1. A detailed microscopic study of the life-cycle of this parasite made by blood smears taken hourly from 4 patients inoculated with malaria about 3 years ago.

2. The significant finding was the total absence of gametocytes (the sexual forms of the malarial parasite). This agrees with contentions of Gerstmann.[2] The asexual cycle of this strain of malaria is identical with the asexual cycle of "natural" malaria. From a biological point of view it is interesting to note that our malarial strain has apparently become "sterile", which means that it has lost its capacity for sexual propagation. The absence of gametocytes would make it impossible to infect mosquitos, thus limiting the life-cycle to man alone. Incidentally, we have failed in our efforts to cultivate this strain of malaria in artificial media.

3. A strain of malaria free from gametocytes is of considerable practical value in the treatment of general paralysis in that:

(a) It eliminates the possibility of the transmission of malaria to other members of the community.

(b) It precludes the occurrence of malarial relapse following adequate quinine administration.

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Spectrophotometric Analysis of Dye Penetrating Nitella from Methylene Blue.

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When living cells of *Nitella* are placed in methylene blue solution the rate of penetration of dye into the vacuole is more rapid at pH 9.2 than at pH 5.5. The penetration at pH 5.5 is too slow for satisfactory spectrophotometric analysis but at pH 9.2 this is possible. The dye in the vacuole gives an absorption curve characteristic of a dye consisting chiefly of azure B and a trace of methylene blue with an absorption maximum at 655mμ, while the external solution gives a curve characteristic of methylene blue with an absorption maximum at 664mμ. The presence of methylene blue in the sap is not due to contamination because the sap is extracted by cutting the end of the cell which is wrapped in dampened absorbent cotton and kept

[2] Gerstmann, S., *Die Malariabehandlung der Progressiven Paralyse.* J. Springer. 1925.

outside the solution. The same result is obtained whether the analysis is made immediately or several hours after extraction of the sap from the vacuole.

This result shows that the dye in the sap of *Nitella* has more methylene blue than that of *Valonia* (the primary absorption maximum of the latter being at 650mμ). This difference may be due either to the difference in the conditions of cells brought about by experiments or due to the difference normally existing. As soon as cells are injured more methylene blue penetrates.

The sap of *Nitella* is incapable of changing methylene blue to azure B even in 20 hours, when a sample of methylene blue is dissolved in the sap.

The rate of penetration of dye from pure azure B solution at pH 9.2 is much more rapid than from methylene blue solution at pH 9.2. The dye which has penetrated is found to be azure B with an absorption maximum of 650 mμ, which is identical with that of the external solution. At pH 5.5 the penetration is too slow for satisfactory analysis. These results agree with those on *Valonia*.

These results support the theory that the dye which penetrates the vacuole of living cells rapidly is the one which is quickly absorbed by the non-aqueous layer of the protoplasm and extracted from this non-aqueous layer by the sap. Azure B penetrates at a greater rate than methylene blue at pH 9.2, because in the form of free base it is rapidly absorbed by the non-aqueous layer. It is rapidly extracted by the sap on account of the fact that as soon as it comes in contact with the sap (which is at pH 5.5) it is transformed to salt, which is not readily absorbed by the non-aqueous layer. Since the dye salt cannot diffuse out it accumulates in the sap. Methylene blue which exists only in the form of salt, on the other hand, is only slowly absorbed from the solution at pH 9.2 by the non-aqueous layer and given up to the sap. The rate of penetration, therefore, depends on the partition coefficient of each form of dye at each respective boundary between the non-aqueous and aqueous phases.

These results do not show that methylene blue does not penetrate the protoplasm. They merely point out the danger of drawing any theoretical conclusion from such experiments unless we are certain of the nature of the dye penetrating.