Sensitive Biological Tests for Medical Grade Plastics
I. Toxicity of Organo-Tin Stabilizers

By MARCEL NIMNI

Tissue implantation and the measurement of inflammation by the dye extravasation technique are sensitive and rapid methods for screening plastics for toxicity. The subcutaneous and the intramuscular implantation of polyvinyl chloride plastics containing organo-tin stabilizers produces severe local tissue reaction. The degree of extravasation of trypan blue at the site of the injection of a suitable plastic extract is also an indication of compatibility with animal tissues. Mineral oil was found to be a suitable, nonirritating agent for extracting the plastic. The same techniques are suitable for screening raw materials before incorporation into the plastic formulation. Tin stabilizers, such as dibutyltin dilaurate, caused irritation in dilutions as great as 1:10,000.

PLASTIC MATERIALS, because of their versatility and low cost, find increasing usage in the pharmaceutical and medical fields (1). To keep pace with varying needs in diversified applications, the plastic industry has developed many different formulations (2, 3). It has been of primary importance to investigate carefully among other properties, the biological inertness of the plastics which contact drugs or tissues. Sensitive bioassay procedures are used to detect any toxic effects of the plastic resins themselves, as well as of the ingredients which may be added to the basic polymers for purposes of stability, flexibility, or coloring. The effectiveness of the subcutaneous or intramuscular implantation in animals, as a means of detecting acute and chronic toxicity of plastics, has been reported previously (4–8).

Herewith are described our results obtained using the rat and the rabbit as test animals: (*a*) tissue reactions caused by the subcutaneous injection of small amounts of plastic additives or implantation of plastic materials (polyvinyl chloride) containing such additives, and (*b*) a sensitive technique for rapidly detecting an inflammatory response by measuring the increased capillary permeability of intravenously injected dye at the site of the intradermal injection of a plastic extract.

EXPERIMENTAL

Tissue Implantation.—Wistar rats and New Zealand white rabbits were used as test animals. Rats were implanted subcutaneously with test samples under light ether anesthesia. After shaving the dorsal area, a transverse incision through the skin was made in the lumbar region. Using the blunt end of a forceps, the skin was separated from the underlying connective tissue, and the plastic sample was positioned using another forceps in the subcutaneous tissue, not closer than 30 mm. from the wound. The incision was sutured with 11-mm. nickel-silver wound clips.

Rabbits were anesthetized with thiopental sodium (20 mg./Kg.) and ether. For routine testing, intramuscular implants were made with an automatic pellet implanter[1] at the level of the dorsal paravertebral musculature. In some instances, larger sections were implanted surgically. In all instances, the procedures were performed in an aseptic manner.

At the end of the experimental periods, either 4 days, 1 week, 2 weeks, or 2 months, the animals were sacrificed and the areas observed for macroscopic changes. The plastic specimen and its surrounding tissue were fixed in neutral formalin solution for histologic examination.

A 1% solution of trypan blue in normal saline (0.9% sodium chloride in distilled water) was used to measure local inflammatory response by the degree of dye extravasation. Extracts of plastics were prepared by autoclaving (121° for 60 minutes) 10 Gm. of plastic, cut into small particles, in 10 ml. of mineral oil. Normal saline extracts were prepared in a similar way. The dorsa of the rabbits were carefully clipped to remove hair and 0.1 ml. of the extract was injected intradermally with a 27-gauge needle. A 20% ethanol solution was used as a control irritant. Fifteen minutes after the last intradermal injection, the 1% dye solution was ininjected intravenously at a level of 1 ml./Kg. Response was graded on the basis of amount of dye accumulation at the injection site and classified as none, mild, moderate, and marked (9). The response with 20% ethanol was considered as marked inflammation.

Commercially available dibutyltin dilaurate and other organo-tin stabilizers from various sources were tested. Dilutions of these compounds were made prior to injection, using mineral oil, and were sterilized before use.

RESULTS

The tissue reaction, from a subcutaneous implant of polyvinyl chloride containing a tin stabilizer, is shown in Fig. 1. This specimen had been maintained *in situ* for 1 week. At that time, the animal exhibited swelling of the area as well as all the other signs which accompany an inflammatory reaction. On exposing the subcutaneous tissue, a large capsule was visible surrounding the implant; there was evidence of increased vascularization and fluid accumulation. Intramuscular implantation of similar plastic caused tissue reactions evidenced by a whitish zone of necrotic tissue surrounding the sample. These reactions have been described and illustrated by previous investigators (7, 8). Figure 2 shows a transverse section of a tin stabilized plastic sample surrounded by necrotic epidermis and fat. There is an abscess formation walled off by a thick layer of proliferating fibrous tissue. The white area is caused by retraction of the plastic during the process of fixation.

Figure 3 shows the subcutaneous tissue of a rabbit at a site of injection of 0.1 ml. of dibutyltin dilaurate.

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[1] Manufactured by Sterol Derivatives, Inc., Los Angeles, Calif.

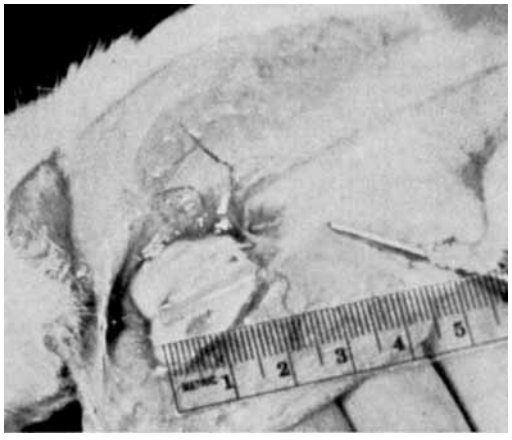

Fig. 1.—Fibrous connective tissue capsule walling off a tin-containing plastic material which has caused a significant inflammatory reaction, 1 week following its subcutaneous implantation.

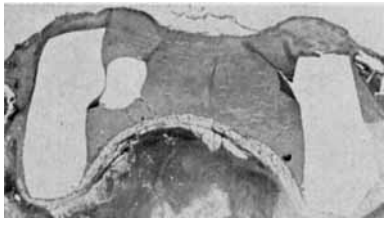

Fig. 2.—Photomicrograph showing a plastic formulation stabilized with an organo-tin compound, surrounded by proliferating granulation tissue, abscess formation, necrotic epidermis, and fat.

After 24 hours, the area showed signs of necrosis, edema, hemorrhage, multiple cystic spaces, and fibrinous exudate. Eosinophils with occasional polymorphonucleocytes surrounded the area. The whole picture is suggestive of an acute allergic reaction.

As a part of this investigation, the changes in capillary permeability caused by the intradermal injection of different extracts of the plastics and stabilizers were also determined. Vital dyes, when injected intravenously, become bound to plasma proteins, and their accumulation in the treated skin sites reflects the degree of inflammation. Table I summarizes the observations made in this connection.

It can be seen that the irritating plastic containing a tin stabilizer gave a marked positive reaction soon after the injection of the dye when the extract was made using mineral oil as a solvent. Using normal saline, the reaction was scarcely visible and would be termed nonirritating. Plastics without tin gave no signs of tissue reaction irrespective of oil or saline extraction.

Dibutyltin dilaurate caused a marked inflammatory response even when considerably diluted in mineral oil. Dilutions of 1:10,000 still gave evidence of inflammation. It should be pointed out that this mineral oil solution is insoluble in body fluids; presumably, therefore, the tissue reaction occurs with those molecules of solute which are on the periphery of the infiltrating fluid. This makes these concentrations in a mineral oil vehicle comparable to that in a plastic formulation which is maintained in contact with tissues.

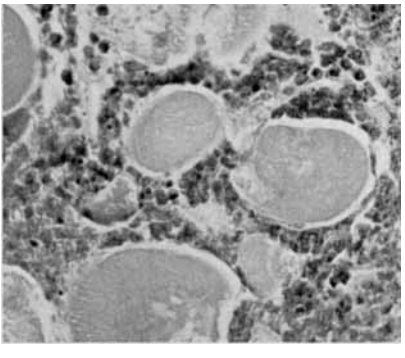

Fig. 3.—Subcutaneous and muscular tissues of a rabbit following the injection of 0.1 ml. of dibutyltin dilaurate. The response is indicative of an acute allergic reaction.

TABLE I.—INFLAMMATORY RESPONSE[a]

Sample	Dilution	Degree of Inflammatory Response[b] Recorded at Different Time Intervals				
		10 Min.	20 Min.	30 Min.	60 Min.	120 Min.
Mineral oil (MO)	...	0	0	0	0	0
Normal saline (NS)	...	0	0	0	0	0
Tin-containing plastic in MO	...	++	+++	+++	++++	++++
Tin-containing plastic in NS	...	0	0	+	+	+
Tin-free plastic in MO	...	0	0	0	0	0
Tin-free plastic in NS	...	0	0	0	0	0
Dibutyltin dilaurate	1:50	++	+++	++++	++++	++++
	1:100	++	+++	+++	+++	+++
	1:1000	++	++	+++	+++	+++
	1:5000	++	++	++	++	+++
	1:10000	0	0	+	++	++
Tin-containing stabilizer A	1:1000	++	++	+++	++++	++++
Tin-containing stabilizer B	1:1000	0	+++	+++	++++	++++
Tin-containing stabilizer D	1:1000	0	++	+++	++++	++++
Tin-containing stabilizer E	1:1000	++++	++++	++++	++++	++++
Tin-containing stabilizer F	1:1000	++	++	++	++	++
20% ethanol	...	++++	++++	++++	++++	++++

[a] Measured by the degree of dye extravasation caused by the subcutaneous injection of plastic extractables and of different concentrations of organo-tin stabilizers. [b] Degree of inflammatory response: 0, none; +, slight; ++, moderate; +++, marked; ++++, severe.

DISCUSSION

The presence of organo-tin compounds in a plastic formulation, added for stabilization purposes, renders the plastic irritating to animal tissues. Its subcutaneous as well as intramuscular implantation in rats or rabbits causes a severe tissue reaction, reflected by edema, cellular necrosis, proliferating granulation tissue, as well as encapsulation by fibrous tissue.

These results confirm the observations of previous investigators which indicate that the tissue implantation technique is one sensitive means of determining the suitability of plastic formulations for medical applications. Our findings indicate that the rat is a satisfactory animal for routine testing of plastics as well as the raw materials proposed for their formulation.

Implantation reveals tissue reaction, if any, within 2 to 4 days, whereas inertness is reflected by excellent tolorance during many weeks of implantation.

The subcutaneous implantation of solid materials and the injection of liquid constituents are valuable in screening the ingredients which are compounded for the different formulas. Macroscopic as well as microscopic examination of the areas of contact are of immediate importance in determining the nature of the response.

The degree of dye extravasation, at the site of intradermally injected extracts to the rabbit, was a rapid and sensitive method for detecting toxicity. Extracts, prepared by autoclaving the plastics in test with mineral oil, increased the sensitivity of the method. Using this method, it was determined that dilutions of 1:10,000 of organo-tin stabilizers still were able to cause an inflammatory response. In this connection it should be mentioned that dibutyltin dilaurate has recently shown to be teratogenic when injected into the yolk sac of fertile eggs prior to incubation (10). Because of this reactivity in tissue, plastics with organo-tin compounds should not be used in applications involving intimate and/or prolonged contact with tissue, in view of other effective stabilizers that do not cause reaction.

In addition to the test described, it is also recommended that all plastic materials which shall be in contact with tissue or body fluids be screened for the presence of pyrogens, vasomotor activity, antigenicity, ocular irritation, hemolytic effects, chronic toxicity in various species, and *in vitro* with isolated muscle systems.

Additional work continues in this area. The author would like to stress the importance of biologically screening the individual constituents that are used in making up all formulations as well as testing the completed plastic formulation.

REFERENCES

(1) Autian, J., *Am. J. Hosp. Pharm.*, **18**, 329(1961).
(2) Autian, J., This Journal, **52**, 1(1963).
(3) *Ibid.*, **52**, 105(1963).
(4) Turner, F. C., *J. Natl. Cancer Inst.*, **2**, 81(1941).
(5) Oppenheimer, B. S., Oppenheimer, E. T., Stout, A. P., and Danishefsky, I., *Science*, **118**, 305(1953).
(6) Russell, F. E., Simmers, M. H., Hirst, A. E., and Pudenz, R. H., *J. Natl. Cancer Inst.*, **23**, 305(1959).
(7) Brewer, J. H., and Bryant, H. H., This Journal, **49**, 652(1960).
(8) Lawrence, W. H., Mitchell, J. L., Guess, W. L., and Autian, J., *ibid.*, **52**, 958(1963).
(9) Hopper, J. O., Alexander, E. B., and Miles, L. C., *ibid.*, **39**, 147(1950).
(10) McLaughlin, J., Jr., Marliac, J. P., Verrett, M. J., Mutchler, M. K., and Fitzhugh, O. G., *Toxicol. Appl. Pharmacol.*, **5**, 760(1963).

Thimerosal as a Preservative in Biological Preparations I. Application of Polarography to the Determination of Thimerosal in Aqueous Solutions and Vaccines

By J. BIRNER and J. ROS. GARNET

Thimerosal, in the low concentration commonly used as an antiseptic and bacteriostatic agent in biological preparations such as vaccines, may be determined polarographically and rapidly and with a degree of accuracy comparable to that obtained by the commonly used agar-cup technique.

It has been observed that the concentration of thimerosal[1] added to vaccines and other biological preparations diminishes with storage (1). The loss has been attributed to absorption of the substance by the rubber with which the containers are often sealed or to physical or chemical interaction with one or another of the components of the rubber. Although there is general agreement that reduction of its concentration does occur, methods for the precise measurement of the extent of the reduction have been largely dependent upon biological techniques.

In some earlier studies in these laboratories, the bacteriostatic activity of antiseptics was measured by the well-known agar-cup assay method. With this technique, the growth of selected microorganisms (*Staphylococcus aureus*, strain B313, and *Bacillus subtilis*, strain 8236, of the National Type Culture Collection) on nutrient agar is inhibited within a zone, the diameter of which is approximately proportional to the concentration of antiseptic placed in the cup.

A comparison of the clear zone formed by a chosen volume of the sample and equal volumes of standard concentrations of the thimerosal affords a measure of the concentration of the antiseptic in the sample—hence, a measure of its diminution.

However, the present authors have used for some time a polarographic method (2) which they consider preferable for speed and accuracy to the rather cumbersome biological method. It is being applied successfully with routine samples having a thimerosal content of not less than 20 p.p.m.

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[1] Thimerosal, sodium ethylmercurithiosalicylate, is official in the "British Pharmacopoeia" as Thiomersal. It is trademarked as Merthiolate.