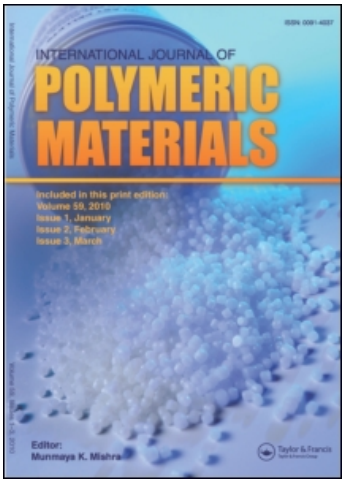

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

Evaluation of Elastomers for Biomedical Applications Utilizing *in vivo* and *in vitro* Model Systems—State of the Art and Future Trends

Z. Oser[ab]; R. A. Abodeely[a]; R. G. Mcgunnigle[a]
[a] Research Division, Ethicon. Inc., Somerville, N.J. [b] Patient Care Division, Johnson and Johnson, New Brunswick, N.J.

To cite this Article Oser, Z. , Abodeely, R. A. and Mcgunnigle, R. G.(1977) 'Evaluation of Elastomers for Biomedical Applications Utilizing *in vivo* and *in vitro* Model Systems—State of the Art and Future Trends', International Journal of Polymeric Materials, 5: 3, 177 — 187
To link to this Article: DOI: 10.1080/00914037708075205
URL: http://dx.doi.org/10.1080/00914037708075205

Intern. J. Polymeric Mater., 1977, Vol. 5, pp. 177–187

Printed in Reading, England

Evaluation of Elastomers for Biomedical Applications Utilizing *in vivo* and *in vitro* Model Systems—State of the Art and Future Trends†

Z. OSER,‡ R. A. ABODEELY and R. G. McGUNNIGLE

Research Division, Ethicon, Inc., Somerville, N.J. 08876

(*Received July 1975*)

The increased use of natural and synthetic materials for biomedical applications has given emphasis to the need for determining the biocompatibility of materials in a rapid and reproducible manner.

This report summarizes classical *in vivo* techniques and describes the evaluation for biocompatibility of a series of thermoplastic and crosslinked elastomers as well as a series of phthalic acid esters utilizing three recently developed *in vitro* methods of tissue culture.

INTRODUCTION

Classical methods

Classical *in vivo* screening of potential biomedical materials is commonly utilized to assay toxicity and tissue compatibility. These state of the art methods include those that can be conducted directly on the material of potential interest, such as intramuscular implantation in rabbit muscle, subcutaneous implantation in rats, and hemolysis testing, as well as methods which use extracts prepared from the material of potential interest. These include solvent extractions of the material with saline, ethanol, cottonseed oil, and polyethylene glycol. The duration and temperature of extraction depend on the heat resistance of the plastic. Extractions are usually carried out as one of the following three times and temperatures: 50°C–3 days, 70°C–24 hours, or 121°C–1 hour.

†Presented at the Symposium on Elastomers in Medicine at the 105th Meeting of the Rubber Division, American Chemical Society, Toronto, Canada, May 9, 1974.

‡To whom requests for reprints should be addressed; *present address:* Patient Care Division, Johnson and Johnson, New Brunswick, N.J. 08903.

Generally, the extracts are evaluated for changes in pH, surface tension, and absorption spectra as well as utilized directly for systemic injection in mice and intracutaneous injection in rabbits. In addition, the extracts may be analyzed for hemolytic potential by *in vitro* hemolysis techniques.

It should be noted that the use of ethanol and polyethylene glycol as extractants is considered by many workers in the field to be not as relevant as the saline and cottonseed oil extraction procedures.

Shown below are some of the organizations presently concerned with the setting of standards and test procedures for biomaterials for specific end uses.

End use	*Organization*
Plastic containers and accessor devices for parenteral products	The Pharmacopoeia of the United States of America, XVIII
Surgical implants	ASTM Committee F-4 on Surgical Implants
Dental materials	Council on Dental Materials and Devices
Anesthetic devices	Z-79 Section, Committee of the U.S.A. Standards Institute
Medical implants and instrumentation devices	Association for the Advancement of Medical Instrumentation

Recently developed methods

Since the classical *in vivo* methods outlined above are time consuming and expensive, especially as screening procedures, the need for rapid, relatively inexpensive *in vitro* methods for biological screening has become increasingly apparent in recent years. One of the most promising recent developments in this area has been the use of *in vitro* tissue culture techniques (1, 2, 3, 4). *In vitro* tissue culture is defined as the growth and propagation of living human or animal cells outside the living body. The specific methods of tissue culture employed in this study for the evaluation of potential biomedical materials include agar overlay, direct contact, and suspension methods.

Figure 1 is a diagrammatic representation of the agar overlay method used to determine the toxicity of candidate elastomers to cells. The agar overlay method utilizes mouse L-cells which have been allowed to form a monolayer on a Petri dish and are, in turn, overlaid with a thin layer of agar augmented with 2X Minimal Essential Medium containing a vital stain (neutral red). Solid elastomer samples are placed directly on the agar overlay and the plates incubated for 24 hours in a 5% CO_2 atmosphere at 37°C.

The second method used in this study is the direct contact method and is shown diagrammatically in Figure 2. This technique is used to determine the

biocompatibility of materials by observing whether cells will attach, grow, and proliferate directly on the surface of the material being evaluated. Suspensions of mouse L-cells (3×10^5 cells/ml) are added to Petri dishes containing a cast film of the material under evaluation and allowed to settle into direct contact with the surface. Compatibility of the cells with the test material is measured as a function of cellular attachment, growth and time required to achieve a complete monolayer.

The third method used in this study is that of suspension culture. This method utilizes an enzymatic assay for ATP levels in cells; ATP levels are determined on the DuPont Luminescence Biometer. The toxic effect of materials on cells is determined by measuring changes in ATP levels of cells in suspension in the presence of the material under study.

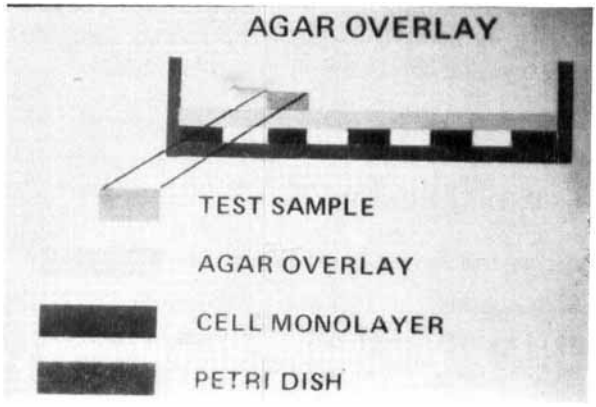

FIGURE 1 Agar overlay method of tissue culture.

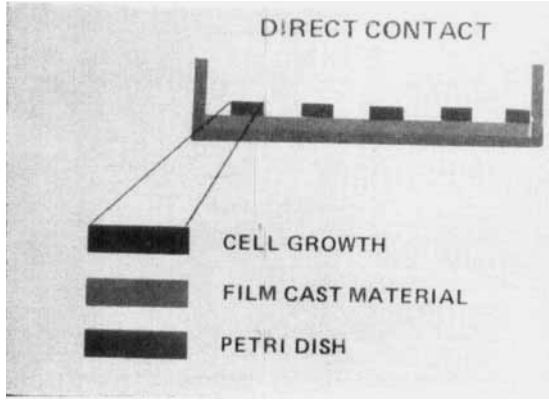

FIGURE 2 Direct contact method of tissue culture.

MATERIALS TESTED

The materials tested by the agar overlay method were selected from those commercially available and included both thermoplastic and crosslinked elastomers. The thermoplastic elastomer group consisted of polyester and polyether polyurethane. The crosslinked elastomer group included natural rubber, polyisoprene, carboxylated butadiene/acrylonitrile, butyl rubber, silicone rubber, and polyether polyurethane.

Solutions or suspensions of some of the aforementioned elastomers were used to cast films (0.5 mm – 1.0 mm in thickness) on carefully cleaned glass Petri dishes. The resulting film surface was employed for *in vitro* testing by the direct contact method.

The final group of materials tested consisted of 1% suspensions of a series of phthalic acid esters commonly used as plasticizers for plastics. The phthalic acid esters ranged from diethyl through didecyl and were used in the suspension cell culture assay for ATP concentration as well as in the agar overlay method described above.

RESULTS AND DISCUSSION

The agar overlay method of tissue culture

The results obtained when the agar overlay method was used to evaluate the relative toxicity of a series of elastomers are given in Table I.

TABLE I

The relative toxicity of a series of elastomers as measured by the agar overlay method of tissue culture

Material	Result
Thermoplastic group	
Polyester polyurethane	–
Polyether polyurethane	–
Crosslinked group	
Silicone	–
Natural rubber	+
Cis-polyisoprene	+
Polyether polyurethane	+
Carboxylated butadiene acrylonitrile	+
Butyl rubber	+

– = Nontoxic.
+ = Toxic.

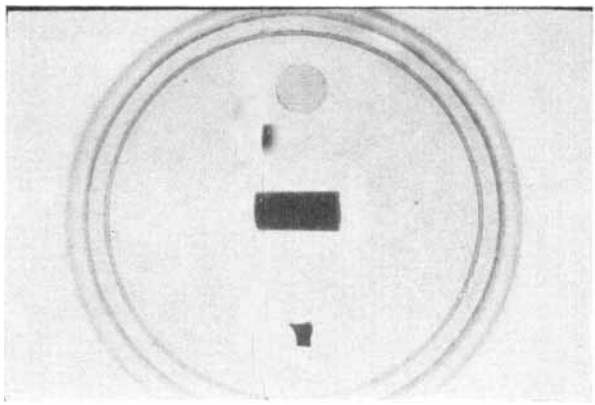

FIGURE 3 Agar overlay toxic response obtained with natural rubber sample

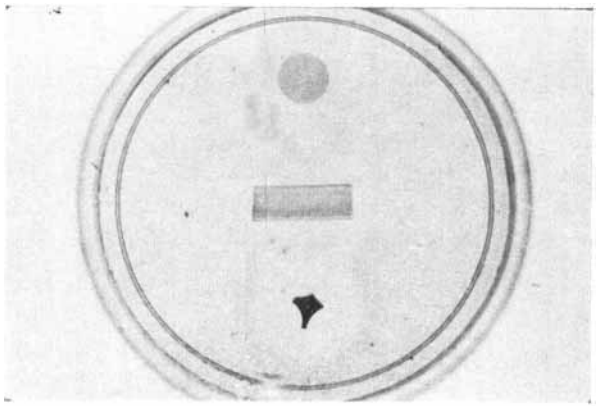

FIGURE 4 Agar overlay non-toxic response obtained with thermoplastic polyether polyurethane.

Figure 3 is an actual photograph of the agar overlay test result obtained with a natural rubber sample. It demonstrates the obvious toxic zone surrounding the sample in the center of the picture. In addition, the toxic response to the positive control at the top of the picture and lack of toxic response to the negative control at the bottom of the picture should be noted. This photograph is representative of those samples that elicited a toxic reaction as shown in Table I.

Figure 4 is an actual photograph of the agar overlay test result obtained with a sample of a thermoplastic polyether polyurethane. It is representative of the nontoxic or negative responses obtained with materials as shown in Table I.

Figures 5 and 6 are photomicrographs of the areas immediately surrounding the samples on the agar overlay plate. In Figure 5, the cells surrounding the natural rubber sample are dead, as indicated by lack of vital stain and by

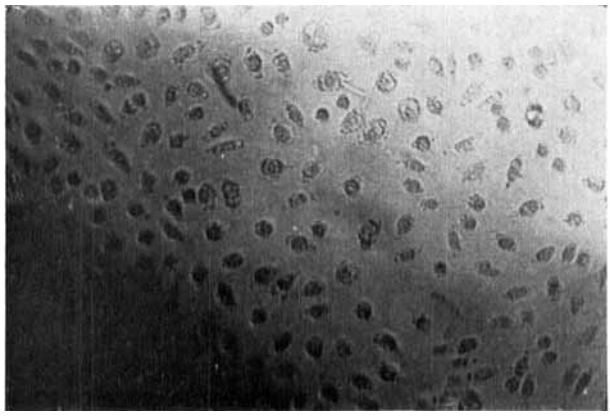

FIGURE 5 Photomicrograph of area surrounding natural rubber sample (agar overlay)

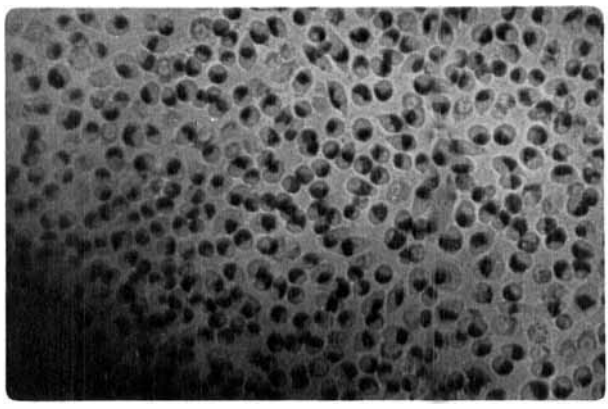

FIGURE 6 Photomicrograph of area surrounding thermoplastic polyether polyurethane (agar overlay).

rounded cell morphology. In contrast, it is apparent in Figure 6 that the cells surrounding the polyether polyurethane sample are viable, have retained the vital neutral red stain, and exhibit fibroblastic morphology.

Table II and Figures 7 and 8 show the results obtained from a series of tests with various phthalic acid esters. Table II reveals that toxicity as measured by zone size, is dependent not only on the specific phthalate, but also on the concentration. Dimethyl phthalate at 50% exhibited the largest toxic zone of the series. Also, toxicity was found to be inversely related to the length of the phthalate ester side chain, as seen by the decreasing zone size from dimethyl through dibutyl phthalate. No toxicity was associated with the series di-n-amyl

TABLE II

Toxicity of phthalate esters to L-cells as measured by the agar overlay method

Phthalate esters	Toxic zone in mm			
	50%	10%	5%	1%
Dimethyl phthalate	14.2	10.5	7.2	4.1
Diethyl phthalate	12.1	9.5	6.5	—
Di-n-propyl phthalate	8.9	3.2	—	—
Dibutyl phthalate	4.0	2.8	—	—
Di-n-amyl phthalate	—	—	—	—
Di-n-heptyl phthalate	—	—	—	—
Dioctyl phthalate	—	—	—	—
Dinonyl phthalate	—	—	—	—
Didecyl phthalate	—	—	—	—

— = Nontoxic.

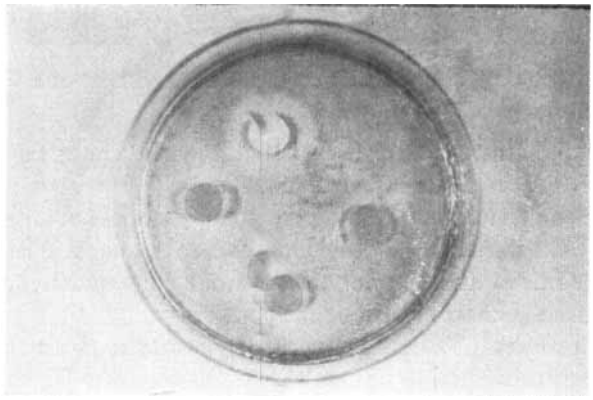

FIGURE 7 Agar overlay non-toxic response obtained with didecyl and di-n-propyl phthalate.

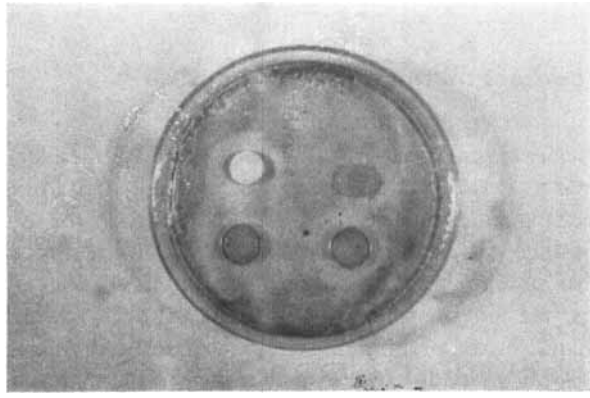

FIGURE 8 Agar overlay toxic response obtained with dimethyl and diethyl phthalate.

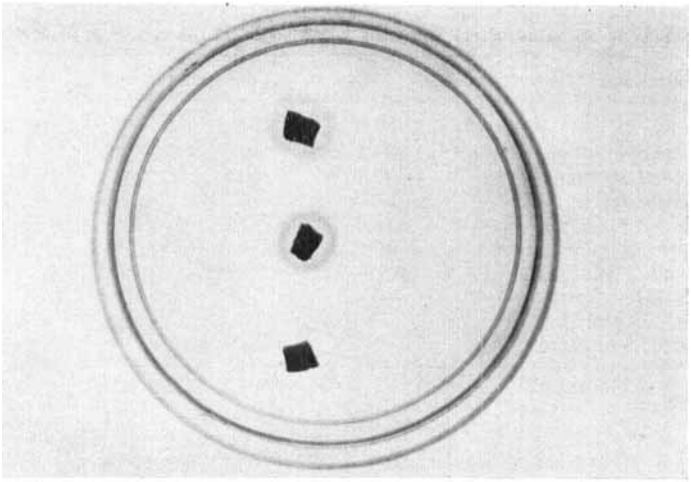

FIGURE 9 Agar overlay reduced toxic response of cis-polyisoprene after water extraction.

through didecyl at the concentrations tested. Figure 7 shows the cell response obtained with didecyl and di-n-propyl phthalate.

It should be noted that the agar overlay technique does not take into account possible solubility differences of leachable toxic components. These differences may affect the rate of diffusion into the agar and, consequently, the size of the toxic zone observed at any specific time period.

Figure 9 illustrates the reduced toxic response observed with a sample of cis-polyisoprene after water extraction. This result supports the theory held by many workers in this field that the toxicity or lack of biocompatibility exhibited by some materials is due to toxic small molecules present which are leached out of these materials by body fluids.

The direct contact method of tissue culture

The direct contact method is a sensitive technique for assaying materials for compatibility with specific cell types. The compatibility of the material with the cell is measured by the degree and rate of attachment to and proliferation of the cells on the sample material. The photomicrographs show the different cellular responses obtained with different materials using the direct contact method. The absence of attachment or growth of L-cells in direct contact with natural rubber can be seen in Figure 10. The relatively poor growth of L-cells on thermoplastic polyester polyurethane can be seen in Figure 11. At seven days, the cells have only partially covered the surface of the material. In contrast, Figure 12 shows the excellent cell growth on thermoplastic polyether poly-

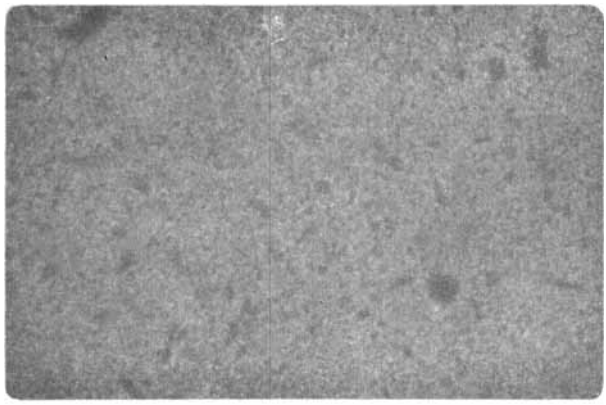

FIGURE 10 Direct contact method (photomicrograph)--no attachment or growth on natural rubber.

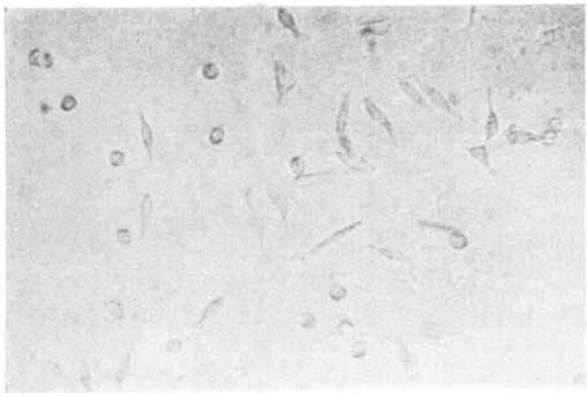

FIGURE 11 Direct contact method (photomicrograph)—poor attachment and growth on thermoplastic polyester polyurethane.

urethane. The L-cells have formed a complete monolayer and exhibit fibroblastic morphology.

These results suggest the possibility of developing specialized materials which would exhibit optimum compatibility with specific cells or tissue types.

The suspension method of tissue culture

As described earlier, the suspension tissue culture method utilizes an enzymatic assay for the ATP level in cells. The toxic effect of materials on cells is determined by measuring changes in ATP level using the DuPont Luminescence Biometer.

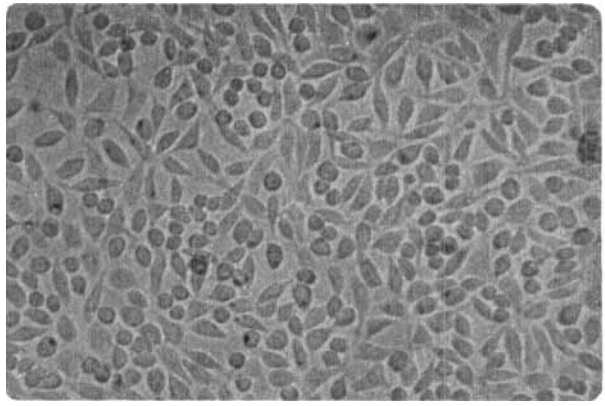

FIGURE 12 Direct contact method (photomicrograph)—good attachment and growth on thermoplastic polyether polyurethane.

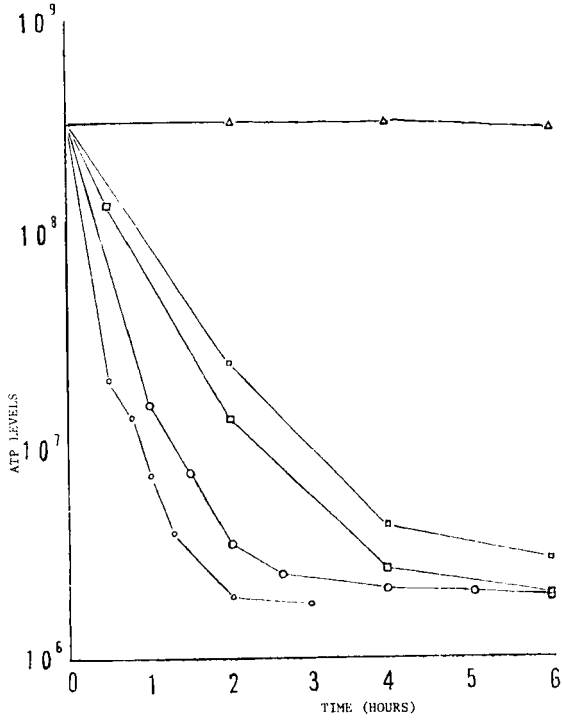

FIGURE 13 ATP level vs. time for a series of phthalic acid esters suspension method of tissue culture.

○ Diethyl; ○ Dipropyl; ▯ Dibutyl; □ Diamyl; △ Diheptyl, Dioctyl, Dinonyl, Didecyl, control

Figure 13 shows the results obtained when a series of phthalic acid esters was tested with L-cells and changes in cell ATP levels were measured. Diethyl phthalate exhibited the most rapid reduction in ATP level and was followed by dipropyl, dibutyl, and diamyl. These results suggest that reduction of ATP level in L-cells with time is inversely related to the length of phthalic acid ester side chain. The diheptyl through didecyl esters showed no variation from the control after six hours. These results correlate well with those obtained by the agar overlay method described above. The disappearance of ATP is thus related to destruction of the cells.

FUTURE TRENDS

In the future, *in vitro* tissue culture methods for screening of potential biomaterials will undoubtedly find more extensive application. These methods will be expanded to include actual cell types found at the anatomical site at which the specific biomedical device is to be used.

The use of tissue culture methods for determining specific toxic effects induced by a given material on a biochemical reaction ongoing at the subcellular level is also envisioned. Hopefully, this will ultimately lead to the elucidation of the mechanism of the *in vitro* toxic response of certain candidate biomaterials.

References

1. W. L. Guess, S. A. Rosenbluth, B. Schmidt, and J. Autian. Agar diffusion method for toxicity screening of plastics on cultured cell monolayers, *J. Pharm. Sci.* **54,** 1545–1547 (1965).
2. R. Johnson and A. F. Hegyeli, Tissue culture techniques for screening of prosthetic materials, *Ann. N.Y. Acad. Sci.* **46,** 193–202 (1968).
3. S. A. Rosenbluth, B. Schmidt, and J. Autian. Tissue culture method for screening toxicity of plastic materials to be used in medical practice, *Amer. J. Hosp. Pharm.* **54,** 156–159 (1965).
4. R. F. Sisca, J. C. Thonard, D. A. Lawer, and W. A. George. Responses of epithelial-like cells in tissue culture to implant materials, *J. Dent. Res.* **46,** 248–252 (1967).