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Cellulose Digestion by Organisms from the Termite Gut.

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The termite thrives in an environment which is most unusual and which requires specialized adaptation for survival. Its galleries are largely cellulose as also is its diet. Relatively little combined nitrogen is found in its food other than that derived from consumption of fecal materials of its fellows. That sector of the nitrogen cycle which includes the metabolism of the termite is as yet unexplored.

It is probable that the energy requirements of the termite are derived from the destruction of cellulose. In addition to the energy required by the animal for its ordinary life processes an additional large amount must be available should it develop that there is any fixation of nitrogen connected with its metabolism. The question of cellulose splitting within the gut of the termite thus is of fundamental importance.

Cellulose digestion is not unknown among the invertebrates. Miller and Boynton[1] have demonstrated the appearance of glucose within the gut of Bankia, the Northwest shipworm. Werner[2] has shown that the digestion of cellulose within the intestine of *Potosia cuprea,* the rose chafer, is bacterial in character. Cleveland,[3] however, believes that the enormous numbers of bacteria found within the gut of the termite have no function as cellulose splitters within the intestinal canal of *Reticulotermes flavipes,* and his series is reported to have included at least 100 individuals.

Inasmuch as opportunity presented itself, through the courtesy of Dr. S. F. Light, to obtain termites from a variety of sources and of a number of different genera, we decided to make a further attempt to demonstrate the splitting of cellulose by bacteria derived from termite gut content. The technique was that usually followed in soil bacteriology. Flasks were prepared containing medium of the following formula: K_2HPO_4—1 gm.; $MgSO_4$—1 gm.; Na_2CO_3—1 gm.; $(NH_4)_2SO_4$—2 gm.; $CaCO_3$—2 gm.; tap water 1000cc. A 2-inch circle of filter paper was added to each flask and the material then was autoclaved.

[1] Miller, R. C., and Boynton, L. C., *Science,* 1926, xliii, 524.

[2] Werner, E., *Cent. f. Bakt. u. Parasit.,* 1926, Abt. 2, lxvii, 297.

[3] Cleveland, L. R., *Biol. Bull.,* 1924, xlvi, 177.

Gut content was prepared by immersing the termite in tincture of iodine (U. S. P.) for 45 seconds. The animal afterwards was washed in each of 2 changes of sterile physiological saline. The gut was then exposed by use of fine forceps and with a sterile glass slide and its contents were placed as an inoculum within a flask of medium. Incubation took place under aerobic conditions at 20°-28° C. Evidence of cellulose digestion was offered as it occurred by disintegration of the filter paper. Control uninoculated flasks were placed with all series thus inoculated in order to make certain that mere physical disintegration was not concerned here.

Our series has included 85 flasks and of these 11 preparations have shown definite destruction of the cellulose. The distribution of these is indicated by the following table:

TABLE I.

Form	Number Tested	Number Showing Cellulose Digestion
Reticulotermes hesperis	8	2
Reticulotermes humilis	20	0
Porotermes froggatti	2	1
Kalotermes minor	4	3
Kalotermes hubbardi	20	0
Amitermes californicus	21	2
Termopsis angusticollis	6	1
Neotermes malatensis	4	2
	85	11

Cellulose digestion took place slowly in positive instances. The period of time necessary for disintegration to appear was of varying length and extended from 10 days to 3 months. The agent causing this digestion of the cellulose is transmissible since inoculums of disintegrating filter paper placed in other flasks of the medium induced the same change in them. Aerobic conditions are necessary inasmuch as anaerobic technique including the use of a paraffin-vasaline seal inhibits all action. The flora is made up of gram negative rods together with some micrococci. The dark field shows no spirals nor protozoa from these cultures.

All attempts to isolate in pure culture the organisms including this change were futile. The usual synthetic agar formula including precipitated granular cellulose within its structure yielded a variety of colonies aerobically although none appeared anaerobically in Burri tubes. No clear zone surrounded any of them and moreover mass inoculation into flasks of cellulose medium was of no effect. The addition of 0.02% sodium nitrate, of 0.1% glucose or of both to media solid or fluid did not improve the outcome. Rather, cellu-

lose destruction in the flasks proceeded better with neither of these compounds present.

Thus by methods probably somewhat unsuited to the material at hand but nevertheless adapted from procedures followed in soil bacteriology we have shown that there is a bacterial flora within the gut of certain individual termites which can destroy cellulose. In crude culture the action is slow. It is very possible that with media built up more closely in accordance with the environment of the intestine, this flora may appear more often and may show greater velocity in reactions produced. Our biochemical knowledge of this portion of the animal's anatomy, however, is as yet too fragmentary to proceed much farther at this moment.

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Duck Disease Studies I: Blood Analyses in Diseased Birds.

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It has long been observed that ducks and other migratory birds which settle about inland marsh lands and shallow lakes reputed to contain "alkali waters" develop characteristic symptoms resulting frequently in heavy mortality. A systematic study of this disease has been proposed through the co-operation of the California Fish and Game Commission, the Hooper Foundation for Medical Research, and the Department of Pharmacology of the University of California Medical School. This report is the first of a series to be made in connection with this study.

This report deals with blood chemistry studies on diseased birds from the San Joaquin Valley district in comparison with normal healthy birds. Four blood constituents, non-protein-nitrogen, uric acid, blood sugar, and chlorides, have been studied on 13 normal and 15 diseased ducks of the pintail or sprig species *(Dafila acuta)*. Blood samples were taken directly from the heart and determinations made on protein-free filtrates according to the Folin and Wu system of analysis. Rectal temperatures were obtained preliminary to taking the sample.

The results indicate an average increase of 50% in non-protein-nitrogen, and an 80% increase of uric acid, in the diseased birds. In the chlorides, computed as sodium chloride, a barely significant