that found for control animals fed the basal diet alone. These data would appear to support the conclusion that orally administered iodoacetic acid exerts an influence on the sulfur metabolism of the organism.

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## Effect of Chlorin-e-Rhodin-g on Experimental Tuberculosis.*

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Inhibition of growth of human type tubercle bacilli (H 37) and avian tubercle bacilli in cultures on glycerol broth and Sauton's medium by certain chlorophyll compounds was reported by Daly, Heller and Schneider.[1] The compounds used included chlorin-e and chlorophyllin-a, the relationship of which to chlorophyll is described in the article cited. Unpublished experiments by the same authors indicated that rhodin-g, a compound related to chlorophyll-b in the same way that chlorin-e is related to chlorophyll-a, has bacteriostatic properties for tubercle bacilli similar to those of chlorin-e.

Chlorin-e and rhodin-g occur in combination in the proportion of approximately 2.75 to 1 in the breakdown of chlorophyll, preserving the same relationship that chlorophyll-a and chlorophyll-b have in the green plant. Since they have similar bacteriostatic effect, separation seemed unnecessary for further experiments on their inhibitory effect on tubercle bacilli.

Our own experiments have confirmed those previously reported. Human type (strain DT) and bovine type (strain 523) tubercle bacilli were inhibited in culture in glycerol broth and Long's medium by the sodium salt of chlorin-e-rhodin-g in concentrations from 0.025 to 0.01%, the effect of the higher concentration being greater than that of the lower.

An attempt was next made to determine if the sodium salt of chlorin-e-rhodin-g inhibits the development of tuberculosis *in vivo*.

Preliminary trial showed that guinea pigs were not suitable. Parenteral administration was desirable, and experiment showed

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1 Daly, S., Heller, G., and Schneider, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 74.

that the pigment was poorly absorbed when given intraperitoneally or subcutaneously. Accordingly the method chosen was intravenous injection in rabbits.

*Method.* Twenty-three young male rabbits were infected with 0.01 or 0.001 mg Ravenel strain bovine type tubercle bacilli and given daily injection of 55 mg pigment dissolved in water. The treatment was well tolerated. Twenty rabbits were infected with the same dose of bacilli but not treated with the pigment. Seven rabbits received the pigment but were not infected. The total amount of pigment injected in the tuberculous rabbits ranged from 2.4 g in the animals killed at 72 days to 6.7 g in a rabbit killed at 169 days. A few animals died of intercurrent disease in the course of the experiment, but the majority were killed at intervals. The tuberculous rabbits, both pigment-treated and nontreated, were killed in 3 main groups, viz., at about 2½ months, 3½ months and 5 months.

Necropsy was performed on all animals, and the gross condition of the organs noted. Microscopic sections, stained with hematoxylin and eosin and by the Ziehl-Neelsen technic for tubercle-bacilli, were cut routinely of the lungs, liver, spleen, one kidney and bone marrow of the femur. Serial blood counts were made on a number of rabbits of each series.

*Results.* In a few pairs of rabbits dying or killed early in the course of the experiment the amount of tuberculosis appeared to be less in the pigment-treated than in the control infected, untreated rabbits. However, the difference was slight and after a brief initial period no significant differences were noted between the infected pigment-treated and infected control animals. In practically all animals of each series the lungs, spleen and kidneys were grossly tuberculous, the extent of involvement increasing with the duration of the disease. No constant differences in the state of these 3 organs in the 2 series were seen. Microscopically the bone marrow was found tuberculous in a large percentage of cases in each group. In the sections examined tubercles were found in 12 of 22 examined bone marrows in the pigment-treated animals, and in 6 of 19 examined cases in the nontreated animals. The difference in the two groups is probably not significant. The sections were small, and where tuberculosis was found in the bone marrow it was not more severe in the pigment-treated than the control animals.

No differences were observed in the 2 series in weight. In each group, as well as in the pigment-treated noninfected group, an initial increase in weight, normal for the age of the animals, occurred. This lasted 2 months. During the next month a drop occurred. A

sharp secondary increase in weight occurred in each group during the fourth month. After this a rapid loss in weight occurred.

The chief change, other than the tuberculous involvement, was the heavy pigmentation in the pigment-treated animals. The liver and spleen were grossly much darker than in the untreated rabbits, and the bone marrow had a deep chocolate color. Microscopic sections showed large numbers of phagocytic cells filled with golden-brown granules in the bone-marrow and spleen. The pigment was readily differentiated from blood pigment by the Prussian blue reaction. In both the spleen and bone marrow the pigment stood out more conspicuously in Ziehl-Neelsen than in hematoxylin and eosin stains. In the liver the pigment was frequently found in the Kuppfer cells, but was much less granular than in the spleen and bone marrow, the cells often having a homogeneous brown color. Pigment was frequently visible as a dark brown mass in the bile canaliculi.

Pigment was commonly observed in phagocytic cells around tubercles. However, the pigmented cells did not take part in the palisade of epithelioid cells forming the true border of tubercles. Pigment and acid-fast bacilli were occasionally found in the same phagocytic cell, but this was the exception. Whether the 2 cells were fundamentally of the same origin or not, a distinct difference in functional activity was evident, large numbers of cells containing pigment and other large numbers containing bacilli.

*Summary.* Chlorin-e-rhodin-g inhibited the growth of tubercle bacilli *in vitro,* but had no effect on the course of experimental tuberculosis in rabbits. The pigment accumulated in large quantity in phagocytic cells in the spleen and bone marrow. Pigmented cells were frequently concentrated on the border of tubercles, but did not appear to function as epithelioid cells and rarely were found containing tubercle bacilli.