TABLE I

The species of trypanosomes	The species of animal from which the trypanosome was isolated	Cultures	
		No. of days maintained *in vitro*	No. of subcultures made
Tr. americanum (*Tr. theileri*)	Cows (Near Ann Arbor, Mich.)	1,010	61
Tr. avium (*Tr. paddae*?)	*Passer domesticus* (English sparrow)	868	42
Tr. avium (*Tr. noctuae*)	Screech owl	883	46
Tr. cruzi	From guinea-pigs experimentally infected with intestinal contents of *Triatoma Geniculata*	619	34
Tr. duttoni	*Mus Musculus* ("Common house mice")	231	14
Tr. lewisi	*Rattus norwegicus* (Laboratory white rats)	1,224	56
Tr. lewisi	*Rattus norwegicus* (Rats)	224	12
Tr. melophagium	*Melophagus ovinus* (Sheep keds)	375	28
Tr. rotatorium	*Rana pipiens* (American frogs)	834	44

americanum, *Tr. cruzi* and *Tr. duttoni* resemble more closely the *B. typhosus* colonies. Although the colonies resembled bacterial colonies, in all the microscopical examinations they revealed a solid mass of flagellates with healthy protoplasm. As a rule the individuals in the colonies were somewhat round and pear-shaped, but quite active, and those in the water of condensation were more active and usually had the morphology of crithidia or herpetomonas.

Infectivity and Spontaneous Attenuation of Trypanosomes in vitro

A strain of *Trypanosoma lewisi* (No. 201) was isolated on November 22, 1930, and was kept *in vitro* until the time of writing (March 31, 1934). Although this culture was infective for rats during its first few months *in vitro*, at the end of two years it had completely lost this power. Normal young rats were splenectomized and several cultures were inoculated in these animals, but no infection was produced. A recently isolated strain of *Tr. lewisi* cultivated *in vitro* for four months is still capable of producing an infection in rats. As a rule *Tr. lewisi* during its first year of subcultivation *in vitro* is infective for rats, but after about a year it gradually begins to lose this power. It is a definite fact that *Tr. lewisi* when kept in culture for two years on N.N. media is completely innocuous for rats.[3,4]

The initial culture of *Tr. cruzi* was obtained on July 20, 1932. After maintaining it *in vitro* for 468 days, it was inoculated into *Mus musculus*, into hairless *P. m. gambelii* (American deer mice) and into a guinea-pig. All these animals contracted the infection, trypanosomes were demonstrable in scanty numbers in their peripheral circulation and were recultured. Thus this species of trypanosome is still infective after 586 days' cultivation *in vitro*.

Tr. duttoni was cultured *in vitro* on March 2, 1933; 135 and 229 days after initial cultivation it was still infective to *Mus musculus*.

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AN ARTIFICIAL SYMBIOSIS

In order to keep tissue cells *in vitro* alive and healthy it is the practise of tissue culturists to renew the medium at frequent intervals. Such a procedure is supposed to increase the supply of oxygen and decrease the concentration of harmful by-products of metabolism. The three most common methods are: (1) Cutting out a fragment of tissue from the old culture and transferring it to fresh medium (usually a mixture of embryonic or other tissue extract and plasma); or (2) bathing the clot containing the culture in physiological salt solution (Tyrode's solution) for part of an hour, adding a small quantity of new medium before resealing; or (3) continuous perfusion of the culture chamber with a renewable fluid. Now if oxygen and food materials could be delivered to the very doorway of the cells and the by-products of metabolism be carried away, much as is done by the capillary circulation in the intact animal, there should be an improvement in health of tissues *in vitro*. Like the capillary circulation, the interchange should be continuous and relatively rapid.

There are many animals (protozoans, coelenterates, flatworms, etc.) which can live without feeding, provided they harbor green algae. The animal lives in the light, dies in the dark. The animal obtains carbohydrates and oxygen from photosynthesis and has its carbon dioxide and nitrogenous wastes removed. The alga gets nitrogen compounds and carbon dioxide from animal respiration. The common, unicellular green alga, *Chlorella*, is frequently found thus living symbiotically. A sterile culture of *Chlorella* grows very well on an inorganic salt agar medium.

Cultures were made combining the green algae with embryonic chick connective tissue cells and macro-

[3] F. G. Novy, W. B. Perkins and R. Chambers, *Jour. Infect. Dis.*, 11: 411, 1912.
[4] A. C. Behrens, *Jour. Infect. Dis.*, 15: 24, 1914.

phages. Control cultures were made of algae alone and others of chick tissue alone. Other cultures were made with amphibian cells instead of chick cells. The medium consisted of embryonic chick extract, chicken plasma and Tyrode's solution.

There was a very marked effect on the alga. In cultures containing algae alone, there was slow growth; the individual cells gradually became pale and the chloroplast appeared greatly shrunken. In the cultures of algae with tissue, the plant cells grew abundantly until there were numerous colonies. There was a marked difference, even here, between the algae immediately around the tissue and those a considerable distance away from the tissue. This was particularly true in the slow-growing amphibian cultures. Thus in cultures of adult amphibian heart, the algae became very dense about the beating heart, but showed little growth over the control in the periphery of the drop of medium. Not only did the algae multiply more rapidly in the mixed cultures, but they were larger, greener and the chloroplast did not shrivel up as in cultures of algae alone. The tissue cells grew around many of the algae colonies and individual cells. Many colonies were thus embedded in the tissue, and these remained green and increased in size, even through subcultures. Macrophages and fibroblasts took up algal cells in great numbers. Some of the tissue cells could be seen with included algal cells in all stages of disintegration, indicating digestion. It appeared that in some cases, after a macrophage accumulated a number of algae, it died, and the algae became a free colony, traces of the animal cell still adhering.

Macrophages engulfed algae in great numbers, and digestion probably occurred. These algae-filled macrophages seldom became fatty, as is characteristic of macrophages after a few days in cultures of tissues alone. The fibroblasts were also unusually devoid of fat. At the same time that the cultures of tissues alone showed almost every cell gorged with fat droplets, the cells of the cultures with algae rarely had fat droplets. In a few cultures in which there were algae on only one side of the explant, only the cells farthest from the algae produced fat. In rate of growth, as determined by increase in surface area, the mixed cultures were far superior to the cultures of tissue alone. Further, mixed cultures of algae and tissues grew and remained in a healthy condition for at least twice as long as cultures of tissue or algae alone.

Although it has not yet been demonstrated that the plant cells really do use the nitrogenous wastes and carbon dioxide of tissue respiration in vitro, it is true that they live and grow better under these conditions in the presence of animal cells. Similarly, although we have not shown that the animal cells really use the oxygen and carbohydrates produced by photosynthesis of the algae, it is true that they appear to be much healthier as evidenced by marked absence of fat and increased rate of growth (as increase in surface area). It is possible that the mutual benefit may be due to a decrease in toxicity of the medium, such as a mutual hydrogen-ion adjustment or a change in the physical structure of the plasma clot. However, we believe that whatever the mechanism, this is a case of an artificial symbiosis.

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PURE CULTURES OF PARAMECIUM

IN September, 1933, Glaser and Coria[1] described a method for the culture of *Paramecium caudatum* free from living microorganisms. The medium employed was a liver extract broth, into each tube of which was placed aseptically a bit of fresh unheated rabbit kidney and a suspension of heat-killed yeast cells. The medium was quite simple in composition and, for any one accustomed to working aseptically, easy to prepare. In May, 1934, Hetherington[2] reported that he had been unable to cultivate *Paramecium caudatum* free from bacteria in the medium of Glaser and Coria. In fact he found the medium as prepared by him toxic for *Paramecium*. He concluded that "Glaser and Coria probably did not have sterile paramecia."

Glaser has supplied me with three presumably pure strains of *Paramecium caudatum* and three of *Paramecium multimicronucleatum* growing in his medium. He also supplied some of the sterile medium which I used for transplants of the cultures. The cultures were examined microscopically, unstained and stained by various methods, for the presence of bacteria, but none were found. Detached cilia and certain needle-like crystals were easily distinguished from bacterial cells. All the cultures were inoculated onto the surface of slants of meat infusion agar, infusion agar plus ascitic fluid and Sabouraud agar, which were incubated at 22°C. and 37° C. aerobically and anaerobically. They were also inoculated into the depths of deep tubes of infusion agar and infusion agar plus glucose and ascitic fluid incubated at 22° C. and 37° C. These media were examined grossly and microscopically at intervals for two weeks and showed no evidence of growth of any kind. In transplants of the *Paramecium* cultures into the medium supplied by Glaser no evidence of toxicity was noted. All the cultures grew, and actively motile paramecia were seen for as long as two months after inoculation of the media.

[1] *Jour. Parasit.*, 20: 33, 1933.
[2] SCIENCE, 79: 413, 1934.