Mean pct. germination	Correlation between colloidal index and (1) pct. germination	(2) height	(3) green weight
55.8	.634 ± .037	.680 ± .034	.693 ± 0.33
76.5	.498 ± .051	.534 ± .049	.547 ± 0.48
86.5	.374 ± .026	.341 ± .027	.313 ± .027

According to this the colloidal index test is increasingly accurate as the sweet corn becomes more inferior. This is a very decided advantage.

A number of students in the laboratory of plant physiology at the University of Illinois have used the nephelometer with decided success in measuring the reduction of vitality as affected by disease or following treatment with various chemical and physical agents. It is possible that these tests may prove useful in determining viability in grains and in other seeds having a fairly large endosperm.

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SPECIAL ARTICLES

EXCYSTATION IN VITRO OF HUMAN INTESTINAL PROTOZOA[1]

FOR many years it was believed that the cysts of intestinal protozoa would not excyst until subjected to the digestive juices peculiar to the normal host of the species. Recent experimental work, however, indicates that moisture and a temperature of about 37° C. for several hours are the only factors necessary to stimulate excystation in the intestinal protozoa of man. Darling[2] (1913) noted the disappearance of cysts and the appearance of trophozoites in feces containing cysts of *Endamoeba histolytica* that were kept in a moist chamber. It is not at all certain, however, that the trophozoites observed came from the cysts, since amoebae of other species often appear in fecal material kept under similar conditions. Yorke and Adams[3] (1926) observed the process of excystation in this species; Allen[4] (1926) describes what she believes to have been excystation in the form named by Kofoid and Swezy[5] (1921) *Councilmania lafleuri*; and Smith[6] (1927) has observed, and shown to the writer, excystation in *Iodamoeba williamsi*. The writer[7] is now able to add to this list *Endamoeba coli, Endolimax nana* and the flagellate *Chilomastix mesnili*; he has also observed early stages of what appears to be excystation in vitro in *Giardia lamblia*. The other intestinal protozoa of man are *Trichomonas hominis* and *Endamoeba gingivalis*, which have no cyst stage, and *Embadomonas intestinalis, Tricercomonas intestinalis* and *Dientamoeba fragilis*, which are rare species not easily obtained for study.

Endamoeba histolytica. Excystation in vitro in this species has been described by Yorke and Adams (1926). Material containing cysts was sealed under a cover glass and examined in a warm microscope chamber. Pseudopodia were formed inside of the cyst; then a break appeared in the cyst wall through which the amoeba escaped. Moisture and a temperature of about 37° C. seemed to be the essential factors in excystation. Cysts that had been in Locke-egg-serum medium at 37° C. for two hours proved more satisfactory than unincubated specimens.

Councilmania lafleuri. Allen (1926) saw what she believed to be the last of eight amoebulae to escape from the cyst wall of this species. According to her observations the entire amoeba does not leave the cyst, but the eight amoebulae into which it divides pass out one by one through a pore in the cyst wall.

Iodamoeba williamsi. Excystation in this species has been observed by Septima C. Smith (1927). She found that cysts fifteen hours old, when placed in an incubator at 37° C. for two hours and then in a warm chamber at about 40° C. for three hours, would excyst in a saline medium. Minute pseudopodia were noted within the cyst; then followed a break in the wall and the escape of the amoeba. In some cases the amoeba emerged part way and then returned, escaping only after several passages back and forth. The newly excysted organisms were very active. She concluded that the stimuli necessary for excystation are moisture and a temperature of about 37° C. for several hours.

[1] From the Laboratory of Protozoology, Johns Hopkins School of Hygiene and Public Health. The writer is greatly indebted to Mr. Conrad Bauer for his valuable assistance.

[2] Darling, S. T., 1913. "Observations on the cyst of *Entamoeba tetragena*." Archiv. Int. Med., 11: 1–14.

[3] Yorke, W. and Adams, A., 1926. "Observations on *Entamoeba histolytica*. I. Development of cysts, excystation and development of excysted amoebae, in vitro." Ann. Trop. Med. and Parasit., 20: 279–302.

[4] Allen, E. A., 1926. "Excystment of *Councilmania lafleuri* Kofoid and Swezy in culture in vitro." Univ. Cal. Pub. Zool., 29: 175–178.

[5] Kofoid, C. A. and Swezy, O., 1921. "On the free, encysted and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine." Univ. Cal. Pub. Zool., 20: 169–198.

[6] Smith, Septima C., 1927. "Excystation in *Iodamoeba williamsi* in vivo and in vitro." SCIENCE, 65: 69–70.

[7] Hegner, Robert, 1927. "Excystation and infection in the rat with *Giardia lamblia* from man." *Amer. Journ. Hyg.* (in press).

Endamoeba coli. Excystation of *E. coli* was observed many times by the writer in material obtained by washing infected feces in water. This material either in water or in weak saline solution was sealed under a cover glass and placed on the stage of a microscope confined in a warm chamber. The protoplasm within the cyst is at first finely granular and the eight nuclei are usually clearly visible, but later the nuclei become invisible and a number of larger granules of various sizes appear. The first evidence of activity preceding excystation is the movement of the cytoplasm in the center of the cyst. No large free area exists between the cyst contents and the cyst wall such as described by Smith (1927) in *Iodamoeba williamsi.* Pseudopodia first appear through an opening in the cyst wall. This opening is small and the protoplasm streams through it rapidly in a thin strand. The amoeba does not leave the cyst wall at once, but usually, after from one half to three fourths of the protoplasm has escaped, movement begins in the opposite direction and most or all of the animal streams back again into the cyst. This egress and return of the protoplasm may occur as often as ten times before complete escape is effected and the liberated amoeba moves away from the deserted cyst wall.

After excystation the amoeba moves at first slowly but soon flows across the field by means of rapidly forming pseudopodia. These pseudopodia are somewhat similar to those of *E. histolytica,* being formed rapidly and more or less explosively and being at first free from granules although not so clear and hyaline as those of *E. histolytica.* In every case the entire contents of the cyst emerged as a single amoeba. Excysted amoebae were watched for more than six hours, but no division stages were observed.

Endolimax nana. Excystation could not be studied as easily in *Endolimax nana* as in *Endamoeba coli* because of its minute size. So far as could be observed, however, the process was similar in every respect. The first evidence of activity was movement in the cytoplasm; this was followed by a minute break in the cyst wall through which the cytoplasm protruded; then after flowing in and out several times the organism separated from the cyst wall as a single amoeba.

Chilomastix mesnili. Excystation of this flagellate was seen in only one case. The details were not clearly made out, but the essential features were observed. Movement of the protoplasm within the cyst was followed by a break in the wall at the anterior end and the rapid emergence of the organism, which soon took on approximately the shape of a typical trophozoite. One large cystostome was present. Whether the excysted specimen contained one or two nuclei was not determined. In this case the cyst was in a saline medium and excystation occurred after three hours and forty minutes at about 37° C.

Giardia lamblia. Complete excystation of *Giardia lamblia* in vitro has not been observed, but movement within the cyst can be brought about by the same method as that shown to be effective with other protozoa. Washed cysts from two to four days old were used. Material was sealed under a cover glass and kept in an incubator at 37° C. for two hours; it was then placed on the stage of a microscope in a warm chamber at approximately 39° C. Within from ten to fifteen minutes movement began in some of the cysts. The contents seemed to contract and expand, due probably to bending movements of the axostyle such as were observed in cysts recovered from the small intestine of the rat (Hegner, 1927). The protoplasm of the organism was seen to shrink away from the cyst wall and after from one to four hours became quiescent.

It seems safe to conclude from these observations that, as suggested above, moisture and a favorable temperature (about 37° C.) for a sufficient period (several hours) are the essential factors in excystation. It, therefore, follows that the digestive juices of the host that ingests the cysts of intestinal protozoa are unnecessary in bringing about excystation. They may be helpful, but on the other hand it is possible that they are harmful. If the latter is true, then the cyst wall probably protects the cysts from the secretions encountered in the stomach. In this connection it may be noted that no excystation nor protoplasmic movements were observed within the cysts of *Giardia lamblia* that were injected into the stomach of the rat, although cysts hatched in the small intestine of this animal (Hegner, 1927). Further details of excystation in these intestinal protozoa will be published in a later communication.

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ISOTOPES OF CALCIUM

THE writer has recently studied the selective reflection of several carbonates at about 6.5 microns. Polarized light was used so that bands due to vibrations along the different directions in the crystal would not be superimposed. In the case of calcite ($CaCO_3$) three small maxima were observed. The wave lengths were 6.36 μ, 6.54 μ, and 6.62 μ. When several bands overlap, it is difficult to calculate the true intensity of the separate bands as there is no zero line of reference. However, using the band at 6.54 μ as the standard, the band at 6.36 μ is about