ously contained large amounts of non-viral lung material. The procedure which was finally adopted and which led to a highly active product was one which combined the methods of adsorption on and elution from chicken red cells[12,13] and differential centrifugation. Four week-old mice from the colony of the Department of Animal and Plant Pathology of the institute were inoculated in a special chamber with an extremely fine spray of a 1 per cent. suspension of infected mouse lung[14] in 0.1 M phosphate buffer at pH 7. After about 72 hours, the mice were placed under deep ether anesthesia and their lungs were perfused with 0.85 per cent. saline and removed. The lungs were ground in 9 times their weight of 0.1 M phosphate buffer at pH 7 in a Waring Blendor, and the resulting suspension was angled in a low-speed centrifuge to remove gross particles. Such clarified 10 per cent. suspensions were accumulated and stored at about −70° over the course of 3 months until somewhat more than a liter had been obtained. The frozen material was then thawed, mixed thoroughly, and centrifuged to remove insoluble matter. The supernatant liquid possessed 113 standard CCA units[15] per ml and 50 per cent. infectivity endpoints in mice[16] and in chick embryos[17] at $10^{-6.6}$ ml and $10^{-8.7}$ ml, respectively. To 1,380 ml of cold clarified suspension were added 14 ml of chicken red cells. After standing overnight, the red cells were removed by centrifugation. The virus was then eluted from the red cells by incubation at 37° for 90 minutes in 200 ml of 0.1 M phosphate buffer at pH 7 and the resulting product was freed from blood elements by 2 cycles of differential centrifugation. This process yielded a product possessing more than 130 times the CCA activity of the starting material on a mg of nitrogen basis, but the value, 11,200, was still low compared to the CCA activity of chick embryo virus. Hence the adsorption and elution process was repeated followed by 2 additional cycles of differential centrifugation. The final product possessed about 28,000 CCA units per mg of nitrogen, a value which compares favorably with that of PR8 virus preparations obtained from allantoic fluid and which is about 17 times that of the best preparations obtained from mouse lungs by centrifugation alone. In so far as determined, the physical, chemical and biological properties of the purified material obtained from infected mouse lungs are indistinguishable from those of purified preparations of PR8 virus obtained from allantoic fluid. The preparation was highly infectious for both mice and embryos and gave 50 per cent. infectivity endpoints at 10^{-11} and 10^{-13} grams of nitrogen in mice and in chick embryos, respectively. In contrast to the bulk of material in preparations obtained from mouse lungs by centrifugation alone, the product obtained by the combined methods was soluble in 0.1 M phosphate buffer at pH 7 and gave a good boundary in the analytical ultracentrifuge. The sedimentation constant corrected for the viscosity of the preparation was 683S.[8] Electron micrographs revealed approximately spherical particles which were indistinguishable from those of the virus produced in chick embryos. The material was isoelectric at pH 5.4, contained about 10 per cent. of nitrogen and 7 per cent. of carbohydrate and precipitated strongly with antiserum to PR8 virus grown in chick embryos. As will be described in greater detail elsewhere, it is believed that a preparation of PR8 influenza virus has been obtained from infected mouse lungs that possesses essentially the same physical, chemical and biological properties as the virus preparations obtained from the allantoic fluid of chick embryos infected with PR8 influenza virus. The facts that the 2 hosts from which highly active preparations of virus have now been obtained are of such decidedly different nature and the sites of infection are so dissimilar, add to the significance which the findings have regarding the nature of viruses which infect animals and the use of heterologous sources of virus in the production of prophylactic vaccines.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

STERILITY TEST FOR PENICILLIN EMPLOYING CYSTEINE FOR INACTIVATION

CAVALLITO and Bailey[1] recently noted that cysteine was capable of readily inactivating a number of antibiotics including penicillin. This fact might be used to advantage in testing for the sterility of penicillin preparations.

For a preliminary test of the method, a penicillin solution containing 1,830 International Units and 10

1 C. J. Cavallito and J. H. Bailey, SCIENCE, 100: 390, 1944.

mg of $NaHCO_3$ per ml and a cysteine solution containing 2 per cent. cysteine hydrochloride and 2 per cent. $NaHCO_3$ were prepared. Each solution was sterilized by Seitz filtration. To 6 ml of the sterile penicillin solution was added 3 ml of the sterile cysteine solution. This resulted in a penicillin to cysteine hydrochloride ratio of 183 I.U. to 1 mg. This mixture was incubated for 1 hour at 37° C aseptically, after which time a cylinder plate assay against *Staphylococcus aureus* showed no activity.

The incubated solution was immediately tested for its ability to inhibit the growth of *Staphylococcus aureus* A.T.C.C. No. 6538, *Bacillus subtilis* N.R.R.L. No. 558, and the anaerobe, *Clostridium botulinum* Type A, A.T.C.C. No. 7948, in Brewer's fluid thioglycollate medium.[2] Also studied was the possibility of including cysteine in Brewer's medium so that the penicillin solution need merely be added to the sterility test medium for both inactivation and the sterility test: The experimental data are presented in Table 1.

TABLE 1

GROWTH RESPONSE OF SEVERAL ORGANISMS TO PENICILLIN AND CYSTEINE SOLUTIONS ADDED TO BREWER'S THIOGLYCOLLATE MEDIUM*

Organism	Penicillin solution,	Cysteine solution,	Penicillin-cysteine solution,	Day showing growth			
				1	2	3	7
	ml	ml	ml				
None	1	0	0	–	–	–	–
"	0	1	0	–	–	–	–
Staph. aureus	1	0	0	–	–	–	–
" "	0	0	1	+	+	+	+
" "	1	1	0	+	+	+	+
B. subtilis	1	0	0	–	–	–	–
" "	0	0	1	+	+	+	+
" "	1	1	0	+	+	+	+
Cl. botulinum	1	0	0	–	–	–	–
" "	0	0	1	+	+	+	+
" "	1	1	0	+	+	+	+

* Test medium consisted of 15 ml of Brewer's thioglycollate medium in 18 × 250 mm tubes.

Further studies were made in which both Brewer's fluid thioglycollate medium (Table 2) and this medium containing an added 0.2 per cent. cysteine hydrochloride (Table 3) were employed. These solutions were sterilized by autoclaving. The penicillin sodium solution for these studies contained 8,350 I.U. per ml. The cysteine solution was prepared from a 2 per cent. cysteine hydrochloride solution neutralized to pH 7 with $NaHCO_3$. These solutions were sterilized by Seitz filtration. The penicillin-cysteine solution was prepared by mixing 6 ml of the penicillin solution with 3 ml of the cysteine solution aseptically. This mixture was incubated at 37° C for 1 hour before use. The penicillin to cysteine hydrochloride ratio was about 835 I.U. to 1 mg. Bacteriological test data are

[2] Difco Laboratories, Inc., Detroit, Michigan.

TABLE 2

GROWTH RESPONSE OF SEVERAL ORGANISMS TO PENICILLIN AND CYSTEINE SOLUTIONS ADDED TO BREWER'S THIOGLYCOLLATE MEDIUM*

Organism	Penicillin solution,	Cysteine solution,	Penicillin-cysteine solution,	Penicillin : Cysteine ratio	Day showing growth			
					1	4	6	7
	ml	ml	ml	I.U. : mg				
None	0	0	0	....	–	–	–	–
"	1	0	0	....	–	–	–	–
"	0	1	0	....	–	–	–	–
"	0	0	1	....	–	–	–	–
Staph. aureus	1	0	0	....	–	–	–	–
" "	0	0	1.2	835 : 1	–	+	+	+
" "	1	1	0	418 : 1	+	+	+	+
B. subtilis	1	0	0	....	–	–	–	–
" "	0	0	1.2	835 : 1	–	–	–	–
" "	1	1	0	418 : 1	–	+	+	+
Cl. botulinum	1	0	0	....	–	–	–	–
" "	0	0	1.2	835 : 1	–	+	+	+
" "	1	1	0	418 : 1	+	+	+	+

* Test medium as for Table 1.

modified Brewer's medium containing cysteine was employed.

With the ratio of penicillin to cysteine hydrochloride at 835 I.U. to 1 mg, the organisms which grew

TABLE 3

INACTIVATION OF PENICILLIN AND SUPPORT OF BACTERIAL GROWTH BY CYSTEINE CONTAINING STERILITY TEST MEDIUM*

Test organism	Penicillin solution,	Penicillin : cysteine ratio,	Day showing growth			
			1	4	6	7
	ml	I.U. : mg				
None	0	....	–	–	–	–
Staph. aureus	1	278 : 1	–	+	+	+
B. subtilis	1	278 : 1	–	+	+	+
Cl. botulinum	1	278 : 1	+	+	+	+

* Test medium consisted of 15 ml of Brewer's fluid thioglycollate medium containing 0.2 per cent. cysteine hydrochloride in 18 × 250 mm tubes.

given in Table 2, where Brewer's thioglycollate medium was employed, and in Table 3, where the were delayed in starting and *Bacillus subtilis* showed no activity in 7 days at the incubation temperature of 37° C. At the ratios of 418 and 278 I.U. of penicillin to 1 mg of cysteine hydrochloride all three test organisms proliferated well. In Table 3 it is evident that the modified Brewer's medium containing cysteine satisfactorily inactivated the added penicillin and supported the growth of all three test organisms.

Brewer's fluid thioglycollate medium employed in these studies was made from a commercial dehydrated powdered product.[2] Although thioglycollate was present in this medium, it is quite possible that the inclusion of cysteine would allow the elimination of the thioglycollate from the medium.

It is evident that should this method prove to be satisfactory for the sterility test, its simplicity would

be quite advantageous over the current sterility test methods.

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A METHOD FOR THE CULTURING OF EXCISED, IMMATURE CORN EMBRYOS IN VITRO[1]

THE culturing of excised, immature plant embryos *in vitro* is a very useful technique for the propagation of otherwise abortive embryos often encountered in hybridization work.[2, 3, 4] A method, adapted from that developed by van Overbeek *et al.*[4, 5] for *Datura*, was found applicable to the culturing of excised corn embryos[6] 8 to 10 days after pollination and 0.3 to 3 mm in length.

The ear of corn was carefully husked, dipped into 70 per cent. ethanol and washed with sterile distilled water. Then the corn kernels were cut from the cob aseptically into a sterile Petri dish. About 6 kernels were held between 2 microscope slides, which had been previously dipped in 70 per cent. ethanol and flamed, and cut with a sterile, thin razor blade. The embryos were lifted from the endosperm by means of sterile, spear-shaped dissecting needles and placed on the surface of the sterile culture medium contained in half-dram shell vials, fitted with cotton plugs. More rapid growth was obtained when the embryos were placed on the surface of the agar medium than when submerged. The vials were then incubated at 30° C.

Unlike *Datura*, 10-day-old proembryos over 0.3 mm in length did not require the addition of coconut milk to the medium described by van Overbeek *et al.*[4] for continued growth. For embryos smaller between 0.3 and 1 mm in length, however, a higher sucrose concentration of 5 per cent. must be used. Otherwise, no growth will result. Excised 10-day-old embryos below 0.25 mm in length did not grow even in the presence of coconut milk. Also, the growth of the embryos, particularly the epicotyl, was accelerated by the addition of 1.5 gm of asparagine per liter of culture medium. Thus, 10-day-old embryos with an initial length of 2 mm grew in the van Overbeek basic medium to a length of 13 mm in 10 days. With the addition of asparagine, comparable embryos in a parallel test grew to 27 mm in the same length of time.

[1] Work supported in part by Grant No. 720 of the American Philosophical Society, to which the authors are indebted.

[2] F. Laibach, *Jour. Hered.*, 20: 200, 1929.

[3] H. B. Tukey, *Proc. Am. Soc. Hort. Sci.*, 32: 313, 1934.

[4] J. van Overbeek, M. E. Conklin and A. F. Blakeslee, *Am. Jour. Bot.*, 29: 472, 1942.

[5] J. van Overbeek, R. Siu and A. J. Haagen-Smit, *Am. Jour. Bot*, 31: 219, 1944.

[6] We are indebted to Drs. J. L. Randolph and R. A. Brink for the suggestion of using corn embryos made at the recent Smith College embryo culture conference.

To give an idea of the rate of growth of corn embryos cultured *in vitro* at 30° C in van Overbeek's basic medium containing 5 per cent. sucrose, plus 1.5 mg asparagine and 0.001 gamma biotin per cc of culture medium, the average growth of 10-day-old corn embryos of different initial lengths is plotted in Fig. 1. Each initial size, with the exception of the largest, is represented by 30 to 60 embryos. The largest group represents the average of eight.

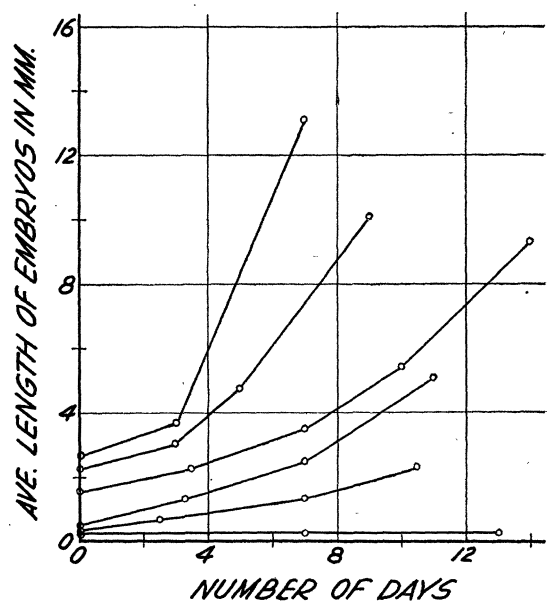

FIG. 1. Growth of excised 10-day-old corn embryos *in vitro*.

From the growth data presented above, it is apparent that the so-called "embryo factors" of coconut milk[4, 5] are not limiting for the survival of the corn embryo. Excised 10-day-old corn embryos above 0.3 mm in length do not require coconut milk for continued growth *in vitro*, while smaller embryos do not survive even with the addition of coconut milk to the medium. It seems likely, therefore, that the growth factors derived from the corn kernel, which are necessary for the growth of the corn embryo, are different from those in coconut milk, which are required by *Datura* proembryos.

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