pyridoxine deficient rats have been studied. In the laboratory of the Poultry Division, hundreds of pyridoxine deficient rats[2] were studied without the appearance of such epileptic-like fits. Recently, however, fits such as described[1] were observed. The fits occurred among pyridoxine deficient rats, each of which was fed 5 cc of water by stomach tube every 3 hours. After the second water feeding 5 out of 16 rats were observed to go into fits which lasted from a few seconds up to about 2 minutes. Others possibly went into fits but were not observed. Fits developed when the rats were startled by noise or were surprised by being suddenly picked up. Others were nervous but were not observed to go into convulsions. The rats had been on the pyridoxine deficient diet about 4 to 5 months.

Since there is a disturbance of water metabolism in human epilepsy[3] we repeated the experiment, but the fits did not recur. The rats were then exposed to the piercing sounds produced continuously by frequencies of 30 to 10,000 cycles after they had been given water by stomach tube, but the convulsions did not recur.

At about this time, fits were observed in pyridoxine deficient rats in the laboratory of the Vitab Corporation. They were few but in sufficient numbers to arouse interest. These rats were also given water by stomach tube without producing any fits.

After the rats were 6 to 8 months old, convulsions occurred spontaneously with increasing frequency. The convulsions were not alike, but they followed a definite pattern. They generally occurred when several rats were together in a can and frequently handled during the time when they were weighed or the vitamin doses fed. One rat in fits will generally set off other rats in convulsions. Without warning the rat will sometimes start convulsively forward in jerks similar to the hopping of a mechanical rat. Often the rat will paddle the air with its paws. The head is often jerked up into an upward tilt and the rats get up on their hind legs and paddle the air. Sometimes they get up so high on their hind legs that they lose their balance and tumble over backwards or on their sides. When paddling or fanning the air with their paws their ears will sometimes wiggle rapidly, appearing much like a fan without a handle. Sometimes their ears are pressed flat against the back of their necks. While rearing on their hind legs, they will often involuntarily hop in the air, sometimes jumping out of the can. The paddling of the air and the fanning of the ears seemed to be motivated from one source as though controllable by one switch. These involuntary hops gave the appearance of being motivated by a hidden spring in the rat resembling a hopping mechanical rat. Mild convulsions were generally limited to a few convulsive hops or a half minute's pawing of the air often accompanied by the wiggling of the ears. Severe convulsions lasted 2 to 3 minutes. A round drop of saliva sometimes involuntarily appeared at the mouth of the rat during a severe convulsion. After a severe convulsion, the rat would sit or lie perfectly still, sometimes holding the wire screen tightly in the front paws. During this period the rat seemed unconscious and did not react when touched. The only sign of life was the heaving in and out of the sides, the forward part of the rat's body slowly moving forward and backward in rhythm with the heaving in and out of the sides. Occasionally the rats lay on their sides with their legs outstretched as though dead. They came out of this coma-like condition without any apparent effort and scratched themselves behind the ears or washed their faces as though nothing had happened. Occasionally after they had regained consciousness they shook themselves, much like a dog just coming out of water.

Convulsions were never observed under $4\frac{1}{2}$ months, and then only rarely. When they reached the age of 6 to 8 months they occurred more frequently. Some rats had fits daily. The duration of the convulsions gradually increased. Out of 13 rats 7 months of age, 6 regularly had fits. Out of 3 rats 9 months of age receiving 5 micrograms daily of pyridoxine, one occasionally had a short mild convulsion.

We can, therefore, confirm the findings of Chick *et al.*[1] that fits will occur in pyridoxine deficient rats and we wish to suggest the possibility, remote though it may be, that disturbed water metabolism may in some way be involved in the production of these epileptic-like fits.

SAMUEL LEPKOVSKY
MYRTISE E. KRAUSE
POULTRY DIVISION, UNIVERSITY OF CALIFORNIA, BERKELEY
MILDRED K. DIMICK
THE VITAB CORPORATION, EMERYVILLE, CALIFORNIA

[2] S. Lepkovsky, *Jour. Biol. Chem.*, 124: 125, 1938.

[3] C. H. Best and N. B. Taylor, "The Physiological Basis of Medical Practice," p. 1460. Baltimore: Williams and Wilkins Company. 1940.

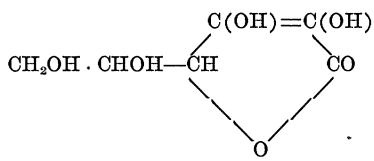
EFFECT OF l-ASCORBIC ACID ON THE ISOLATED FROG HEART[1]

SIMPLE unsaturated lactones like βγ-angelicalactone, αβ-angelicalactone and crotonolactone γ-acetic acid and its esters have a characteristic digitalis-like action upon the frog heart isolated according to the method of Straub. If administered in an adequate concentration and replaced continuously (by means of a special cannula) at a steady rate for a suitable length of time,

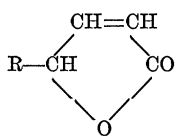
[1] Carried out under the auspices of the University Committee on Pharmacotherapy.

they initially bring about an increase in the amplitude of contraction and eventually lead to an irreversible systolic standstill.[2]

l-Ascorbic acid[3] (I) can be considered as an αβ-unsaturated lactone related in structure to the simple αβ-unsaturated lactones of type (II).



(I)



(II)

When administered into the cannula of the isolated frog heart at an initial concentration of 1:2,000 to 1:50,000 and replaced continuously at a rate of 2–2.5 cc per minute, l-ascorbic acid caused a systolic standstill within a period of from 2 to 5 hours. There was an increase in the rate of the heart. When the ventricle eventually stopped in systole, the auricle continued beating. The initial action of l-ascorbic acid was an increase in the amplitude of contraction. This was observed previously by Urban and Peugnet.[4] They also noted an increase in the diastolic "tonus" but were unable to reproduce this effect consistently.

Our preliminary work indicates that the reaction may be modified by the pH of the physiological salt solution. We have not yet studied how the systolic action is influenced by copper, which Peugnet[5] has found essential for the "beat-strengthening action" of l-ascorbic acid.

Our observations show for the first time that the reported "systolic" action upon the frog heart can be caused by an organic-physiological substance of known chemical structure occurring in the organism of the warm-blooded animal.

R. P. LINSTEAD
HARVARD UNIVERSITY, DEPARTMENT OF CHEMISTRY, CAMBRIDGE

O. KRAYER
HARVARD MEDICAL SCHOOL, BOSTON

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE STERILE CULTURE OF PARAMECIUM MULTIMICRONUCLEATA

DURING the last few years protozoologists have recognized more and more the need for sterile culture methods. When it is impossible to culture an organism in the absence of other living cells the rigid control of conditions so necessary in all experimental studies is not possible. Advances in methods of sterile culture of free-living forms offer new possibilities for similar studies on related pathogenic forms.

The establishment and culture of a strain of *Paramecium* that is bacteriologically sterile has frequently been attempted. The only successful cases that have been reported are those of Loefer[1] and Glaser and Coria,[2] in each of which the presence of other living cells was essential. We have established five strains of *P. multimicronucleata* in a yeast juice medium. These strains have been maintained for ten months through 18 transfers. Their sterility has been confirmed, so we wish to report the method used.

The paramecia were obtained from a live yeast stock culture which has been growing in our laboratory since 1937. The organisms were sterilized by a combination of the Claff[3] migration-dilution technique and the Parpart[4] washing technique.

Many kinds of culture media were tried, including peptone media, yeast extracts, yeast autolysates and unheated disintegrated bacteria, all with and without added growth factors. Success was attained only with the pressed yeast juice of Buchner.[5]

The pressed yeast juice is made as follows. One pound of Fleischmann's baker's yeast is ground with an equal weight of washed, fine, white sand. This is then mixed with 125 gms of diatomaceous earth and reground to a sticky dough. This dough is wrapped in two layers of birdseye diaper cloth, which has been dampened with distilled water, and then placed in a 4½″ perforated screw press cylinder. Pressure, by a manually operated screw press,[6] is applied as rapidly as possible.

[2] O. Krayer, R. Mendez, E. Moisset de Espanés and R. P. Linstead, *Jour. Pharmacol. and Exper. Therap.* In press.

[3] l-Ascorbic acid was donated by Hoffmann-La Roche, Inc., Nutley, N. J., and by Merck and Company, Inc., Rahway, N. J.

[1] J. B. Loefer, *Jour. Exp. Zool.*, 72: 387–407, 1936.

[2] R. W. Glaser and N. A. Coria, *Am. Jour. Hyg.*, 21: 111–121, 1935.

[4] F. Urban and H. B. Peugnet, *Am. Jour. Physiol.*, 123: 207, 1938.

[5] H. B. Peugnet, SCIENCE, 90: 162, 1939.

[3] C. L. Claff, *Physiol. Zool.*, 13: 334–341, 1940.

[4] A. K. Parpart, *Biol. Bull.*, 55: 113–120, 1928.

[5] E. Buchner, H. Buchner and M. Hahn, "Die Zymasegärung," pp. 58–66. Münschen and Berlin: R. Oldenbourg. 1903.

[6] A "ten-ton" press made by the Atlas Press Company of Kalamazoo, Michigan, is used.