AN ATTENUATED SURRA OF MAURITIUS WITH IMMUNITY TESTS AFTER RECOVERY.*

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Surra is so pathogenic for mice that in these animals, according to Laveran and Mesnil (see Nabarro[1]) "the disease, if untreated, always ends fatally." *To this statement no exception was seen while the surra of Mauritius*[2] *now to be described, was preserved in mice.* Under these conditions, injections similar to those in the first outline below, infected on the second or third day and killed on the sixth or seventh, while richer intraperitoneal inoculations infected on the first and usually killed on the fourth or fifth day.

Attenuated Mauritian Surra.—Subsequently, however, the virus was kept for six months exclusively in guinea pigs, and apparently as a result of the guinea pig passages, it became greatly attenuated[3] for mice. This is well shown in the outlines (page 179).

From the outlines we see that the mice[4] inoculated with the

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[1] Nabarro, D., Trypanosomes and Trypanosomiases. Translated from the French of Laveran, A., and Mesnil, F., Chicago, 1907, p. 265.

[2] This virus was placed at my disposal through the kindness of Dr. Felix Mesnil, of the Pasteur Institute, Paris.

[3] As far as I am aware, no one has described as great an attenuation of surra due to guinea pig passage, as that met with among my animals. Nevertheless it is known that the course of infection in mice may be somewhat altered when the virus is obtained from guinea pigs. According to Nabarro,[1] Laveran and Mesnil found that when once the parasites of Indian surra appeared, they never diminished except in a few cases in which the mice were inoculated with guinea pig blood.

[4] The two mice in the outline were controls on a mouse which had previously successfully resisted eleven inoculations with surra of Mauritius. When, however, this animal was tested with the attenuated strain, it was apparently slightly hypersensitive to infection, becoming positive on the fourth and dying on the ninth day after inoculation. The detailed history of this animal will soon be published.

attenuated strains were not visibly infected until the fifth day. From then until its death on the twentieth day, the first of these animals remained continuously infected. The second was for some weeks alternately positive and negative, then apparently recovered completely. In the blood of this animal in daily examinations, trypanosomes were found thirteen times in the first three weeks. Subsequently they were seen only on *the twenty-eighth, thirty-sixth and seventy-second day after inoculation.*

A Carrier of Trypanosomes.—The infection in the second mouse had been so persistent that, after the disappearance of the parasites, a careful watch for their return was kept for over six weeks. During this time trypanosomes were never detected and on the 121st day the mouse was still microscopically negative. As a more delicate test for the presence of the parasites, on this day ten to twelve drops of its blood were inoculated intraperitoneally into a normal mouse. The animal became infected and died, showing conclusively that *the mouse, in which the infection had been so persistent, had remained a carrier*[5] *of trypanosomes for seven weeks after the parasites had last been detected microscopically.*

A Spontaneous Cure.—It was now of considerable interest to determine whether the second mouse would continue to carry trypanosomes or would recover. To determine this point it was bled freely from the tail on the 130th and again on the 141st day, and all of the blood thus obtained was injected intraperitoneally into normal mice (into four at the first bleeding, into two at the second). In none of these animals, however, did infection take place, and three of the six were living 180 days later. In spite of the failure of the animal tests, microscopical examination of the blood of the second animal above was not discontinued until the 190th day, by which time it seemed certain that the mouse had recovered. It lived until the 217th day.

Two Other Spontaneous Cures.—In order to determine whether other animals would recover spontaneously, the following four mice received an injection of the strain of surra of Mauritius employed to infect the last two.

[5] For the production of trypanosome carriers in cattle by passing nagana (?) through rat and dog, the papers of R. Koch and C. Schilling should be consulted.

After incubation periods of seven to sixteen days, all four animals became positive, two died infected and two apparently recovered. Animal inoculation, however, (see test on 50th day above) showed that *one of the last two mice remained a carrier of trypanosomes for over four weeks after the parasites were last seen in its blood.*

No Lasting Immunity after Recovery.—If mice could recover spontaneously, it was of interest to ascertain whether they acquired a lasting immunity. In order to test this point, on the 94th day the last two mice in the experiment above were reinoculated with the surra of Mauritius[6] from which they had apparently recovered. The result showed that when the test was made the animals pos-

Explanation of Outlines.—The outlines are read from left to right, each line representing a different mouse.

SM = an inoculation of surra of Mauritius. To the right above, in each instance, are indicated three points and in the following order: (1) The ***quantity*** of the virus introduced (.2 = two-tenths c.c., .3 = three-tenths c.c.); (2) the ***mode of inoculation*** (s = subcutaneous, i = intraperitoneal); (3) the ***number of parasites*** per field (Zeiss, lens "D," No. 4) in the injected suspension (in physiological salt solution).

The course of controls on the surra of Mauritius inoculations are shown by bold-faced figures to the right of the SM (see second outline, Mice 3 and 4, second inoculation with SM). The 2-7, 4-10 in this position mean that one control was positive on the second day after inoculation and dead on the seventh, and that the second control was positive on the fourth and dead on the tenth day.

° = no parasites in at least twenty fields. To save space some of the negative examinations are omitted.

+ = parasites (few or many) microscopically visible.

The *days* on which examinations were made are indicated by bold-faced figures placed above the zeros and plus signs.

L (large or small) = living.

D (large or small) = dead.

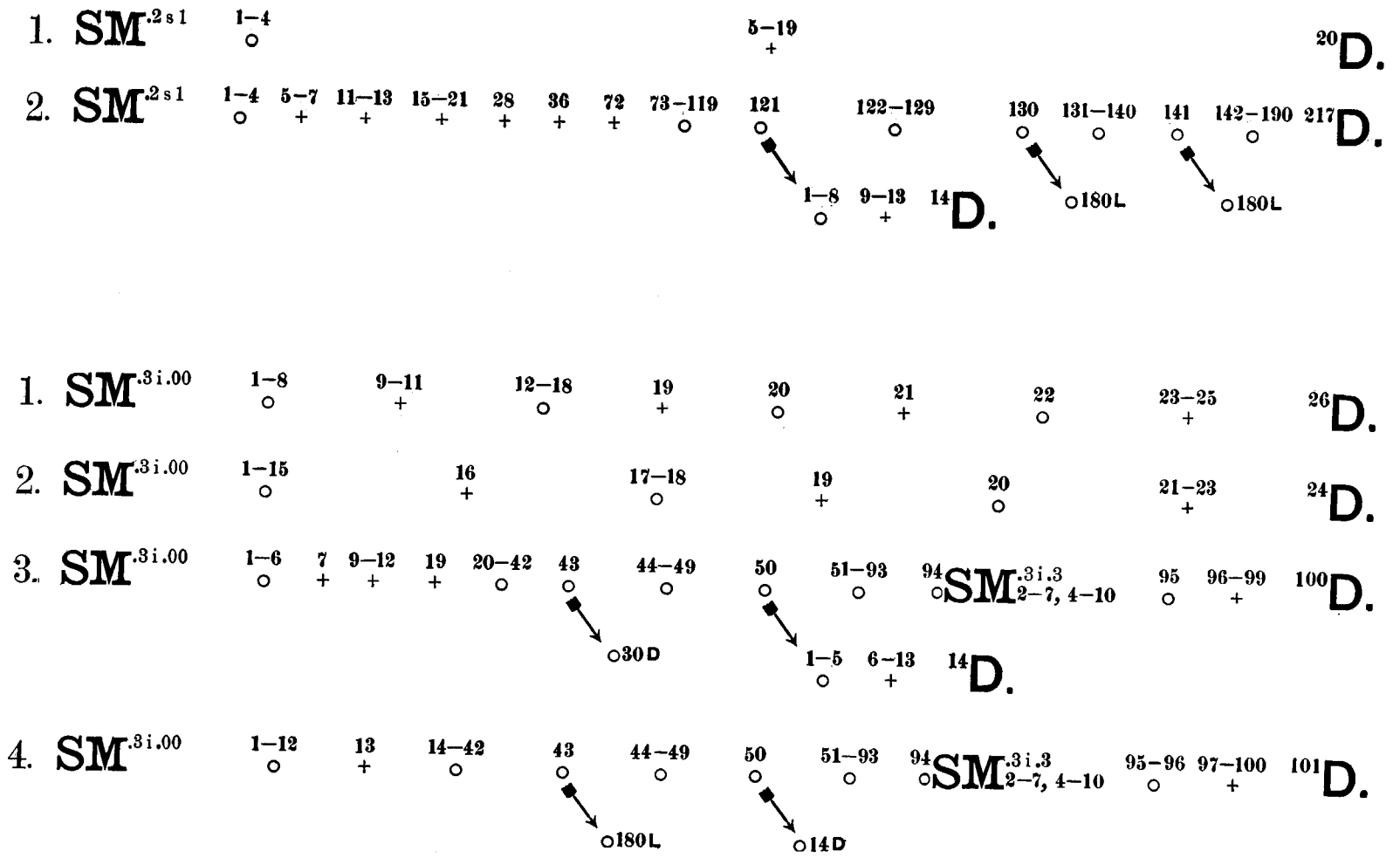
The *days* on which animals died or on which they were still living is shown by the figures placed to the left of the *L*'s and *D*'s.

° 180 *L* = negative and living on the 180th day.

° 30 *D* = remained negative, dead on the 30th day.

An *arrow* indicates a subinoculation and points from the animal supplying the blood to one receiving it. (The number of mice inoculated at one time is not shown. See text.)

[6] As inoculations of surra of Mauritius derived directly from guinea pigs had yielded uncertain results, before employing this virus in the immunity test it was passed through a single mouse to increase slightly its virulence.


1. SM.2s1
1–4
5–19
20D.
2. SM.2s1
1–4 5–7 11–13 15–21 28 36 72 73–119 121 122–129 130 131–140 141 142–190 217D.
1–8 9–13 14D.
180L
180L
1. SM.3i.00
1–8 9–11 12–18 19 20 21 22 23–25 26D.
2. SM.3i.00
1–15 16 17–18 19 20 21–23 24D.
3. SM.3i.00
1–6 7 9–12 19 20–42 43 44–49 50 51–93 94 SM.3i.3 2–7, 4–10 95 96–99 100D.
30D
1–5 6–13 14D.
4. SM.3i.00
1–12 13 14–42 43 44–49 50 51–93 94 SM.3i.3 2–7, 4–10 95–96 97–100 101D.
180L
14D

sessed no immunity, for both became infected promptly. They died six and seven days after inoculation; their controls, seven and ten.

Increased Virulence for Guinea Pigs.—As Laveran[7] had shown that the virulence of surra of Mauritius for guinea pigs might increase when the virus was preserved for a long time in these animals, it was of interest to see whether an increase of virulence for guinea pigs had not taken place in my virus. Since infected guinea pigs pursue very irregular courses, it was not possible to determine this point until the average length of life in groups of these animals had been calculated. As soon as this was done, however, it was perfectly obvious that, *while the parasites were being attenuated for mice, their virulence for guinea pigs had increased,* for the average life, in successive groups containing five or more of these animals, had grown progressively shorter. For example, on dividing the animals into groups of sixes, it was found *that the average life in the first group was 86 days; that in the second, 81; that in the third, 58.*

Virulence for Mice Restored.—From the result of the last experiment and from various published papers,[8] it seemed not improbable that the virulence of the surra of Mauritius parasites for mice could be restored by successive passages through these animals. In testing this possibility three experiments were carried out. In the first was employed the virus from which Mouse 2 (in the first outline) recovered. From this mouse the parasites for the second mouse passage were obtained by subinoculation on the 121st day. As had been expected, the virulence for mice increased in the successive passages, until, in the eighth mouse, the parasites developed as rapidly as before their attenuation. In the other two experiments the restoration of the virulence was even quicker as was well shown by the rapidly decreasing intervals between inoculation and death. In one of these series, for example, the first mouse died on the 16th day; the second, on the 11th; the third, on the 7th; and

[7] Laveran, A., Influence des passages par cobayes sur la virulence de quelques trypanosomes, *Bull. de la Soc. de path. exotique,* 1908, i, 198.

[8] For example, Laveran and Mesnil (see Nabarro[1]) have shown that the virulence of the Indian surra for the mouse may be greatly exalted by passage through rat or mouse.

the fourth, on the 4th day. In this experiment the normal level of virulence was reached after only four passages.

SUMMARY.

Apparently as a result of repeated passages through guinea pigs, the virulence of surra of Mauritius for mice became greatly attenuated. Three mice recovered spontaneously and *two remained carriers of tryanosomes for four to seven weeks after the parasites were last seen in their blood.* The mice that recovered acquired no lasting immunity. While the virulence of the parasites decreased for mice, it increased for guinea pigs. By cultivating the parasites in mice the virulence for these animals was restored after four to eight passages.