centage following vitamin C medication. On the other hand, iron therapy was followed in most cases by a significant increase in hematocrit, erythrocyte and hemoglobin values, although, as expected, the plasma vitamin C concentration remained subnormal as before in all instances. The average increase of hematocrit was from 32.8 to 37.3%, that of erythrocyte count from 3.20 to 3.74 million per cu mm, and that of hemoglobin from 9.2 to 12.1 g per 100 cc. The mean corpuscular volume and the mean corpuscular hemoglobin showed insignificant variations, while the mean corpuscular hemoglobin concentration evinced a definite increase after the iron administration. No distinct rise in reticulocyte count was noted.

Summary. Anemia appears to be common in association with vitamin C deficiency among the inmates of a municipal relief institution studied. Therapeutic results indicate that the anemia is not due to lack of vitamin C *per se,* but related in all probability to a concomitant iron deficiency.

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Attempts to Grow *Leishmania donovani* in Tissue Cultures.

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The parasites of kala-azar, *Leishmania donovani,* live as non-motile forms only in the cells of the mammalian hosts. When they are cultured in the ordinary artificial media at 37°C, no growth occurs, but at room temperature, 22-25°C, or when brought to the digestive tract of certain insects, they develop into motile flagellates. It seems, therefore, that in order to cultivate the oval non-motile form of the parasites *in vitro,* an opportunity must be given them to grow in the living cells, and the tissue-culture method naturally suggests itself.

Gavrilov and Laurencin[1] have already made use of this method to cultivate these parasites, using the tissue of the infected hamsters. They found that the oval forms survived in the cells for only about 10 days. The following experiments were undertaken to determine whether it is possible to get a more successful growth of these parasites in the tissue cultures.

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[1] Gavrilov, W., et Laurencin, S., *Ann. Soc. Belge Med. Trop.,* 1938, **18,** 1.

Two series of experiments were made with the tissue cultures kept at 37°C. In the first series, 51 explantations by the hanging drop method were made of the spleen and the calf muscle of chicken embryos, the spleen, the omentum and the subcutaneous tissue of normal adult hamsters and hamsters infected with kala-azar, and the spleen and the subcutaneous tissue of a human fetus. For the chicken embryonic tissues, chicken plasma and embryonic tissue juice were used as media. For the hamster and human tissues, hamster serum and human serum from the umbilical cord respectively were added to the above mentioned media. After 3 to 7 transplantations, a drop of the suspension of *Leishmania donovani* (obtained from the spleen of an infected hamster) in Tyrode's solution was introduced into, and thoroughly mixed with, the media except in cases in which the tissue used was already infected. Transfers were subsequently made every 2 to 3 days, whole cultures were fixed and stained with iron hematoxylin at different intervals. It was found that within 24 hours the parasites were already taken up by large roundish phagocytes which contained from one to 15 parasites in their cytoplasm (Fig. 1). In some cultures the parasites began to degenerate on the seventh or eighth day of cultivation; in others they remained normal in appearance even on the thirteenth day, but by the fifteenth day all the parasites were found to be degenerated. It was also found that frequent transfer hastened the degeneration of the parasites and that different kinds of tissues yielded practically the same results.

In the second series of experiments only the calf muscle of the chicken embryo was employed. Altogether 65 explantations were made. In order to keep the tissue culture growing at a slower rate, the tissue, after being infected with *Leishmania donovani* in the hanging drop culture, was transferred into a Carrel flask prepared according to the method of Fischer and Parker[2] in which was used a solid medium composed of about 0.5 cc of chicken plasma and 1 cc of Tyrode's solution with only a very small amount of embryonic tissue juice to hasten the coagulation. The cultures were subsequently washed with Tyrode's solution and fed with heparinized plasma every 2 or 3 days. At different intervals the cultures were sectioned and stained for histological study. It was found that the parasites began to show degeneration after 20 days of cultivation; but some of them were morphologically and tinctorially still normal on the 35th day; by the 43rd day, however, only the degenerated forms were seen. Evidence of multiplication of the

[2] Fischer, A., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 585.

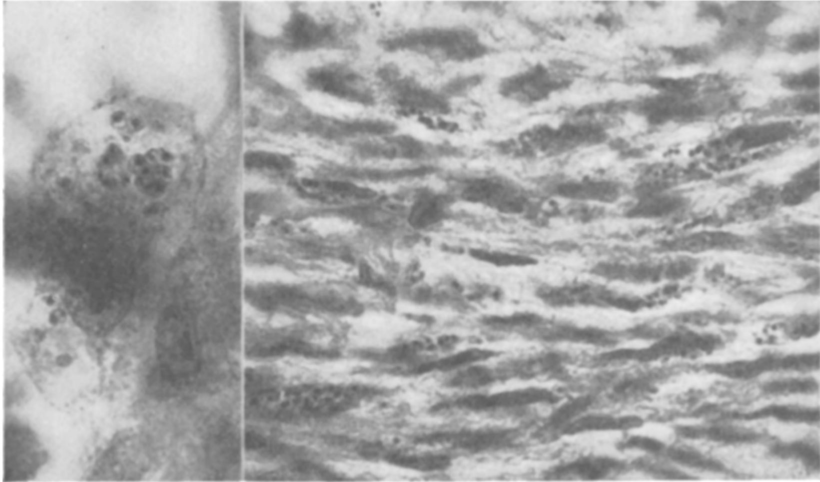

FIG. 1. FIG. 2.

FIG. 1. Photomicrograph showing the still well preserved *Leishmania donovani* in a phagocyte, 12 days after cultivation in a hanging drop culture. Iron hematoxylin stain. × 1000.

FIG. 2. Photomicrograph showing the normal appearing *Leishmania donovani* in elongated fibroblast-like cells, 35 days after cultivation in a Carrel flask. Tissue was sectioned and stained with hematoxylin and eosin. × 745.

parasites was at no time observed. In the older cultures the parasite-containing cells had transformed into elongated fibroblast-like cells (Fig. 2).

Summary and Conclusion. Attempts to grow *Leishmania donovani* were made in cultures of chicken, hamster and human tissues. It was found that the parasites were taken up by the phagocytes within 24 hours. In the hanging drop cultures parasites were all degenerated by the 15th day. In the slowly growing cultures in flasks prepared according to Fischer and Parker's method they were all degenerated by the 43rd day. Multiplication of the parasites has not been observed.

It is concluded that the parasites may survive for various lengths of time but cannot grow in the tissue cultures according to the methods described, and that the length of survival is in inverse ratio with the rapidity of the growth of the tissue cultures.