using a spray solution stronger than 10 p.p.m.; (2) applying the spray at an earlier date with reference to fruit maturity; and (3) combining the 2,4 dichlorophenoxyacetic acid with Carbowax and with naphthaleneacetic acid, or with both.

L. P. BATJER
P. C. MARTH

AGRICULTURAL RESEARCH ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE,
BELTSVILLE, MD.

INFECTIOUS MYXOMATOSIS IN MALNOURISHED RABBITS

THOMPSON[1] and Parker[2] have demonstrated that infectious myxomatosis is modified in rabbits exposed to high external temperatures. In preliminary attempts to determine whether or not chemically induced fevers have a similar modifying action, we studied the action of dinitrophenol in 12 rabbits. Six of these rabbits were injected with myxoma virus and sustained at fever temperatures by the subcutaneous injection of alpha dinitrophenol (20 mg/Kg of a 3 per cent. sodium salt) twice daily at 12-hour intervals. Two of these rabbits (as seen in Table 1) showed a delayed and modified infection. Since these two rabbits had a distinct loss of weight we decided to investigate further the effect of malnutrition on infectious myxomatosis.

Twelve rabbits were kept on minimal amounts (5 to 20 grams) of stock rations for a period of 10 days prior to the injections of virus and during the course of the disease, except where premature death was expected. Myxoma virus was titrated intradermally[3] and temperatures, weights, tumor measurements and clinical signs were recorded daily. Thirteen tumor biopsies were made serially on starved and well-fed rabbits and examined microscopically to determine the progressive pathology.

Microscopic study of the tumor biopsies revealed that the process of tumor formation was retarded in the malnourished rabbits. Table 1 presents data on 9 malnourished rabbits; 3 others died before any significant observations could be made. Tumors in the malnourished rabbits were delayed in appearance, and were definitely smaller than those in the controls in this series or in any of the well-fed animals in our previous study.[3] In one malnourished rabbit only a single minimal lesion appeared in one of the 16 injected sites.

TABLE 1

INFECTIOUS MYXOMATOSIS IN MALNOURISHED RABBITS

Number of rabbits	Treatment	Weight loss in per cent.	Days after virus injection: Lacrimal discharge	Secondary lesions	Death	Gross pathology of tumors*
1	Malnourished	16	0	0	7	+
1	"	22	8	0	13	+
3	"	23–26	7–9	0	10–11	+++
1	"	28	8	8	11	++
1	"	29	0	0	8	+
1	"	30	8	0	10	+
1	"	34	0	0	8	+
4	Dinitrophenol	0	6–8	6–9	9–11	++++
1	"	10	9	0	9	+++
1	"	25	0	0	7	+
7	None	0	6–8	5–8	8–13	++++

* ++++ = typical, large, cyanotic, raised; +++ = delayed, typical but smaller; ++ = delayed, only slightly raised; + = small, not raised or edematous.

Strict comparisons of the 50 per cent. end-points could not be made because of the atypical appearance of many of the dermal lesions in the starved rabbits. The delay in the appearance of lacrimal discharges—or the complete absence of such—and the absence of secondary lesions in 8 of 9 rabbits suggests that there was less generalization of the virus than in the controls. The known mortality rate of about 100 per cent. and the duration of the infection were not modified. It should be noted that 4 of the starved rabbits died showing an atypical clinical picture of infectious myxomatosis.

RALPH B. HOULIHAN

UNIVERSITY OF VIRGINIA
SCHOOL OF MEDICINE

W. ANSELL DERRICK

CITY LABORATORY, KINGSTON, N. Y.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS FOR GROWING MICROORGANISMS ON A FLOWING MEDIUM[1]

THE work of Shwartzman[2] describing the effect of Cellophane on penicillin production prompts us to publish the description of an apparatus used for growing microorganisms on cellulose tubing. We similarly used cellulose to increase the surface-volume ratio, but also to make a pathway through the growing fungus for the medium to pass into and out of the culture.

The apparatus (Fig. 1) consists of 50 feet of quarter-inch cellulose tubing[3] wound in a coil on

[1] R. L. Thompson, *Jour. Infect. Dis.*, 62: 307, 1938.
[2] R. F. Parker and R. L. Thompson, *Jour. Exper. Med.*, 75: 567, 1942.
[3] R. B. Houlihan and G. McL. Lawson, *Jour. Infect. Dis.* (in press).
[1] Journal Article No. 746 (n.s.) from the Michigan Agricultural Experiment Station.

[2] Gregory Shwartzman, SCIENCE, 100: 390, 1944.
[3] Blood transfusion tubing manufactured by Visking Corporation, Chicago, Illinois.

supports and suspended in a large Pyrex glass cylinder. The end of the tubing passes out through an aluminum cover held on the cylinder by means of metal strips passing underneath. Glass tubes are attached to the cellulose tubing where it passes through rubber stoppers in the aluminum top. This

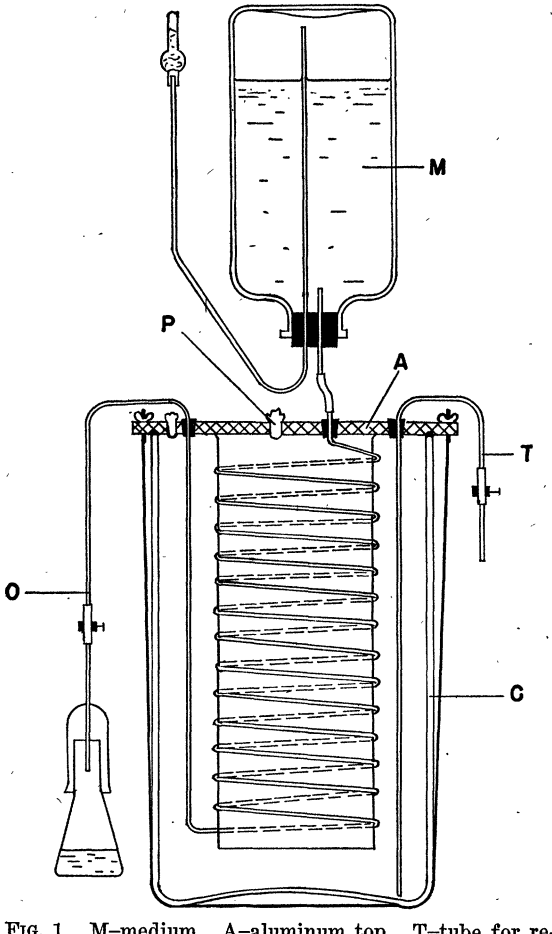

FIG. 1. M–medium. A–aluminum top. T–tube for removing seepage liquid. O–outlet tube. P–cotton plug. C-glass cylinder.

part of the apparatus is covered with a cloth hood, the outlets wrapped and sterilized in the autoclave.

A three-gallon bottle of sterile medium was prepared with an outlet assembly. It was then attached by a rubber tube to the cellulose tube inlet and supported so the medium would flow down into the cellulose tubing. The outside of the tubing was sprayed with a suspension of spores by reaching through the extra holes in the top. All necessary precautions were taken to prevent contamination.

A special atomizer was constructed by increasing the length of the two outlet pipes of a common DeVilbiss atomizer to 12 inches. A small Erlenmeyer flask was used in place of the regular fluid container. Sterile air was obtained by forcing air through water and then through a column of sterile cotton. The whole atomizer was constructed so that it could be autoclaved.

After inoculating with *Penicillium notatum*, the fungus grew readily and in two or three days the tubing was covered with mycelium. Usually the medium in the tubing was changed each day by draining through the outlet tube. The fungus continued rapid development and was covered with spores on the fourth or fifth day. When growth was well established, the medium was allowed to drop from the outlet tube at varying rates, usually about a liter per day. On the sixth to eighth day after inoculation, depending upon conditions, penicillin could be detected in the run-off medium. The penicillin titre increased within a few days so that it equaled or surpassed that of a flask culture. Unfortunately, the experiment could not be carried past the sixteenth day because leaks developed in the cellulose tubing owing to the action of the fungus.

An attempt was made to overcome the breakdown of the tubing by securing a thicker tube of cellulose and by the use of other materials. Thicker tubes of small diameter were not available. Cellulose nitrate films were found to be much more resistant to the fungus action. Attempts to coat the cellulose tubing with a cellulose nitrate film, either inside or outside, failed to give good results. Porcelain tubes with and without a coating of cellulose nitrate have so far failed to give the desired results, although they have overcome the problem of disintegration of the tubing.

Bacillus brevis was grown in this apparatus for three weeks without any apparent breakdown of the cellulose tubing.

The authors believe the apparatus as described can become a useful laboratory tool for studying certain organisms and that if a better tubing can be secured, it may be adaptable to the production of antibiotics and toxins.

R. W. LEWIS
E. H. LUCAS

MICHIGAN STATE COLLEGE

THE ESTIMATION OF PENICILLIN IN BODY FLUIDS

DURING the course of numerous investigations requiring the determination of penicillin activity in various body fluids, the need for a simple method of estimating penicillin levels of a lower order than that readily measured with accuracy by the agar cup plate method became apparent.

A 4-hour turbidimetric assay employing *Staphylococcus aureus* was investigated but was found of little value with blood serums because of an apparent stimulation of the microorganisms by the presence