inbred and the F_1 heterotic hybrid exhibited considerable response. White[6] concluded that this vitamin did not improve the growth of excised tomato roots, while glycine had a marked beneficial effect.

In the experiments referred to above a liquid culture medium was used. The writer has determined the effect of pyridoxine on excised tomato roots in agar culture in experiments carried on at the New York Botanical Garden. The nutrient medium consisted of a modified Pfeffer's solution containing 1.0 per cent. sucrose and 0.5 per cent. purified agar to which thiamin, pyridoxine, nicotinamide, neopeptone, glutamic acid and glycine were added in various combinations.

The strain of excised tomato roots was that originally isolated by Robbins and Schmidt.[7] Fragments of roots which had grown for 47 or more successive passages in a mineral-sugar solution containing thiamin or a mineral-sugar solution containing the thiazole intermediate of thiamin were used as inoculum. The inoculum was therefore in all probability free of any material other than that synthesized by the roots or contained in the solutions given. Uniform pieces of the roots growing in these liquid cultures were transferred to the agar medium in Petri dishes. These were incubated in a moist chamber at 25° C. in the dark. In subsequent passages at eight- to ten-day intervals strongly growing and uniform appearing root tips 1.0 cm in length were transferred from the medium in one Petri dish to the same medium in another. Growth was determined at the end of each passage by measuring the increase in length of the main root. Growth of branch roots was not included in these measurements. In each experiment from 12 to 31 root tips were grown on a particular medium.

In the basal medium the roots seldom grew for more than two passages. With the addition of thiamin similar root tips grew about 2.0 mm daily. In the same experiments, where pyridoxine was added to the agar medium containing thiamin, similar root tips generally showed a daily increment of 5.0–6.0 mm, or in several passages as much as 8.0 mm. Supplementing this medium with nicotinamide had no appreciable effect on the rate of growth. The further addition of neopeptone decreased the rate of growth to two thirds of that in the medium containing thiamin and pyridoxine. The addition of glutamic acid to the agar medium containing thiamin decreased the rate of growth. The addition of glycine to the agar medium containing thiamin had little or no effect.

Although the growth of the roots in the agar medium was less rapid than in the same medium without the agar, they appeared healthy and vigorous where suitable growth-substances were present. In one experiment the roots in the agar medium containing both thiamin and pyridoxine have grown for more than twenty passages during more than two hundred days with no diminution in rate of growth. The roots in the agar medium containing both thiamin and pyridoxine showed the characteristic hooks and curls noted by Robbins and Schmidt.[2]

Pyridoxine was of distinct benefit to the excised tomato roots in these experiments. Neither glutamic acid nor glycine in the amounts used appeared able to replace it.

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[6] P. R. White, *Am. Jour. Bot.*, 27: 811–821, 1940.

[7] W. J. Robbins and M. B. Schmidt, *Bot. Gaz.*, 99: 671–728, 1938.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE PREPARATION OF STERILE PROTEINS IN THE "LYOPHILED" STATE[1]

IN the preparation of certain proteins (fibrinogen, thrombin) and proteinaceous material (chick embryo extract) in the lyophiled state, for uniform tissue culture media, it was noted that it was possible to obtain sterile preparations without the application of the elaborate and costly precautions necessary for aseptic technique. The sterility of these preparations was at first attributed to an empirical treatment of the dry protein with sodium-dried toluene for ten minutes at 60° C. The subsequent discovery that the method of removal of toluene after this treatment left traces of the toluene still in the material made it seem possible that it was the action of the compound over a period of some days that was effective. This was confirmed by the observation that mass cultures of bacteria in the lyophiled state were killed by the application of dry toluene for a period of four weeks, but not by treatment with toluene for ten minutes at 60° C.

The role of the lyophile process itself in the sterilizing action was not at first suspected, since this is a common method of preserving bacterial cultures.[2]

The publication of Heller's[3] study of factors involved in survival and death of bacteria in the desiccated state made it clear that lyophiling itself reduced considerably the number of organisms in a bacterial culture.

Because of the practical importance of being able to prepare, relatively easily, large quantities of sterile

[1] Aided by grants from the Rockefeller Foundation and from the Research Board of the University of California.

[3] George Heller, *Jour. Bact.*, 41: 109, 1941.

lyophiled protein for tissue culture studies, we made quantitative investigations of bacterial survival after certain combinations of chemical treatment and lyophiling.

The test medium chosen was Brewers thioglycollate broth[4] because it supports the growth of anaerobes as well as aerobes.

A local contaminant (gram positive spore-former) was used as a test organism, since previously it had been shown to be more resistant to chemical and thermal treatment (toluene, ether, chloroform, acetone, propylene glycol, various degrees of heat) than any of four standard test organisms,[5] *Staph. aureus, Staph. albus, Ps. pyocyanea, B. subtilis.* The organism was always used as a 24-hour culture of a 24-hour culture.

The results of these experiments are summarized in Tables I and II.

TABLE 1

STERILIZING EFFECT OF CHEMICAL ADDED TO THE DRY RESIDUE AFTER LYOPHILING. NUMBERS EQUAL VIABLE ORGANISMS/ML.

	Incubation 20 hours 37° C.	Lyophiled 24 hours	Lyophiled 24 hours plus Toluene	Relyophiled
Experiment 1	2,900,000	100,000		
Experiment 2	2,800,000		130,000	30

TABLE 2

STERILIZING EFFECT OF CHEMICALS ADDED TO THE AQUEOUS MEDIUM BEFORE LYOPHILING

	Incubation 20 hours 37° C.	Lyophiled 24 hours
Inoculated medium	1,100,000	53,000
Inoculated medium plus toluene	Less than 2,000	0
Inoculated medium plus propylene glycol	Less than 2,000	0

These results indicate that reasonably clean handling of the proteinaceous material, plus toluene treatment and lyophiling, will result in a sterile product.

The material from which proteins intended for subsequent sterile use are to be prepared should be handled in a clean way to reduce sources of contamination. Sufficient toluene should be added to saturate the original material, which then should be worked up in a cool room as quickly as possible to avoid multiplication of the contaminants present. If the product is not sterile, after lyophiling, it may be dissolved in the amount of distilled water removed, toluene again added and relyophiled. In a planned experiment, using organisms in prime condition and using a selective medium, the organisms became non-culturable.

Preparations of fibrinogen, thrombin and plasma, all from slaughter-house beef blood, and of chick embryo extract handled in the open air in non-sterile containers, have been obtained in the lyophiled state in sterile condition in this manner.

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[4] J. H. Brewer, *Jour. Bact.*, 30: 10, 1940.

[5] M. S. Marshall, J. B. Gunnison, M. P. Luxen, *Proc. Soc. Exp. Biol. Med.*, 43: 672, 1940.

REMOVING FROZEN PLUNGERS OF GLASS SYRINGES

THE method of removing the plungers of frozen glass syringes suggested by McCoord[1] was formerly used in our laboratory exactly as he describes it. However, the difficulty in some cases due to the leakage of the water around the rubber gasket caused me to devise a somewhat surer method.

The same principle is used as that suggested by McCoord in which a small tuberculin syringe is utilized to give the necessary hydraulic pressure. Instead of connecting the two syringes by a hypodermic needle and rubber gasket, we have made use of a connector fashioned from the hubs of two broken hypodermic needles. These are brazed together so that they can be used to join the two syringes. In order to make certain that the hole in the needles has not been plugged up in the process of brazing they are subsequently drilled out with a small metal drill. It is important that all air be removed from the system before exerting pressure. This can be accomplished in the "frozen" syringe by using a fine neeedle which will pass freely through the nipple.

This method has been used in our laboratory for the past five years and has never failed to release the plungers even though they are sealed by coagulated blood.

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[1] SCIENCE, Vol. 94, p. 170.

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