These curves show varying degrees of curvature; however, so do many of the curves from other patients with heart disease, but with sinus rhythm. In no instance does the curve obtained in paroxysmal tachycardia show the tendency to inscribe a circle or oval, such as has been seen in flutter and fibrillation. For purposes of comparison, Fig. 2 illustrates the axes for 34 consecutive 1/100 seconds, taken in the horizontal plane, from a patient with mitral stenosis and atrial fibrillation; they inscribe a varying circus movement, clockwise in direction.

*Comment.* Further study of similar cases must be made when opportunity offers, but the contours of the curves of the momentary electrical axes in these cases of paroxysmal tachycardia resemble those obtained in normal sinus rhythm; only their direction is different. On the basis of our present experience, and using a method which appears capable of showing a circus movement if it be present, we are forced to conclude that our evidence points to the presence of an ectopic pacemaker, rather than a circus movement, in paroxysmal tachycardia.

## 14440

## The Excystation, Cultivation and Encystation of *Endamoeba coli.*

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Although much experimental work has been done on the cultivation of the intestinal amebæ, most of the time and effort has been spent on the pathogen, *Endamœba histolytica.* This organism has been cultured *in vitro* for two decades, but the closely related organism, *Endamœba coli,* has presented difficulties in cultivation. Many protozoologists have found that the latter species tends to die out after a few transfers and have concluded that it cannot be maintained in culture indefinitely. Dobell[1] says, *Endamœba coli* is indeed more difficult to cultivate properly than any other amœba living in man." From the present study it has been found that in the majority of cases cysts of *E. coli* from man will excyst and reproduce *in vitro* and can be maintained in culture for many months. The original strain which was planted from cysts in March, 1942, is still in cultivation after a period of 19 months and shows no indication that it will not continue to live and reproduce in the test tube.

Twenty-six strains of *Endamœba coli* were used for this series of culture experiments. Two were in the trophic and 24 in the cystic stage. All were from human material and none had any associated amebæ. All diagnoses were checked by members of the Department of Tropical Medicine, Tulane University. Examinations were made by the use of unstained, iodine-stained, and iron-hematoxylin-stained preparations.

*Method Used to Obtain Maximum Number of Organisms for Culture.* In order to obtain a maximum yield of cysts from a stool specimen, a modified combination of the zinc sulphate centrifugal floatation method (Faust *et al.*[2]) was used with the Lane[3] superimposed cover-glass technic. The specimen was thoroughly emulsified with tap water, 10 cc portions were strained through cheese cloth into Wassermann tubes and centrifugalized for 70 seconds at 2600 r.p.m. The supernatant fluid was poured off the tubes, the packed sediment broken up, washed and centrifugalized again. This process was repeated until the supernatant fluid became clear. Zinc sulphate of the specific gravity 1.180 was then added to

[1] Dobell, C., *Parasitol.*, 1936, **28**, 541.

[2] Faust, E. C., *et al.*, *Am. J. Trop. Med.*, 1938, **18**, 169.

[3] Lane, C., *Trans. R. Soc. Trop. Med. and Hyg.*, 1924, **18**, 278.

the brim. A square 22 mm cover glass was superimposed on the full tube and held in place by 4 metal fingers attached to the metal tube. The preparation was recentrifugalized, the cover-glass removed rapidly but carefully and the material which had collected thereon was washed off into a container with a small amount of physiological salt solution. After the cysts had been collected, they were washed 6 times in physiological salt solution in an effort to remove the zinc sulphate and the associated bacteria.

*Excystation.* Seven combinations of culture media were used at the beginning of the problem. Five of these utilized as a base coagulated whole egg slant; one, coagulated egg albumin; and the other, inspissated human serum. Various liquid overlays were tested, including Locke's serum solution, plain Locke's solution, Ringer's (without sugar) and Ringer's serum solution, egg-albumin Ringer's solution and liver extract broth, also a small quantity of starch. Locke's egg-serum medium plus starch was the only one in which excystation occurred. The initial pH value of this medium was 7.8-8.0 and the final pH was 6.8-7.0. The optimum temperature proved to be 37°C. In most cases the amebæ excysted within 72 hours, although in some instances questionable excystation was observed within 48 hours. Of the 24 strains of *Endamœba coli* that were tested, 15 excysted and were maintained in culture for varying lengths of time from 4 to 19 months. Four of these strains are still in culture. No strain that was started from trophozoites grew in subculture.

The failure of some of the strains to excyst in culture was thought to be due to one or more of several factors, namely, the freshness of the specimen used, the size of the cysts, the degree of the infection, the particular bacteria present or the occurrence of supernucleated cysts. It was found that excystation occurred most readily when the cysts were planted from 1 to 5 days after they were obtained from a freshly passed stool. The extremely large, including supernucleated, cysts would not excyst. The heavier the inoculum the greater was the chance of a positive culture. Finally, certain bacteria presumably must be present in the inoculum before excysted amebæ can be recovered.

Excystation in culture, as observed in iron-hematoxylin preparations, consisted of the massing of the protoplasm around the nuclei, and a general loss of staining capacity of the cyst. The multinucleated metacystic ameba which escaped from the cyst wall was a large trophic ameba containing 5 or more nuclei, some of which were apparently degenerate. The cytoplasm was still massed around the nuclei and one by one the amebulæ were split away from the metacyst. These amebulæ each contained a large nucleus with an eccentric karyosome and heavy plaques of chromatin at the periphery. A small amount of cytoplasm surrounded the nucleus. Starch granules and bacteria were readily ingested and mature trophozoites soon developed.

*Cultivation.* For maintaining cultures over long periods of time Locke's egg serum plus starch proved to be optimum. However, after excystation occurred the trophozoites were maintained for short periods of time in media utilizing the whole egg base and various liquid overlays such as plain Locke's solution, Ringer's solution with sugar, and 0.5% solution of liver extract plus sterile rice starch.

The optimum time for subculturing was 72 hours but it was not detrimental to prolong the time to 4 or 5 days. In some cases cultures were found positive after 10 days. The pH for optimum growth was found to be 7.0 to 7.4 at an incubation temperature of 37°C.

The technic for transfer of the cultures was a precise one. Utmost care was used to prevent the introduction of extraneous bacteria. To guard against such contamination, media and pipettes were sterilized before being used. Capillary pipettes were found to be very satisfactory for the transfer of positive material. The amebæ grow in the deposit of starch in the little pocket formed at the butt of the slanted egg base. In transferring, it is this material that is carried from one tube to the other.

When large numbers of organisms were needed the technic of cultivation was slightly altered. Erlenmeyer flasks of 125 and 250 cc volumes were used. The egg medium was poured into the flasks to a depth of one-half

of the height of the flask and to this 6 loopfuls of sterile rice starch were added. After having been well shaken the entire supernatant fluid from a positive 72-hour culture tube was added to a prepared 125-cc flask. After 72 hours most of the supernatant fluid from this flask was decanted off, leaving only a small amount of liquid and all of the sediment which lay on the solid egg base. Amebæ were to be found growing abundantly in this sediment, which was then well mixed and pipetted into the prepared 250-cc flask and incubated for 72 hours. This method resulted in the development of masses of amebæ which grow vigorously and abundantly.

The cultured trophozoites which have been studied had large nuclei each with a large eccentric karyosome and a nuclear membrane heavy with plaques of chromatin. The pseudopods were typically those of *Endamœba coli:* they were usually blunt, rounded, and granular, at no time hyaline. The cytoplasm was heavily granular, with many bacteria and starch inclusions, was often distinctly vacuolated and exhibited a very sluggish movement.

*Encystation.* Encystation in culture occurred 4 times spontaneously and was not produced at will. The medium in 3 of these instances was Locke's egg serum plus starch. In the fourth instance encystation occurred in cultures growing in a medium consisting of an egg base with a liquid overlay of a saline extract of the mucosa of the large bowel of man. This medium contained no starch. Subsequent experiments using the same media did not result in encystation. The pH value in each successful case was 6.4-6.6.

Working on the assumption that the possible cause of this encystation was the associated bacteria, the writer tried to isolate and identify the organisms present in the tubes where encystation occurred. The only ones found were *Escherichia coli communis* and an, as yet, unidentified species belonging to the *Colon-Aerogenes-Proteus group.* When these organisms, singly or together, were reintroduced into cultures containing *Endamœba coli* trophozoites, encystation did not occur.

*Summary.* Twenty-six strains of *Endamœba coli* were used for culture experiments, 2 strains in the trophic stage and 24 in the cystic stage. The former did not reproduce in subculture but 15 of the latter excysted and grew abundantly. Four of these are still being cultivated. A modified Locke's egg serum medium plus starch was the only one in which excystation occurred and proved to be the optimum one for continued growth of the organism. The medium was incubated at 37°, had an initial pH of 7.8-8.0 and a final pH of 6.8-7.0. Encystation occurred spontaneously but was never produced at will.

## 14441

## Studies on Guinea Pigs After Repeated Administration of Paraldehyde.

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Cervello[1] introduced paraldehyde as a hypnotic in animals and then on February 13, 1882, he ventured to try four 0.5 g doses of the drug on himself. The drug was introduced as a therapeutic agent by Morselli[2] at the Royal Asylum of Turin and within a few years, it had been employed by clinicians in both Europe and the United States. Paraldehyde gained a rather general use in most branches of medicine and especially in obstetrics and psychiatry.

Because of the wide acceptance of paraldehyde as a safe drug and because of the controversy as to whether it loses its efficacy on

[1] Cervello, V., *Arch. per le Scienze Med.*, 1882, **6**, 177.

[2] Morselli, Enrico, *Gaz. d. ospital*, 1883, **4**, 28.