knife across the block quickly. With a camel's hair brush in the left hand, quickly remove the section from the knife before it melts. If the section melts, one is likely to damage the tissue in brushing it from the blade.

Place the section in a syracuse dish of Ringer's or physiological salt solution. When a sufficient number of sections have been made, they may be stained.

By means of a small glass rod, headed on the end like a balsam dropper, remove the section from the solution. Let the section wrap around the beaded portion; it has been hardened enough in formalin to hold its shape fairly well. The section may be held up slightly in the solution by means of a dissecting needle while the glass rod is being slipped under it. With the section remaining on the glass rod, dip it into the stain for ten seconds (the stain I found best for this tissue was polychrome methylene blue). From the stain place the section into Ringer's or physiological salt solution. It is all right to let the section drift in this solution, for the stain gives it added rigidity so that it may easily be picked up again with the rod.

Now, still by means of the glass rod, transfer the section to a slide on which is a drop of Brun's Glucose Medium. By rolling the rod through the drop of liquid the section can be made readily to come off of it. (Glycerine alone takes the stain from the tissue.) A cover slip may now be placed over the tissue. This must be done carefully, for the tissue is often wrinkled in the placing of the cover slip.

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SPECIAL ARTICLES

THE CULTIVATION OF A SPECIES OF TROGLODYTELLA, A LARGE CILIATE, FROM THE CHIMPANZEE

THE examination of several chimpanzees at this laboratory recently revealed that one was infected with a large ciliate of the genus *Troglodytella*. Reports of such an infection are not numerous and the organism seems to have been very little studied. Although this species has not been definitely determined it has been studied alive and stained and found to answer very closely the description of *T. brassarti* var. *accuminata* Reichenow.[2,3] Attention was at once directed to it in the hope that it might be cultivated and be used for further studies.

At the time of the discovery, *Balantidia* from other individuals of the same group of chimpanzees were being cultivated. In this preliminary work the *Troglodytella* has been cultivated for a short time following the same technic that was used in the cultivation of the *Balantidia*. The medium which has proven to be most satisfactory in the writer's experience for the *Balantidia* has also given the most encouraging results for the *Troglodytella*. This medium is that of Tanabe and Chiba[4] for the cultivation of *E. histolytica*. Pig serum only has been used in these experiments.

Greatest success was obtained in an experiment in which six test tubes containing 10 cc of solution were inoculated with about 1 gram of fecal material containing *Troglodytella* and a few *Balantidia*. These tubes were incubated at 37.5° C. The results were as follows:

Tubes	Serum	24 hrs.	48	72	96	120	144	168	172
1	5 per cent.	+	+	+	+	+	+	+	–
2	5 " "	+	+	+	+	+	+	+	–
3	5 " "	+	+	+	–				
4	10 " "	+	+	+	+	+	+	+	–
5	10 " "	+	+	+	–				
6	10 " "	+	+	+	–				

In 24 hours many very active *Troglodytella* and a few *Balantidia* could be seen swimming in all the tubes. Subcultures were made at this time from tube 4, which seemed to contain the most ciliates. In 48 hours the *Troglodytella* had apparently increased in numbers and a number of dividing individuals were seen at this time. The ciliates were very active and some could be seen boring into the agar slant. The *Balantidia* had also increased in numbers. At the end of 96 hours all ciliates in three tubes had died and in the other three the *Balantidia* had multiplied much more rapidly than the *Troglodytella* and outnumbered them. The latter, however, were still active and both *Troglodytella* and *Balantidia* could be seen

[1] From the department of protozoology, Johns Hopkins School of Hygiene and Public Health. The writer wishes to express his appreciation to Drs. Van Volkenburgh and Long and the Committee on Cold Research, from whose chimpanzee the material for this work was secured.

[2] E. Reichenow, "Den Wiederkäuerinfusorien verwandte Formen aus Gorilla und Schimpanse," *Arch. f. Prot.*, 41: 1–33, 1920.

[3] J. Buisson, "Les infusoires ciliés du tube digestif de l'homme et des mammifères," Trav. Lab. Parasit. Fac. Méd., Paris, 1923.

[4] M. Tanabe and E. Chiba, "A New Culture Medium for *Endamoeba histolytica*," *Acta Med. in Keijo*, 11: 1–4, 1928.

boring into the slant. At about this time unfavorable conditions set in in the tubes and multiplication of both ciliates seemed to cease, and at the end of the seventh day, all of both types were dead.

Five tubes were inoculated for the first subculture. Only two of these were positive at the end of 48 hours. In these two the *Troglodytella* seemed to have multiplied considerably at the end of 48 hours and dividing individuals could be seen. The *Balantidia* multiplied much more rapidly, however, than the *Troglodytella* and at the end of 72 hours far outnumbered them. Both types remained active until the end of the sixth day when they all died. Second subcultures were made at the end of 48 hours. In these, twenty-four hours later, the *Balantidia* outnumbered the *Troglodytella,* and, although the latter were alive at the end of 96 hours when subcultures were again made, only the *Balantidia* survived this transfer.

This experiment was repeated several times with the same result and then several other media[5,6,7] were tried but without success. It is possible that the failure of continued cultivation of the ciliate may not have been due so much to any fault of the medium as to the fact that such frequently repeated transfers does not give time for sufficient multiplication as it does in the case of the *Balantidia,* a smaller ciliate. This problem is being worked on at the present time in this laboratory.

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THE EXPERIMENTAL TRANSMISSION OF ANAPLASMOSIS BY DERMACENTOR VARIABILIS

ABOUT a year ago the experimental transmission of bovine anaplasmosis by the brown dog tick, *Rhipicephalus sanguineus,* was reported by the writer[1] from this laboratory. This tick, although very widely distributed throughout all tropical countries of the world, is only known to occur in Texas, Louisiana, Mississippi, Florida, Kansas, Ohio, Pennsylvania and New York so far as this country is concerned, whereas anaplasmosis occurs in an area which, while not yet well mapped, certainly extends not only beyond the zones within which the related disease, piroplasmosis (cattle tick fever), is now confined, but also outside the original range of enzootic bovine piroplasmosis. It was pointed out, therefore, that if anaplasmosis is tick-borne throughout its entire range, it should have, judging from the distribution of ticks in the United States, at least two other species of ticks as its carriers. The experimental evidence presented in the present paper incriminates, as a carrier of anaplasmosis, another tick, *Dermacentor variabilis,* a species important because of its wide distribution and host range. This tick occurs on the Pacific coast and appears to be wide-spread east of a line extending south from the middle of the Canadian border of North Dakota to about Corpus Christi, Texas. It is not known to be present in the Rocky Mountain States. There is grave danger, therefore, that this tick may carry this destructive disease into many areas that hitherto have been considered outside the enzootic range of anaplasmosis.

EXPERIMENTAL CONDITIONS

Since not only the adults but also the larvae and the nymphs of three-host ticks drop to the ground after engorging, it is necessary in experimental work to devise means of retrieving the engorged ticks so that they will not be lost in the stable litter. When available, therefore, bulls have been used in preference to cows since the ticks could be held in a bag which was attached by means of adhesive tape to the bull's scrotum. For the experiments reported in the present paper, the bulls were purchased late in the fall in an area in Colorado where nothing resembling anaplasmosis is known to occur and were shipped immediately to Jeanerette, Louisiana, where they were confined in a screened barn. A description of this barn in which the bulls were kept out of contact with biting flies and other ectoparasites except the ticks used in the experiments has been published by the writer.[1] Bulls that have been held in this barn and not yet used for experiments were checks on the work; during the work herein reported five such checks were held and all of them remained uninfected. Furthermore, during the past three years, 68 head of cattle have been held in this barn, some of them as long as 18 months; except when experimentally transmitted, not a single case of anaplasmosis has been noted.

EXPERIMENTAL PROCEDURE

Many unengorged and partly engorged adults of *Dermacentor variabilis* were taken from cattle in Mississippi and Florida during April and May, 1931, by tick inspectors under the direction of Dr. Hartwell Robbins, of the U. S. Bureau of Animal Iudustry, and by Dr. T. W. Cole, of the U. S. Bureau of Animal Industry. These ticks were sent alive to the laboratory at Jeanerette, Louisiana, where they were used as follows: (1) Adults were allowed to engorge on a bull with clinical anaplasmosis, the females

[5] E. Schumaker, "The Cultivation of *Balantidium coli,*" *The Amer. Jour. Hyg.,* 13: 1, 281–295, 1931.

[6] Sidney Margolin, "Methods for the Cultivation of Cattle Ciliates," *Biol. Bull.,* 59: 3, 1930.

[7] A. Schourenkova and V. Nossine, "Le culture du *Balantidium coli l'ovigen humaine,*" La presse medicale, 10: p. 1686, 1930.

[1] C. W. Rees, "The Experimental Transmission of Anaplasmosis by *Rhipicephalus sanguineus,*" *North American Veterinarian,* September, 1930.