varied environment. I wish now to bring the results up to date (May 26, 1909).

A culture of *Paramecium aurelia* (*caudatum*) was started on May 1, 1907, with a "wild" individual isolated from a laboratory aquarium, and during the twenty-five months which have elapsed since that time it has been under daily observation. Infusions of hay and grass together with any material that may be found in the normal habitat of *Paramecium* have been employed as a culture medium. The possibility of contamination by cysts or "wild" *Paramecia* has been eliminated by boiling the infusion. Daily isolation of an individual from each of the various lines of the culture has enabled an accurate record of the division rate to be kept and has precluded the possibility of endogamous conjugation.

So far the culture has attained the 1,185th generation. The average rate of division for the entire period is over one and a half divisions per day. The average rate has not fallen during any ten-day periods as low as one division in two days, while during several ten-day periods it has averaged over two and a half divisions per day. Marked physiological depression has not been indicated by the rate of division and consequently special stimuli have not been employed to "rejuvenate" the organisms.

The results thus far obtained certainly show that the life cycle of *Paramecium* when subjected to a varied environment may be of very great duration, and, I believe, strongly suggest that the life history may be of unlimited duration.

63 (401)

Immunity to various species of trypanosomes induced in mice by the cure of experimental infections.

By **B. T. TERRY.**

[*From the Rockefeller Institute for Medical Research.*]

When properly treated a temporary immunity was secured against the organisms of surra of India, surra of Mauritius, caderas, dourine and nagana. The immunity was specific in the sense that it was active against the species cured but not against any other. The immunity varied with the virus, the medicament, the time at which the immunity tests were begun, the number of the tests, the

intervals between them and the natural variations in the mice employed.

Against the parasites of surra of India, surra of Mauritius, caderas, nagana, and a toluidin blue resistant strain of nagana, an immunity was produced which was strong enough to prevent one or more subsequent inoculations of virus from infecting. The immunity to dourine was less strong.

In producing immunity to surra of India a single injection of dichlorbenzidine plus amidonaphtol disulphonic acid, 1, 8, 3, 6, or "Cl," was more efficient than one of acetylatoxyl or arsenophenylglycin.

Even when the medicaments apparently prevented infection an immunity was produced. It is interesting that two injections of a mixture of "Cl" and acetylatoxyl has completely protected a normal mouse against an inoculation with surra of India given six days after the second treatment. The mixture protected about twice as long as either of its two constituents used alone had done.

The immunity reaction distinguished with sharpness organisms supposed to have had a common origin, namely, surra of India and surra of Mauritius. Occasionally, however, these two species immunized against each other. With equal clearness the reaction distinguished between organisms known to have had a common origin, *e. g.*, the toluidin blue resistant strain and the parafuchsin resistant strain. When mice were immunized to the toluidin blue trypanosomes, an inoculation with the same virus failed completely, while the tests with the parafuchsin resistant strain and with all others, infected and killed.

By means of the immunity reaction it was apparently possible to separate in purity organisms that had been mixed *in vitro*. By inoculating a mixture of surra of India and surra of Mauritius into mice immune to surra of India an infection with surra of Mauritius was obtained. The surra of India was separated by inoculating the mixture into mice immune to surra of Mauritius. In a similar way caderas and surra of India were separated.

When mice infected with a mixture of two species of trypanosomes were cured, an immunity to both was produced.

In securing prolonged specific immunity, frequent injections of the virus at close intervals were of value. A mouse cured of

caderas by a single injection of trypanred acquired an immunity that lasted 50 days. Another mouse cured of surra of Mauritius by acetylatoxyl and "Cl" had a strong immunity. In the 46 days that followed treatment it received eleven inoculations of virulent surra of Mauritius trypanosomes but did not become infected. When next tested for its immunity (311th day), it was apparently hypersensitive to infection. It was killed in 8 days by a greatly attenuated surra of Mauritius strain. The attenuation was apparently due to long continued passage through guinea pigs. One of the two controls inoculated with this virus died on the twentieth day. The other carried the trypanosomes for 111 days, recovered spontaneously, and is still alive (184th day).

64 (402)

The leucin fraction of proteins. II.

By **DONALD D. VAN SLYKE** and **P. A. LEVENE.**

[*From the Rockefeller Institute for Medical Research, New York.*]

In a previous communication[1] we have described a method for quantitatively separating leucin and isoleucin from valin, the leucin isomers being precipitated as normal lead salts. It has since been found that from the specific rotation of the mixture of leucin and isoleucin, as obtained analytically pure from the lead salts, the percentage of each can be accurately calculated. The rotation of d-leucin in 20 per cent. hydrochloric acid is + 36.8°, of l-leucin + 15.6°. Consequently the proportions are calculated by the formulæ:

$$\text{per cent. d-isoleucin} = \frac{100\,(R - 15.6)}{21.2},$$

$$\text{per cent. d-leucin} = \frac{100\,(36.8 - R)}{21.2}$$

(R representing specific rotation in 20 per cent. hydrochloric acid). The specific rotations of the isomers are unaffected by the boiling acid used for hydrolysis, but are affected by boiling with alkalies. Consequently the method is not applicable to products of alkaline hydrolysis, which however is seldom used.

[1] These Proceedings, 1909, vi, 54.