functions to perform in addition to serving as a building block for body proteins. Although the changes in tryptophane deficiency are not identical with those seen in other conditions causing epitheliodystrophic cataracts, the similarity of the pathological picture is striking and suggests that some common metabolic path is interrupted in these disturbances.

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A VIRUS INACTIVATOR FROM YEAST

THE ability of certain bacteria to inactivate tobacco mosaic virus was reported by Mulvania.[1] Johnson and Hoggan[2] found that a number of fungi as well as bacteria could cause inactivation of tobacco mosaic virus and suggested that this inactivation was most likely due to the utilization of the virus constituents by these organisms. The high rate of inactivation by *Aerobacter aerogenes* and *Aspergillus niger* later led Johnson[3] to suspect that a virus inhibitor was produced by these organisms. Broth cultures of the organisms were found to contain a growth product (or products) capable of inactivating several plant viruses *in vitro* and in dried-leaf tissue. Efforts to separate the active substance from the culture medium yielded discouraging results.[4]

Johnson and Hoggan[2] grew a yeast, *Saccharomyces sp.*, in a medium containing filtered tobacco mosaic virus and broth for 8 days, but could not detect a reduction in the concentration of virus due to the action of the yeast in this mixture. Extraction of an inhibitor from the yeast was not attempted by these workers.

In the work reported below a virus inactivator has been extracted from yeast by autolysis and by autoclaving. A simple method for extracting this substance is as follows: one kilo of frozen baker's or brewer's yeast cake is mixed with 4 liters of distilled water and autoclaved for 30 minutes at 15 pounds pressure. The autoclaved material is filtered through a pad of Celite No. 505. The pale yellow, somewhat opalescent filtrate is treated with two volumes of acetone or alcohol and the resulting bulky, white precipitate is separated from the liquid by centrifugation. The precipitate is dissolved in a volume of distilled water equal to that of the original filtrate. The precipitation and solution in water may be repeated several times, but a small amount of an electrolyte such as NaCl must be added to effect complete precipitation. This partially purified substance was used for much of the preliminary work. Later, for more exact work, the inactivator was further purified by clearing with safranine and neutral lead acetate, followed by heating in 2N HCl. In more detail, 33.3 cc of a 1 per cent. solution of safranin per liter of inactivator were used and the neutral lead acetate (saturated) was added dropwise until no further precipitation took place. Potassium oxalate and $CaCl_2$ were used to free the solution of lead and oxalate, respectively. After precipitating with acetone the supernatant liquid was discarded, the precipitate dissolved in 2N HCl and heated on a boiling water bath for ½ hour. Six to seven volumes of acetone were required to effect the final precipitation. The white precipitate was dried for several days in a desiccator, then ground into a powder before weighing.

TABLE 1

THE INACTIVATION OF PURIFIED TOBACCO MOSAIC VIRUS BY DIFFERENT CONCENTRATIONS OF A PURIFIED INACTIVATOR FROM YEAST

Milligrams of inactivator per 100 cc of a suspension containing 5 mg of virus	Local lesions on 20 half leaves		Per cent. of virus remaining active	Per cent. of virus inactivated
	Treated virus	Untreated virus		
0.303	493	1520	32.3	67.8
0.625	209	1359	14.8	85.2
1.25	164	1614	10.05	89.95
2.50	44	1398	3.14	98.86
5.00	22	1547	1.42	98.58

Each step in the purification process and the effect of different treatments were followed by mixing a solution of the test material with a solution of purified virus. The changes in virus concentration brought about by the inactivator were measured by inoculating half leaves of *Nicotiana glutinosa*. In all cases, one half of each of 20 leaves was inoculated with each treated sample and the other halves with corresponding controls. The ultracentrifugally purified tobacco mosaic virus used in these experiments was kindly supplied by Dr. W. M. Stanley, of the Rockefeller Institute for Medical Research, at Princeton, N. J.

The reaction between the inactivator and the virus is rapid. The results of a typical experiment tabulated above show that with each doubling of inactivator concentration a halving of active virus concentration takes place. This strongly suggests a chemical reaction between one unit of inactivator and one unit of virus rather than an adsorption phenomenon. Furthermore, the inactivator does not combine with heat-denatured virus. The virus-inactivator combination can be broken if the mixture is heated to 99° C. for 10 minutes.

Scions of rose, peach and pear taken from virus-

[1] M. Mulvania, *Phytopath.*, 16: 853–871, 1926.
[2] James Johnson and Isme A. Hoggan, *Phytopath.*, 27: 1014–1027, 1937.
[3] James Johnson, SCIENCE, 88: 552–553, 1938.
[4] James Johnson, *Phytopath.*, 31: 679–701, 1941.

infected parent plants were placed in a solution of the inactivator for several days and later grafted on healthy stock. This treatment did not prevent the appearance of typical symptoms of each disease. Sets of detached leaves from mosaic and healthy plants were placed with their petioles in a solution of the inactivator and in distilled water for 9 days. A study of different combinations of juices from these leaves showed that although the inactivator had entered the leaves evidence for its entrance into the living cells and the destruction of the virus therein was not conclusive.

The activity of the substance is destroyed by heating with 1N NaOH on a boiling water bath but is unaffected by 2N HCl under the same conditions. In some cases an increase in activity was detected after the acid treatment. Treatment with trypsin or emulsin does not impair its activity. The usual protein tests are negative. Microchemical analysis of the purified substance gave: N—negligible, C—39.70 per cent., H—5.85 per cent., S—0 per cent., Chlorides—1.40 per cent., and ash—negligible. Fehling's solution is not reduced, but a strongly positive Molisch's alpha-napthol test is shown. On the basis of this test and the ratio of C to H it is suggested that the substance is a polysaccharide.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW PETRI DISH COVER AND TECHNIQUE FOR USE IN THE CULTIVATION OF ANAEROBES AND MICRO-AEROPHILES

THIS Petri dish cover, which has been designed to work in combination with a solid medium containing a reducing agent, makes possible the surface cultivation of anaerobes and micro-aerophiles without the use of anaerobe jars, petrolatum seals or chemicals other than those included in the medium itself.

Any good infusion agar containing a satisfactory reducing agent is poured into the usual Petri dish and allowed to harden. Either a pour or streak plate may be made. After the agar has solidified, the Petri dish cover is replaced by the anaerobic lid (Fig. 1), which

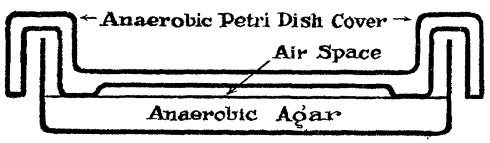

FIG. 1. Cross section showing anaerobic Petri dish cover in use.

is so designed that it touches the agar at the periphery and results in trapping a small amount of air over the surface of the agar. The reducing agent in the medium uses up the oxygen in this small amount of air and an anaerobic condition exists. The glass rim on the lid forms a seal with the moist solidified agar, and no other seal is necessary. If 1 cc of 1:500 methylene blue is added to each liter of agar to act as an indicator, the reduced center of the media in the dish becomes colorless, while the oxygenated periphery for about 5 mm remains blue.

A tentative formula for a suitable agar is as follows:

Infusion agar or blood agar base (containing 1.5 to 2 per cent. agar)	1,000 cc
Sodium Thioglycollate[1]	2 grams
Dextrose	10 grams
Methylene Blue 1 cc of 1:500 solution	
pH 7.5	

This agar should be distributed in about 40 cc amounts if 100 × 15 mm Petri dishes are used and 25 cc amounts if 100 × 10 mm dishes are used. The 40 cc dishes are more satisfactory and may be incubated longer without drying out. The depth of agar in the dish must be sufficient so that the rim of the anaerobic cover rests on the surface of the agar and not on the Petri dish at any point.

We have found that Cl. tetani, Cl. novyi, Cl. septique and Cl. welchii give good surface colonies in 48 to 72 hours and that the plates may be incubated several days longer without drying out. In most cases the growth was much better than that obtained with the same culture in an anaerobe jar. If an unglazed procelain top is used in pouring the plates, better isolation of surface colonies will be obtained. To facilitate opening the dish, the cover should be turned slightly to break the agar seal.

This technique may be used with the usual agar for obtaining partially anaerobic conditions for the cultivation of micro-aerophiles.

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CONTRIBUTION TO THE STEREOCHEMISTRY OF DIPHENYLPOLYENES

IN the series of the diphenylpolyenes, $C_6H_5 \cdot (CH=CH)_n \cdot C_6H_5$, which have been studied especially in the important investigations by Kuhn and Winter-

[1] One gram of sodium formaldehyde sulfoxylate and two grams of sodium thioglycollate seem to give a much quicker reduction.