is actually in excess of the amounts necessary for adult rats. Pyridine nucleotide concentrations which could be correlated with growth changes would probably be obtained when the concentrations are lower than those required by the animal for normal function.

Summary. 1. It has been observed that a pyridoxine deficiency in the rat does not disturb the normal conversion of tryptophan to liver pyridine nucleotides to any demonstrable extent. 2. Niacin fed to pyridoxine-deficient rats spares liver pyridine nucleotide concentrations. 3. Tryptophan fed in excess along with normal dietary protein in a complete ration increases liver pyridine nucleotides concentrations above normal demonstrating that the excess metabolite can be utilized rather than simply degraded and excreted.

†One hundred g of vitamin mixture contained the following vitamins in a sucrose base: thiamine hydrochloride 10 mg, riboflavin 15 mg, pyridoxine hydrochloride 12.5 mg, calcium pantothenate 100 mg, biotin 0.5 mg, folic acid 1 mg, choline chloride 5 g, and i-inositol 0.5 g.

Received January 24, 1951. P.S.E.B.M., 1951, v76.

Toxicity of Rubber Stoppers for Tissue Cultures.*† (18518)

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During studies on the development of a synthetic medium for animal cells in tissue culture(1-3), it was observed that chance contact of the synthetic feeding mixtures with the rubber stoppers used to close the culture tubes often killed the cells within 48 hours. The following experiments were undertaken in an attempt to reduce the toxicity of the rubber stoppers by various methods of chemical treatment and to test the relative toxicity of various types of rubber.

Methods. Chick embryo mesenchyme tissues, derived from skeletal muscle, were cultivated on the inner surface of standard Pyrex test tubes and supplied with a fluid synthetic mixture (No. 199), which was withdrawn and replaced at regular intervals. Full details of the culture methods and of the synthetic mixture have already been reported(1). Unless otherwise specified, the rubber stoppers that were tested were of the standard black laboratory variety, size No. 1, obtained from commercial supply houses. After chemical treatment (Tables I and II), 9 stoppers

TABLE I. Effect of Solvent Extraction on the Toxicity of Black Rubber Stoppers Tested in Tissue Culture.

Exp. No.	Treatment*	Survival (days)†
A	Control. Culture fluid not exposed to rubber	33
B	Control. Culture fluid exposed to untreated black stoppers	2
1	Hot ether (6 hr)‡	6
2	Hot acetone (6 hr)‡	3
3	Hot alcohol (6 hr)‡	3.2
4	Hot ether (2 hr), hot acetone (2 hr), hot alcohol (2 hr)‡	9.2
5	Cold ether (1 wk)§	8.6
6	Cold acetone (1 wk)§	2.5
7	Cold alcohol (1 wk)§	7
8	Cold ether (2 days), cold acetone (2 days), cold alcohol (2 days)§	7.3

* In each treatment, 9 new black stoppers were extracted with 250 ml of solvent, under the conditions specified, and were then boiled in 3 changes of distilled water.

† Each figure represents the average survival time of 6 cultures in each of the fluids tested.

‡ Extracted in a Soxhlet apparatus.

§ Solvent changed daily.

* This work was supported, in part, by grants from the National Cancer Institute of Canada and the Ontario Cancer Treatment and Research Foundation.

† With the technical assistance of Miss B. J. Barrett, Miss E. A. Burr and Miss O. E. Rockett.

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TABLE II. Effect of Treatment with Hot Alkali and Acid on Toxicity of Black Rubber Stoppers Tested in Tissue Culture.

Exp. No.	Treatment*	Survival (days)†
A	Control. Culture fluid not exposed to rubber	33
B	Control. Culture fluid exposed to untreated black stoppers	<3
1	0.5 N NaOH, 0.5 N HCl	3
2	1.0 N NaOH, 1.0 N HCl	3
3	2.0 N NaOH, 2.0 N HCl	3
4	5.0 N NaOH, 5.0 N HCl	4.6
5	1.0 N NaOH, 1.0 N HCl (alkali and acid made up in 95% ethyl alcohol)	2
6	2.0 N NaOH, 2.0 N HCl (repeated twice)	3.8
7	7 days cold alcohol, then 2.0 N NaOH, 2.0 N HCl	2
8	2.0 N NaOH, 2.0 N HCl, then 7 days in cold alcohol	9.7

* In each treatment, 9 new black stoppers were boiled successively for 6-hour periods in 250 ml of alkali, acid and distilled water.

† Each figure represents the average survival time of 6 cultures in each of the fluids tested.

from each batch that was processed were placed in 500-ml Erlenmeyer flasks and autoclaved. The stoppers in each flask were then covered with 50 ml of Mixture 199 and left standing for 1 week at room temperature. At the end of this period, the fluids were removed from the stoppers and used as feeding solutions for the test cultures. The length of time the cells survived served as a measure of the effectiveness of the various treatments. All solvents and chemicals were of reagent grade.

Results. Exhaustive extraction of the stoppers with hot or cold solvents reduced the toxicity only slightly (Table I). The best results were obtained with ether, or with combinations of ether, acetone and alcohol. It was difficult, however, to remove these solvents from the stoppers, which became hard and brittle. Accordingly, solvent treatment was abandoned and various combinations of alkali and acid were studied (Table II). Prolonged boiling in NaOH and HCl, at concentrations sufficient to disintegrate the stoppers, did not appreciably reduce their toxicity. These attempts were also abandoned, therefore, and a comparative study was made of various types of rubber (Table III). The most suitable rubber tested was a gray product for pharmaceutical use prepared from crude rubber of virgin stock. This rubber was also slightly toxic, but the degree of toxicity was attributed mainly to the severity of the assay procedure. In any event, stoppers of this type have now been used successfully on many hundreds of cultures.

Discussion and conclusions. In most laboratories, it is customary to treat new rubber stoppers with hot alkali and acid in order to remove surface impurities. But in the present experiments, based on more than 500 cultures, stoppers that were strongly toxic in the beginning remained toxic even after the most rigorous treatment. It should be emphasized that the toxicity of rubber stoppers is much more evident in cultures maintained in synthetic media than in media containing such naturally-occurring ingredients as blood plasma, blood serum and tissue extracts. For this reason, extreme care should be taken to avoid toxic stoppers in all experiments in which protein-free media are used. And since animal cells cultivated in synthetic media serve as extremely sensitive test objects, the information reported here can almost certainly be applied to other biological systems.

TABLE III. Relative Toxicity of Various Types of Rubber Stoppers Tested in Tissue Culture.

Exp. No.	Type of rubber*	Survival (days)†
A	Control. Culture fluid not exposed to rubber	33
B	Control. Culture fluid exposed to ordinary black stoppers	2
1	Ordinary stoppers, source A	2
2	Ordinary stoppers, source B	1
3	Neoprene stoppers, source A	2.8
4	Neoprene stoppers, source B	3.8
5	Neoprene stoppers, source C	6.2
6	Red stoppers (virgin rubber)	2.2
7	Pure gum-rubber stoppers‡	12
8	Silicone-impregnated stoppers‡	12
9	Gray stoppers (virgin rubber)‡	22

* With the exception of Nos. 8 and 9, which were washed only in distilled water, all types tested were first boiled successively for 6 hours in 2 N NaOH, 2 N HCl, and distilled water.

† Each figure represents the average survival time of 6 cultures in each of the fluids tested.

‡ Obtained from The West Co., Phoenixville, Pa. Stoppers in Exp. No. 9 specified as stock compound No. S-124.

Received January 25, 1951. P.S.E.B.M., 1951, v76.