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.

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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.

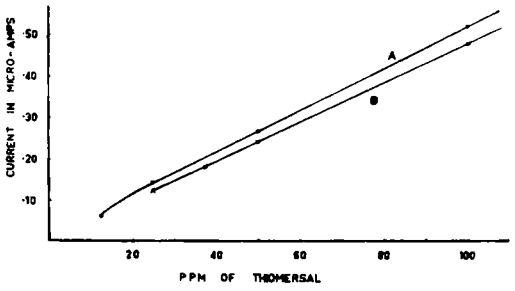

Fig. 1.—Standard curve for thimerosal. Key: A, in water; B, in triple antigen vaccine.

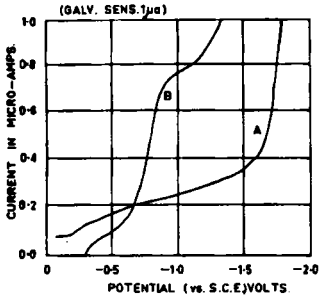

Fig. 2.—Key: A, 10 ml. of water + 2 ml. of 0.1 *N* KNO_3 + 1 ml. of 0.1% gelatin (blank); B, 10 ml. of 100 mcg./ml. of thimerosal in water + 2 ml. of 0.1 *N* KNO_3 + 1 ml. of 0.1% gelatin.

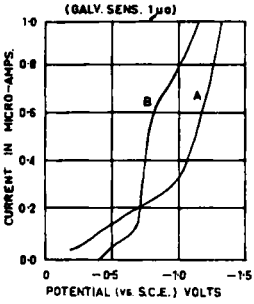

Fig. 3.—Key: A, 10 ml. of triple antigen vaccine + 2 ml. of 0.1 *N* KNO_3 + 1 ml. of 0.1% gelatin (blank); B, 10 ml. of 100 mcg./ml. of thimerosal in triple antigen vaccine + 2 ml. of 0.1 *N* KNO_3 + 1 ml. of 0.1% gelatin.

EXPERIMENTAL

Equipment.—A Tinsley self-recording polarograph was used at damping 3 with an H-type cell against a saturated calomel electrode and a saturated potassium chloride/agar bridge maintained at 25 ± 0.5°, in a thermostatically controlled water bath.

The drop time in open circuit in distilled water was 4.4 seconds per drop. The *M* value of mercury was 2.10 mg. per second.

Procedure.—Estimations were carried out in duplicate in 10-ml. quantities of the sample, to each of which was added 2 ml. of 0.1 *N* potassium nitrate in glass-distilled deionized water to serve as supporting electrolyte and 1 ml. of 0.1% gelatin as maximum suppressor.

Two waves characteristic of thimerosal were registered at −0.75 v. and −1.35 v., respectively, but for our purposes, only the first wave (shown in Fig. 2) was used for measurement. Figure 3 shows the comparable polarogram for triple antigen.[2]

[2] Triple antigen is an alternative name for diphtheria, tetanus, and pertussis vaccine B.P.

Standard curves for thimerosal in aqueous solution and in triple antigen and pertussis vaccines were established in the following manner.

Standard Curve for Thimerosal in Aqueous Solution.—Three reference solutions containing respectively 50, 25, and 12.5 parts of thimerosal per million of deionized water were prepared from a 0.01% solution which in turn was derived from a 1% stock solution.

Ten milliliters of reference solution were pipeted into a dry test tube, and 2 ml. of 0.1 *N* potassium nitrate and 1 ml. of 0.1% gelatin were added. The contents of the tubes were mixed and, after transfer to a dry polarographic cell, freed from oxygen by bubbling in oxygen-free nitrogen.

Polarograms of each of the four standard concentrations were recorded and the standard curve was plotted from the values obtained. (See Fig. 1, curve A.)

Standard Curve for Triple Antigen Vaccine.—A standard curve for a series of comparable concentrations of thimerosal in triple antigen was constructed in the same manner. (See Fig. 1, curve B.)

RESULTS AND DISCUSSION

In constructing the standard curve for thimerosal in triple antigen, it was noted that the values registered for a concentration of 12.5 p.p.m. were less than those to be expected by extrapolation of the curve for higher values. Hence, values for the assay of thimerosal in solutions containing this quantity or less represent only a qualitative indication of the presence of the antiseptic. Values registered in this region of the curve indicate that the "limit" of assay is about 20 p.p.m., and anything less than 12.5 p.p.m. can only be regarded as indicating the presence of a trace.

The standard curve for pertussis vaccine was essentially identical with that derived from triple antigen.

Figure 2 shows two polarograms, curve B representing that of an aqueous solution containing 100 parts of thimerosal per million and curve A the "blank"—a solution of the same composition, except that it lacked thimerosal. In curve B only the first thimerosal wave, appearing at −0.75 v. half-wave potential, is shown.

Figure 3 represents comparable polarograms recorded for solutions in which triple antigen vaccine is substituted for water as the diluent. Curve B represents a solution of thimerosal in the vaccine and curve A the thimerosal-free blank. Replacement of triple antigen by pertussis vaccine furnishes solutions giving an analogous picture.

SUMMARY

The polarographic method of assay makes it practicable to determine quickly low concentrations of thimerosal in aqueous solution or in vaccines, provided the antiseptic is present in an amount not less than 20 p.p.m. Using standard curves, thimerosal concentrations in unknown strength above this limit are capable of being determined to within 5%.

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