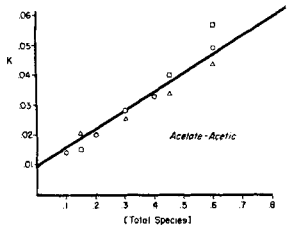


Fig. 3.—Plot of K *vs.* [acid] + [base].

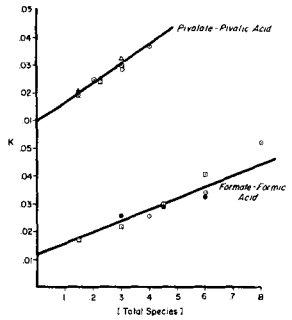
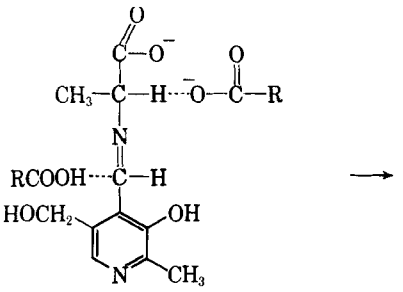
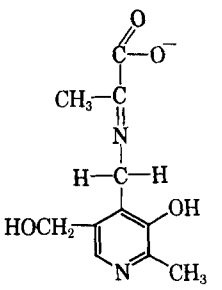


Fig. 4.—Plot of K *vs.* [acid] + [base].





Scheme I

The magnitude of catalysis follows in the order of formic acid < acetic acid < pivalic acid.

Thus, the appearance of equi-acid-base catalysis in the above transamination supports the likelihood of the simultaneous push–pull mechanism of Swain and Brown (11), proposed by Bruice and Topping (6).

Scheme I depicts the rate-limiting step in transamination showing the concerted removal of the α-hydrogen by the base species and the addition of a proton from the acid species.

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Tissue Culture Method for Screening Toxicity of Plastic Materials to be Used in Medical Practice

By S. A. ROSENBLUTH, G. R. WEDDINGTON, W. L. GUESS, and J. AUTIAN

In the past, a number of methods has been used to evaluate toxicities of plastic items for use in medical and related fields. This paper describes a tissue culture method utilizing monolayers of strain L 929 mouse cells in modified Eagle's medium. Samples of plastics are placed in direct contact with cell monolayers. After 24 hours, cells adjacent to the plastic samples are examined for toxic manifestations. Controls include toxic and nontoxic plastic samples. Up to the present time, a large number of plastic samples has been screened by the tissue culture method, and the method has been found to be more sensitive, more rapid, and less expensive than the previously employed intramuscular implantation technique in rabbits.

ONE OF THE most successful tests used in screening of plastic items to be used in medical practice is the implantation method originated by Brewer and Bryant (1). This technique consists of implanting small strips of the plastic sample into the paravertebral muscle of rabbits for a period of 3 to 7 days, at which time the animals are sacrificed and the sites of implants examined both macroscopically and by histopathological methods. Such a method was employed to screen a number of plastic items used in medical practice (2).

Even though the intramuscular implantation technique in rabbits has proven useful to the authors and other investigators in screening plastic items,

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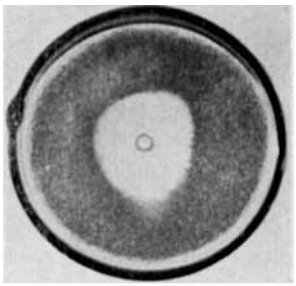

Fig. 1.—Photograph of a monolayer culture of strain L 929 mouse cells exhibiting a positive or toxic response to a plastic sample.

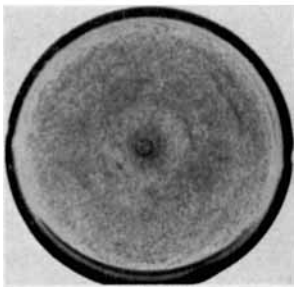

Fig. 2.—Photograph of a monolayer culture of strain L 929 mouse cells exhibiting a negative or nontoxic response to a plastic sample.

considerable time and expense are involved in conducting the tests, particularly if many plastic samples are to be evaluated in a short period of time. For this reason, over 1 year ago a preliminary investigation was initiated to examine tissue culture as a potentially useful tool in the screening of plastics for toxic components. In the past, a number of reports have dealt with tissue culture as a technique for evaluating biological and toxic responses for a great many agents. A selected number of references are given here (3–15).

This paper describes a tissue culture method employed to screen a large number of plastic samples previously tested by the intramuscular implantation technique in rabbits. Evidence will be presented through which the tissue culture method was found to be even more effective as a screening procedure than the implantation technique.

EXPERIMENTAL

Materials

Eagle's medium supplemented with 5% horse serum (16), strain L 929 mouse cells originated by Earle (17) (subcutaneous fibroblasts), and a group of 112 selected plastic samples representing administration devices, containers, catheters, etc., previously

TABLE I.—COMPARISON OF TOXICITY SCREENING RESULTS BY TISSUE CULTURE METHOD OPPOSED TO INTRAMUSCULAR IMPLANTATION METHOD

Total Samples[a] Tested	Positive T.C.[b] Positive I.M.	Negative T.C. Negative I.M.		Positive T.C. Negative I.M.	Negative T.C. Positive I.M.
112	X-2a	X-1	X-182A	X-22	
	X-4	X-1a	X-182B	X-24	
	X-6	X-20	X-182C	X-46	
	X-8	X-23	X-182D	X-47	
	X-17	X-26	X-182G	X-70	
	X-18	X-33	X-182I	X-71	
	X-19	X-35a	X-182J	X-140	
	X-21	X-37HEE	X-185A	X-168HEE	
	X-25	X-39	X-185B	X-168HZ	
	X-34	X-42	X-187A	X-195T	
	X-36	X-43	X-187B	X-196F	
	X-37	X-44	X-187C	X-196T	
	X-38	X-50	X-194A	X-197A	
	X-40	X-52	X-198A	X-197B	
	X-45	X-60	X-198B	X-197G	
	X-48	X-62	X-198C	X-197H	
	X-49	X-68	X-198D	X-197K	
	X-53	X-69	X-198E	X-197L	
	X-54	X-72	X-200A	X-197M	
	X-55	X-73	X-200C	X-197O	
	X-61	X-74HEE	X-200D		
	X-63	X-83			
	X-64	X-148			
	X-65	X-149			
	X-67	X-152HEE			
	X-74	X-154			
	X-113	X-154HEE			
	X-145	X-158			
	X-150	X-159			
	X-152	X-169			
	X-168	X-169HEE			
	X-168EZ	X-175			
	X-168FZ	X-177			
	X-182E	X-178D			
	X-182H	X-179A			
	X-195F				
Totals	36	56		20	0

[a] Individual plastic samples are denoted by the letter X followed by a number. HEE refers to the plastic sample after it was extracted with ethyl alcohol by refluxing for 24 hours. [b] T.C., tissue culture; I.M., intramuscular implantation technique.

TABLE II.—COMPARISON OF TOXICITY RESULTS BETWEEN SELECTED SAMPLES BEFORE AND AFTER HARSH ETHANOL EXTRACTION

Plastic Code No.	Tissue Culture	Intramuscular Implantation	Histopathological Examination of Tissue from Intramuscular Implantation Site
X-37	+[a]	+	+
X-37 HEE[c]	−[b]	−	
X-74	+	+	+
X-74 HEE	−	−	
X-152	+	+	+
X-152 HEE	−	−	−
X-154	−	−	−
X-154 HEE	−	−	−
X-168	+	+	+
X-168 HEE	+	−	+
X-169	−	−	−
X-169 HEE	−	−	−
X-1a[d]	−	−	−
X-175[d]	−	−	−
X-55[e]	+	+	+

[a] +, positive or toxic reaction. [b] negative or nontoxic reaction. [c] HEE, plastic sample after it was extracted with ethyl alcohol by refluxing for 24 hours. [d] X-1a and X-175 were used as control negative plastic samples. These samples have been used repeatedly and have demonstrated that they produce no toxic effect. [e] Sample X-55 represents a positive control.

(or concurrently) examined by other methods were utilized.

Methodology

Preparation of Monolayer Cultures.—Approximately 8×10^6 cells were introduced into 60-mm. glass Petri dishes in 5 ml. of Eagle's medium. The cultures were incubated for 24 hours at 37° with an air mixture containing sufficient carbon dioxide to maintain the medium at a pH near 7.2. Within this time period, cell monolayers had formed on the dish bases, and the total number of cells had multiplied to $1.2–1.5 \times 10^6$.

Preparation of Test Samples.—The plastic test samples, when in the form of tubing, were cut into rings; other types were cut into sizes and shapes which would tend to reduce sample movement during the experiments.

Testing and Evaluating Toxicity.—After 24 hours of incubation, the cultures were removed from the incubator and examined microscopically to assure uniformity. The volume of the culture medium was reduced from 5 to 2 ml. to help avoid excessive sample movement. Three plastic samples were placed gently in contact with the cell monolayers in each dish. Three control cultures were prepared and maintained throughout the experiment: one culture received a known toxic plastic sample; a second culture received a nontoxic sample; and the third received no sample.

After placement of the test samples, all cultures were returned to the incubator and the cells examined microscopically at 24-hour intervals. Death, deterioration, or any observable changes of the cells in relation to control cells were noted as positive or toxic responses. Most positive responses were easily observable within 24 hours, and no additional evidences of toxicity could be attributed to the test samples after the 48-hour reading.

Precautions and Comments.—The indicator, phenol red, is included in Eagle's medium (0.5 mg. %) to reveal excessive deviations in pH, which are potentially harmful to the cells. These may occur when the cultures have been out of the incubator for a long period of time or may be a sign that the carbon dioxide–air system is defective.

Microbiological contamination is minimized through the use of aseptic techniques as well as presterilization of glassware, equipment, and medium. In addition, the medium contains penicillin G potassium (100,000 units/L.) and streptomycin sulfate (50 mg./L.). In initial trials, groups of test samples were exposed to ultraviolet radiation for 10 minutes prior to the experiments. No differences in experimental results were observed between these samples and controls which had not undergone irradiation, nor was evidence of contamination apparent in the cultures. No further attempts to sterilize plastic samples were undertaken.

The test samples themselves, unless from a sealed package, will seldom be sterile when placed on the cells. With the above precautions, however, and the short duration of the experiment, contamination poses no apparent problem. Although sample sterility is not deemed absolutely necessary, ethylene oxide sterilization would likely be suitable if sufficient time is allowed after treatment for elimination of ethylene oxide from samples (18, 19).

Preparation of Cell Layer for Photography.—To prepare a particular dish for photography, the test samples and medium are removed first. Approximately 0.5 ml. of 2.0% crystal violet solution in 20% alcohol is introduced into the dish and rotated over the cell monolayer for 1 minute. After thoroughly washing the crystal violet from the dish, it may be seen that the cells are stained purple and securely fixed to the base of the Petri dish. Unstained areas represent zones of attenuated or dead cells which previously had become detached from the glass dish. Figure 1 is a photograph representing the response of a cell monolayer to a positive plastic sample (tubing) after 24 hours. Figure 2 represents a cell monolayer after 24 hours of contact with a negative sample.

RESULTS AND DISCUSSION

Table I indicates the reaction of the mouse cells to the plastic test samples in relation to activity noted previously by the same samples after intramuscular implantation in rabbits for 1 week. It may be observed that all samples noted as positive by the intramuscular implantation technique appeared as positive to tissue culture. Twenty additional samples demonstrated activity in the tissue culture which had not shown a positive response by the intramuscular implantation method. It is notable that none of the tissue culture negative responses were positive in the intramuscular implantation test. Results in Table I show the close correlation between the two methods and further reveal the greater sensitivity of tissue culture for detecting toxic plastic samples over the intramuscular implantation method.

Among the test samples were six plastics which previously had been extracted by refluxing with 95% ethyl alcohol for 24 hours. The extraction solutions were decanted and the remaining plastic

washed with quantities of ethyl alcohol, then with distilled water. These plastics were referred to as HEE (harsh ethanol extracted). Table II shows the comparative results from plastic samples and their HEE counterparts. Histopathological examinations of rabbit muscle surrounding some of the intramuscular implants were conducted. Table II also contains these evaluations. Excellent agreement may be noted between the results from tissue culture and the histopathological results from rabbit tissue immediately proximal to the plastic implant. These data reveal interesting features. For example each plastic sample (except one) which was shown to induce a reaction by any of the three test methods (intramuscular implantation, histopathological, and tissue culture) produced no reaction after extraction with the alcohol solvent. The only exception was X-168, a plastic sample from a rather toxic urinary collection bag, which apparently retained sufficient concentration of the toxic component or components to give continued reactivity when examined by either tissue culture or by histopathological methods (20). The intramuscular implantation method was apparently not sufficiently sensitive to demonstrate this reactivity.

Among the 112 samples screened, only one, X-154 (an intravenous administration device), was found nontoxic by both methods and later shown to exhibit pharmacological activity by the use of further biological tests (21). Thus, it is important to recognize that occasionally there may be toxic manifestations which will not be revealed by either the tissue culture method or by the intramuscular method (including histopathologial examination). For this reason, depending upon the extent of investigation desired, biological tests may be needed. Suggestions for various tests in evaluating the safety of plastic items to be used in medical practice have been reported previously (22).

The tissue culture method appears to be an excellent screening method for revealing the safety of a plastic item and may eventually supplant the more time-consuming and expensive rabbit implantation technique. Presently, both methods are being used in the screening of all plastic samples arriving at the laboratory. Additional studies, however, have been initiated on tissue culture methods in the hope of making further improvements in this technique, not only as a screening tool but as a research instrument for specific studies on plastics. Results of these added studies with tissue culture will be reported at a later date.

SUMMARY

A tissue culture technique was used to screen 112 plastic samples previously tested by an intramuscular implantation method in rabbits. Results of the tissue culture tests were compared with the results from the implantation tests. These results are summarized as follows: (*a*) 36 samples shown to be toxic by the implantation method were found to be toxic by tissue culture methods; (*b*) 56 samples shown to be nontoxic by the implantation method were found to be nontoxic by tissue culture; (*c*) 20 samples found to be nontoxic by implantation were found to be toxic by tissue culture; (*d*) in no instance was there a nontoxic reaction by tissue culture which previously demonstrated toxicity by the implantation method; (*e*) the tissue culture tests paralleled closely the results obtained by histopathological examinations of the implanted sites.

The results of this study suggest that the tissue culture technique may be substituted for the intramuscular implantation technique for the screening of plastic materials to be used in medical practice, thereby reducing the testing time while increasing both test sensitivity and the number of plastic samples which can be evaluated.

It should be remembered that further biological tests may be necessary for the complete evaluation of plastic items to be used in medical practice.

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