may be the peak of this continuous spectrum radiation due to the bombardment of the photoelectrons against neighboring atoms in the secondary radiator. This theory would explain for instance the fact that the observed radiation is a band much broader than the lines of the primary rays.

[1] These PROCEEDINGS, Dec. 1923.

[2] *Bull. Natl. Res. Council*, Oct. 1922, p. 16: *Physic. Rev.*, May 1923, p. 483, and June 1923, p. 715.

[3] These PROCEEDINGS, July 1923, p. 246.

[4] *Physic. Rev.*, June 1918, p. 505.

[5] *Comptes Rendus, Paris*, Jan. 31: March 29: Sept. 26, 1921; *J. Physique*, Sept. 1921.

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# SYMBIOSIS BETWEEN TERMITES AND THEIR INTESTINAL PROTOZOA[1]

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A correlation between the presence of intestinal protozoa in termites and a wood-feeding habit was postulated by Imms,[2] and this, if true, as Imms pointed out, indicates a possible symbiotic relationship between termites and their intestinal protozoa. After making several microchemical tests on the protozoa, Buscalioni and Comes[3] concluded that the protozoa digest the wood particles which they take into their bodies; and from this conclusion, without stopping to consider whether or not the protozoa can digest wood, these authors claim that they have established symbiosis between termites and their intestinal protozoa. Other investigators, notably Grassi[4,5] and Kofoid,[6] though devoting most of their time to systematics and morphology, have usually regarded the protozoa either as commensals or parasites. Recent investigations by the writer[7,8], together with hitherto unpublished data, are briefly summarized in this paper.

*Examination of Museum Material.*—Careful examination of the intestinal contents of five workers of each species of termites in the U. S. National Museum revealed that wherever protozoa were present wood was also present and, *mutatis mutandis*, protozoa were present only when wood was present; thus confirming Imms' postulatum. Four families of termites are known. Among the 18 genera and 64 species examined from the family Termitidae, protozoa and wood were present in only 1 of the 21 species of the genus *Nasutitermes* and 2 of the 8 species of the genus *Mirotermes;* but protozoa and wood were present in 18 genera and 76 species, or all that were examined, in the other three families (Mastotermitidae, Kalotermitidae, Rhinotermitidae). Thus the correlation between a strict wood-feeding habit and the presence of intestinal protozoa is perfect and positive.

*Experimental Work on the Relation of the Protozoa to their Host: Material.*—Living material of *Reticulitermes flavipes* Kollar, *Kalotermes schwarzi* Banks, *K. jouteli* Banks, *Prorhinotermes simplex* Hagen, *Termopsis nevadensis* Hagen and *T. angusticollis* Hagen was used in the experiments, although *R. flavipes,* owing to its local abundance, was used most extensively. The protozoa present in most of these termites, together with a classification of all the known termite protozoa, may be found in another paper.[7]

*Removal of the Protozoa without Injury to the Host.*—The feeding to termites of wood, antecedently soaked in aqueous solutions of various chemicals (NaCl, $CaCl_2$, $HgCl_2$, KCl, $CuSO_4$, etc.), resulted, as a rule, in killing both host and parasite if either was killed; on the other hand when humus, dirt, and cellulose were fed them, neither was killed. Many other methods were tried out, but cannot be given here.

Finally, however, it was discovered that if the termites were incubated for 24 hours at 36° C. all the protozoa were killed, while the host remained active and apparently uninjured. Termites from which the protozoa have been removed are referred to as defaunated termites.

*Observations and Experiments on Defaunated Termites.*—If given their normal diet of wood, defaunated termites become less active, with considerably flattened abdomens, on or about the 10th day after incubation, and are dead within 3–4 weeks.

What is the cause of death, incubation *per se,* the removal of cellulose digesting bacteria and fungi, or the removal of protozoa? In order to answer this question the three factors involved were studied separately.

Some of the defaunated termites were fed fungus-digested cellulose and some were fed humus. In neither case did death result, as had previously occurred when they were fed wood; they were active and apparently normal after four months, at which time the experiments were discontinued, for it had been shown that incubation *per se* was not responsible for the termites death.

Since incubated termites do not die when fed fungus-digested cellulose, and since cellulose is the principal constituent of wood, the intestinal bacteria and fungi of termites were carefully studied in all the media that have been used in the study and isolation of cellulose digesting microörganisms; but it was never possible, after many trials, to get any of these organisms to grow on any medium where cellulose was the only carbohydrate present. It was, therefore, concluded from this that the intestinal bacteria and fungi of termites do not aid their host in the digestion of cellulose. That unincubated termites are able to utilize cellulose was demonstrated by the fact that they were active and apparently normal in every way after having been fed pure cellulose for four months; whereas when fed nothing, but kept under identical conditions of moisture, light and temperature as when fed cellulose, they died in 3–4 weeks.

Only the protozoa are now left to consider, and it is important here to note that they are very abundant, both in form and number, and *completely* fill the large and much distended gut of their host. In order to determine whether or not the removal of the protozoa by incubation was in any way responsible for the death of their host, several thousand incubation defaunated termites, with their right antennae cut off close up to the head, were placed in jars and vials, 10–50 in each, and were fed either wood or pure cellulose. In half the jars and vials the same number of unincubated termites were placed with the incubated ones. The incubated termites were killed and carefully examined at intervals of 1–30 days to determine whether or not they were being reinfected with protozoa; and it was found that they were regaining a protozoan infection from their associates, the number of protozoa present in them gradually increasing up to about the thirtieth day, at which time, in most instances, the usual number present in a single unincubated host had been attained. The incubated ones were removed from the unincubated at intervals of from 10–30 days and were seemingly normal after having been fed wood or cellulose for three months, at which time the experiments were discontinued, since it was evident that association with the unincubated for ten days or more had in some way restored the ability to utilize wood or cellulose as food.

But how had the incubated termites, by association with the unincubated ones, recovered their ability to live on wood or cellulose? Are the protozoa responsible? If so, how? This was studied in several ways. (1) The termites were wood starved, and it was noticed, after four to five days, that *Trichonympha*, the large and principal wood-ingesting protozoan in many termites, had disappeared entirely; after seven to eight days practically all the wood-ingesting species of protozoa were dead, while the non-wood-ingesting ones were as abundant as ever. These termites, though active and apparently normal, when returned to a wood diet lived but little, if any, longer than the incubation defaunated ones. (2) Several hundred adult individuals of the second form caste were isolated from many different colonies. These when fed wood or cellulose, in every instance, were all dead in 3–4 weeks. Adults of this caste do not harbor protozoa and eat wood rarely, if at all. They are fed salivary secretions by other members of the colony. (3) Adult soldiers harbor protozoa and have wood in their guts, but die when placed to themselves and given wood or cellulose, apparently because they cannot eat the food. They partake principally—entirely unless they eat wood meal—of proctodael food (wood) furnished by other members of the colony. Protozoa, then, it seems must be present in order for a termite to live on a diet of wood or cellulose.

The next question to consider is, how do the protozoa function in enabling their host to live on wood or cellulose? The second form caste individuals that were isolated and studied did not eat the cellulose (Whatman filter paper No. 43), and, so far as I could tell, they never ate the wood either, for none was ever present in their guts. It is possible that the degeneration of the jaw muscles which occurs in this caste does not permit the eating of wood after reaching the adult stage, although it is certain that at an earlier stage in their life-cycle they did eat wood and were infected with potozoa, which are gradually lost, when their host, owing to the degeneration of its jaw muscles, ceases to furnish them with wood for food.

There is thus a very close correlation between the wood-feeding habit and the presence of intestinal protozoa. The protozoa are never present except when wood or cellulose is the principal food of their host. This close correlation of wood with the presence of protozoa, in species, castes, and even in the life-cycle of an individual in nature, strongly suggests that the protozoa in some way are directly connected with the utilization of wood as food, either by themselves or in coöperation with their host.

The protozoa probably use the wood as food and subsist on it largely rather than the intestinal fluids of their host, since they die 10–20 days in advance of the termites during wood starvation. Termites from which the protozoa have been removed by incubation or wood starvation, cannot live on a diet of wood, though they eat great quantities of it as may be seen by watching them or by a microscopic examination of their intestinal contents. In termites that have not been wood starved at all, certainly a very large percentage of the wood which they have eaten may at all times be found in the bodies of their intestinal protozoa; whereas in termites that have been wood starved for a few hours, it is almost impossible to find wood particles free in their intestines, because the protozoa have ingested them. Also when termites are wood starved, the amount of wood present in the protozoa gradually disappears.

All the microchemical tests that have been made to determine whether or not the protozoa digest the wood particles cannot be given here. A large quantity of glycogen is present in the bodies of the wood-ingesting protozoa, while none is present in the intestinal cells of the termites or in the non-wood-ingesting protozoa. This indicates that the wood-ingesting protozoa form the glycogen themselves. When the host has been fed nothing but the purest filter paper (.00006% ash) for three months, the wood-ingesting protozoa still have great quantities of glycogen present in their bodies, which suggests that they possibly split the cellulose into cellobiose, and the cellobiose into glucose from which they build up the glycogen.

The protozoa, but not the termites, it seems have the ability to digest wood or cellulose, which explains why the termites die, though given their normal diet, when the protozoa are removed from them, and why they live indefinitely when the protozoa are restored. It is quite probable, then, though not proved perhaps beyond all question, that the protozoa play a very important rôle in the digestion of the wood which their host eats, and that the relation of termites to their intestinal protozoa is one of symbiosis, the termites furnishing food and lodging for which the protozoa give them in return products elaborated from the wood.

[1] *Acknowledgment.*—Some of the investigations summarized in this paper were carried out after the writer was granted a Fellowship in the Biological Sciences by the National Research Council.

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[6] Kofoid, C. A., and Swezy, O., *Univ. Calif. Publ. in Zoöl.,* **20,** 1919 (1–113).

[7] Cleveland, L. R., *Amer. J. Hygiene,* **3,** 1923 (444–461).

[8] Cleveland, L. R., [In Press] *Biol. Bull.,* May-June (?), 1924.

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## *THE ABSOLUTE WEIGHT OF THE HEART AND THE SPLEEN*[1]

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In some earlier work the authors[2] found that the organ weight ratios $L/H$, $K/H$, $L/S$, $H/S$, and $K/S$ (where $L$ denotes liver weight, $H$ heart weight, $K$ kidney weight, and $S$ spleen weight) had values which suggested that, in persons whose only significant pathological lesions at autopsy were those of tuberculosis, the weight of the heart was relatively low and that of the spleen relatively high. It was determined to make a thorough study of the absolute weight of those two organs, from the same material used in the earlier study. This tedious task has now been completed. In advance of the final publication of the results[3] in a place perhaps not generally accessible to non-medical readers, it seems desirable to make a brief report of the work here.

The method used in this work for comparing different groups in respect of the absolute weights of organs, which change in size with age, as a result both of processes of growth and of senescence, seems worthy of mention. It consists essentially of setting up and measuring as wholes *difference bands*