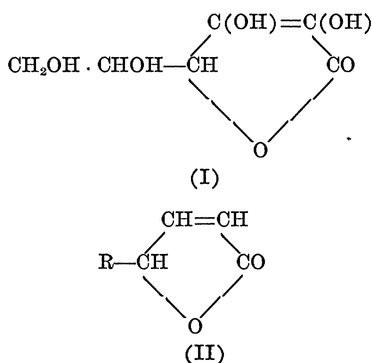
they initially bring about an increase in the amplitude of contraction and eventually lead to an irreversible systolic standstill.[2]

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).



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.

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

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

[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.

[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.

This procedure yields about 120 cc of yeast juice which is caught in an ice-packed flask and then stored in a refrigerator over night. It is then forced through a Seitz bacteriological filter by pressure. This juice is stored in a refrigerator at all times and is used only after complete sterility tests have been made.

For cultures, 5 cc portions of triple distilled water are sterilized in 18 mm Pyrex culture tubes, and, after cooling, 0.5 cc of the yeast juice is added to each aseptically. These are then ready for inoculation. Tests with a wide range of dilutions indicated that 1:10 is near the optimal concentration.

In all, sixty-one cultures of yeast juice were inoculated with sterile paramecia. From these, five strains were established and successfully maintained. Transfers are made at about 14-day intervals. Sterility tests have been made regularly at every transfer. Most of the common test media have been tried at various times in these tests. The standard testing media now employed consist of: 0.5 per cent. Difco yeast extract plus 0.5 per cent. dextrose; 0.03 per cent. beef extract; Difco nutrient agar plus 0.5 per cent. dextrose; and Brewer's thioglycollate anaerobic medium. Dr. C. B. van Niel,[7] of the Hopkins Marine Station, has examined several of these cultures and has confirmed their sterility.

So far it has not been possible to obtain in these sterile cultures a rate of growth equal to the highest rates obtainable in pure-mixed cultures of *Paramecium* and single strains of bacteria. The best fission rate obtained in these cultures to date is approximately 0.5 divisions per day. Fission rates of 1.0–2.0 per day have been reported for pure-mixed cultures. Heated yeast juice will not support the growth of *Paramecium*. Some factor or factors necessary for their growth is apparently destroyed by heat. This is in line with other observations which have been made on *Paramecium* and other ciliates. Most of the normally holozoic forms that have been studied will not grow when furnished with heat-killed organisms or with the ordinary heat-treated culture media as their only source of food.

W. H. JOHNSON
E. G. S. BAKER

DEPARTMENT OF BIOLOGY,
STANFORD UNIVERSITY

THE SIGNIFICANCE OF FIBRINOLYSIS IN THE MECHANISM OF COAGULATION OF BLOOD[1]

WHEN recalcified dog's plasma is shaken briefly with chloroform and allowed to stand 24 hours, and the chloroform thereafter removed, the serum so obtained shows marked fibrinolytic properties. The properties of this so-called chloroform serum will be reported elsewhere.[2]

The precipitate of globulin obtained from this chloroform serum by isoelectric precipitation at pH 6 is soluble in isotonic saline solution. This solution of globulin possesses marked fibrinolytic properties.

The addition of small amounts to a solution of fibrinogen produces no clot but complete lysis of the fibrinogen. In the presence of prothrombin, however, a clot forms which may undergo fibrinolysis. The addition of the globulin solution to oxalated plasma results in coagulation sometimes followed by fibrinolysis. The addition of the globulin solution to prothrombin results in the formation of thrombin in the absence of calcium, thromboplastin or formed blood elements. In its action on blood plasma and fibrinogen the globulin solution resembles the action of trypsin as reported by Eagle and Harris.[3]

The active globulin solution may also be prepared by dialysis of chloroform serum against running water. In this preparation the active fibrinolytic substance is associated with the euglobulin precipitate. The solution in isotonic saline of this precipitate behaves as does the isotonic saline solution of the acid precipitated globulin.

It is to be noted that the methods of precipitation and dialysis are similar to those used in the preparation of "globulin substance" by Patek and Taylor.[4]

HENRY J. TAGNON[5]

[7] We wish to express our appreciation to Dr. C. B. van Niel for first suggesting yeast juice as a possible medium, and for making sterility tests of our cultures.

[1] From the New York Hospital and the Department of Medicine, Cornell University Medical College, New York, N. Y.

[2] Henry J. Tagnon, *Jour. of Lab. Clin. Med.*, in press.

[3] H. Eagle and T. N. Harris, *Jour. Gen. Phys.*, 20: 543, 1937.

[4] A. J. Patek, Jr., and F. H. L. Taylor, *Jour. Clin. Invest.*, 16: 113, 1937.

[5] Graduate fellow of the Belgian American Educational Foundation.

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