blood culture was negative for *Bartonella bacilliformis* on Dec. 21, 1926, and the skin lesions had disappeared.

Macacus rhesus 5-M, also recovering from verruga, was inoculated with 0.5 cc. of malarial blood at the same time as the foregoing animal. Fever began on Dec. 16, 1926, and lasted 5 days (temperatures 104°–106.8°). Plasmodia were present on Dec. 16, 1926, and on Jan. 3, 1927. On Dec. 28, 1926, the temperature rose to 104.4° but fell to 103.6° the next day, and there were no later paroxysms. On Jan. 8, 1927, verruga material was inoculated intradermally. No skin lesions appeared at the old sites, and none developed at the sites of the new inoculations, and the blood remained negative for *Bartonella bacilliformis* by culture. Observation ended Jan. 29, 1927.

Effect of Splenectomy upon Verruga Peruana.

In connection with the problem of the nature of the intracorpuscular bodies known as *Bartonella muris*,[1] which appear in the red cells of rats following splenectomy, it became of interest to determine what effect splenectomy would have upon the course of experimental infection with *Bartonella bacilliformis*.

A monkey splenectomized on November 12, 1926, and inoculated soon afterwards with verruga material, developed the severe type of Carrion's disease (Oroya fever), with marked anemia, extensive edema, emaciation, and skin lesions. Even more violent reactions than the one in this animal (*Macacus rhesus 1-S*) are occasionally observed in *rhesus* monkeys as a result of inoculation with *Bartonella bacilliformis*,[3,4] and hence the severity of the infection in this instance cannot be ascribed to the effect of splenectomy. Moreover, *M. cynomolgus 2-S*, which was subjected to splenectomy and simultaneously inoculated with nodular material from Monkey 1-S, showed a moderate reaction only. Although the blood titer was high (1:100,000), there were no other evidences of systemic infection, and the nodules were of medium size. The control monkey in this instance (*M. cynomolgus 5-T*) reacted more severely than the splenectomized animal, although the blood titer was only 1:10, as was that of a *rhesus* control.

[3] Noguchi, H., *J. Exp. Med.*, 1927, xlv, 175.
[4] Noguchi, H., *J. Exp. Med.*, 1926, xliv, 697.

Protocol 5.—Macacus rhesus 1-S, splenectomized Nov. 12, 1926, was inoculated 3 days later intravenously with 1 cc. of culture of *Bartonella bacilliformis* and 1 cc. of saline suspension of nodular tissue from *Macacus rhesus 2-T*.[5] The same material was injected intradermally. A temperature of 104° to 104.6°F. was recorded on Nov. 11, 24, Dec. 10, 20, and 21, and on Jan. 15, 1927. Except on these 6 days the animal was afebrile. Blood cultures were positive on Nov. 24 (1:10,000), Dec. 8 (1:100,000), and Dec. 28 (1:100). The organisms were sufficiently numerous in the blood on Dec. 3 to be detected by examination of stained films. The nodules developed to moderately large size and persisted for about 2 months. From Dec. 11, 1926, to Jan. 3, 1927, there was general edema, especially marked in the scrotum, and a generalized nodular eruption.

Blood Counts.

	R.B.C.	Hemoglobin (Sahli) *per cent*
Nov. 12	5,456,000	90
Nov. 24	4,424,000	80
Dec. 27	3,080,000	50

The animal had completely recovered by Feb. 7, 1927.

M. cynomolgus 2-S, splenectomized Dec. 1, 1926, and inoculated intradermally with a culture of *Bartonella bacilliformis* and a saline suspension of nodular tissue from *M. rhesus 1-S*. Nodules of moderate size had developed by Jan. 14, 1927. Blood taken on Jan. 6, 1927, yielded cultures of *Bartonella bacilliformis* in a dilution of 1:100,000. The nodules had disappeared by Feb. 9, 1927.

M. cynomolgus 5-T[5] was inoculated in the same way and with the same material as the foregoing animal but was not splenectomized. There was no fever at any time, but enormous cherry-red nodules developed on the eyebrows and abdominal wall, reaching their maximum growth on Dec. 27, 1926. The blood titer was 1:10 on Jan. 6, 1927, but blood culture was negative on Jan. 24.

Splenectomy did not induce relapse of experimental verruga infection in two animals which had recovered (*M. rhesus 40* and *41*[5]), nor did removal of the spleen render recovered animals susceptible to reinoculation with *Bartonella bacilliformis* (*M. rhesus 49, 50, 68*). Observation continued for a period of 1 month after inoculation of *Bartonella bacilliformis* into these animals failed to reveal any skin lesions, and blood cultures were uniformly negative throughout this period.

Effect of Splenectomy and Malarial Infection Together upon Verruga Peruana.

Two animals were subjected to splenectomy as well as to infection with plasmodia and *Bartonella bacilliformis*. One succumbed to the

[5] Noguchi, H., *J. Exp. Med.*, in press.

malarial infection before the verruga lesions had had time to develop; the other had a moderately severe infection with *Bartonella bacilliformis* and while recovering died of tuberculosis.

Protocol 6.—Macacus rhesus 6-M was splenectomized and at the same time inoculated with 0.5 cc. of malarial blood on Dec. 10, 1926. The plasmodia were found in the blood on Dec. 16, and 4 days later the temperature rose and remained high for 3 successive days (104.2–106°). The second paroxysm began on Dec. 29, 1926, and lasted for 8 days (temperatures 104–106.6°). The plasmodia were very numerous on Jan. 3. The animal was inoculated with verruga material on Jan. 8, 1927, but succumbed to the malarial infection on Jan. 14, 1927, before the verruga lesions had had time to develop. *Bartonella bacilliformis* was not recovered in blood cultures.

Macacus rhesus 7-M was splenectomized on Dec. 10, 1926, and at the same time inoculated with 0.5 cc. of malarial blood and 0.5 cc. of verruga cultures and tissue, the latter intradermally as well as intravenously. The first paroxysm occurred on Dec. 20 and lasted 4 days (temperatures 104.2–106°), the second, beginning Jan. 3, lasted 5 days (temperatures 104.6–106.4°). Examinations for plasmodia were positive on Dec. 16, 1926 (+), and on Jan. 3, 1927 (+++). Blood taken on Dec. 21, 1926, yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10,000. The animal died of tuberculosis on Jan. 10, 1927.

SUMMARY.

The experiments reported were designed to determine the influence of malarial infection (*Plasmodium inui?*), splenectomy, or both combined, upon the course and character of experimental infection with *Bartonella bacilliformis* in monkeys (*Macacus rhesus* and *M. cynomolgus*).

Blood withdrawn from a monkey showing spontaneous malarial infection was inoculated intravenously into monkeys (*a*) 1 month prior to inoculation with virulent verruga material, (*b*) simultaneously with the verruga material, and (*c*) during convalescence from verruga infection of moderate severity. All the monkeys contracted the malarial infection and suffered one to three paroxysms during a period of about a month. The verruga lesions appeared in the inoculated animals in due course, were of average size, remained for the usual length of time, and *Bartonella bacilliformis* was recovered in culture from blood which also contained the plasmodia. The lesions in the convalescent animals continued to heal at the normal rate, and blood cultures were negative for *Bartonella bacilliformis*, as is usual during

convalescence. One of the recovering animals was reinoculated with virulent verruga material a month after the injection of the malarial blood, but neither did new lesions arise nor old ones recur. The malarial infection, therefore, had no effect upon the course of verruga or upon the establishment of immunity to *Bartonella bacilliformis*, hence it would appear that malaria and verruga may coexist in the same individual without unfavorable effect of one disease upon the course of the other.

Similarly, splenectomy led to no appreciable aggravation of *Bartonella* infection. One monkey subjected to splenectomy and inoculated with verruga material shortly afterwards had an unusually severe reaction, but another, which was infected with material from the first and simultaneously splenectomized, reacted only moderately, while the non-splenectomized control showed a severer type of cutaneous infection. Even the combination of splenectomy and malarial infection did not appreciably aggravate the experimental verruga. Neither relapse of verruga nor reinfection with *Bartonella bacilliformis* was induced in convalescent or recovered monkeys as a result of splenectomy.