Water-Soluble Extractives of Disposable Syringes
Nature and Significance

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Disposable syringes have found wide application in clinical and laboratory medicine. They are used for many purposes in addition to injection of drugs, such as the transfer of radioactive solutions, sterilization of solutions (when used in conjunction with an ultrafiltering device), withdrawal of blood, etc. It is generally assumed that they are made of materials that are essentially chemically inert. However, two brands of disposable syringes, selected at random, were examined and found to yield water-soluble extractives. The extractives from the two brands were different materials. One extractive has been identified as 2-(methylthio)benzothiazole. Biological activity (fungistatic and insecticidal) has been ascribed to this substance. The second extractive has not been identified but was found to inhibit the *in vitro* hydrolysis of adenosine triphosphate (ATP) by cardiac actomyosin. These findings indicate that at least some types of disposable syringes may not be considered as interchangeable with glass syringes.

DISPOSABLE syringes from two different manufacturers, when used to transfer aqueous solutions, have been found to contribute a substantial amount of contaminant to the solutions. The contaminant was detected by its absorption of ultraviolet (U.V.) light in the range of 200 to 300 mμ. The rubber portion of the plunger was the source of the contaminants, and a distinctly different extractive was found in the two types. The plastic parts of the syringes yielded no extractives in either water or 0.9% NaCl. The brands studied were randomly selected and, therefore, have been designated *A* and *B*. The findings serve as examples of an existing problem from both the standpoint of the undesirable addition of the extractives to injectables and the possible chemical interaction of the extractives with drugs. These problems also apply to the use of these syringes in laboratory investigations.

METHODS

The plungers of type *A* and *B* disposable syringes were soaked in 2 ml. of water or 0.9% NaCl for varying periods of time at room temperature. The extractions were carried out in round-bottom Pyrex glass tubes with an internal diameter of 22 mm. With 2 ml. of solution, the rubber portion of the plungers was completely submerged. In one experiment described below, in which 1 ml. of water was used for the extraction, the rubber portion of the plunger was more than 90% submerged. The syringes examined were of intermediate sizes (5- to 10-ml. range). The data pertain to one size from each manufacturer, although the other sizes tested showed similar results. Water distilled from an all-glass apparatus following a preliminary deionization was used for all the studies. A Bausch & Lomb model 505 recording spectrophotometer was used to analyze the absorption spectra of the solutions. The path length of the cells was 1 cm. Most of the samples had to be diluted (up to fifteenfold in some instances) to keep the absorbance recording within the chart span of the instrument.

Rabbit cardiac actomyosin was prepared as described previously (1). The actomyosin precipitate obtained at ionic strength 0.065 (pH 6.9–7.0) was dissolved in 1 vol. of 2 *M* KCl. The solution was diluted with water to a KCl concentration of 0.44 *M* and centrifuged at 1650 *g* for 10 min. The supernatant solution was diluted with an equal volume of water, and the precipitate was collected by centrifugation at 1650 *g* for 20 min. The precipitate was washed twice with 10 vol. of 0.22 *M* KCl. The protein was collected each time by centrifugation as above and dissolved finally in 0.5 vol. of 2 *M* KCl. Protein concentration of the translucent actomyosin preparation was determined by a previously described modification of the biuret assay (2).

Adenosine triphosphatase (ATPase) activity of cardiac actomyosin was assayed as described before

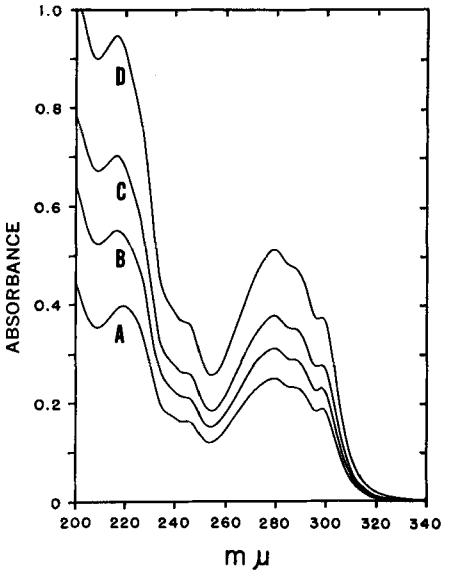

Fig. 1.—Ultraviolet absorption spectra of the progressive extracts of the rubber tip of plunger of type *A* disposable syringe. The plunger was allowed to stand in 2.0 ml. of water for the following periods: curve A, 1 min. extraction; curve B, 1 hr. extraction and extract diluted fivefold for absorbance measurements; curve C, 24 hr. extraction and diluted fifteenfold; curve D, 42 hr. extraction and diluted fifteenfold.

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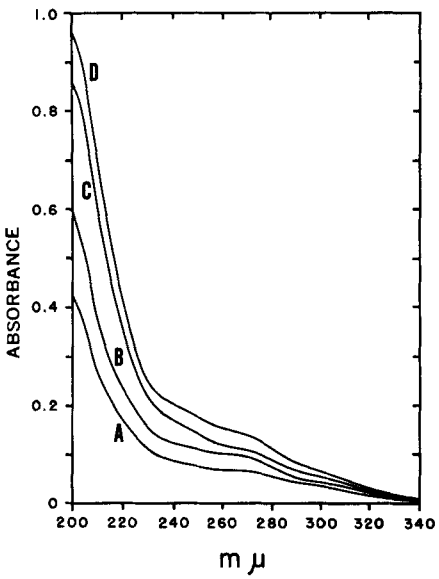


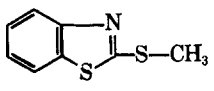
Fig. 2.—Ultraviolet absorption spectra of the progressive extracts of the rubber tip of plunger of type *B* disposable syringe. The plunger was allowed to stand in 2.0 ml. of water for the following periods: curve A, 1 hr. extraction; curve B, 4 hr. extraction; curve C, 24 hr. extraction and extract diluted twofold; curve D, 42 hr. extraction and diluted twofold.

(1) except that the reaction mixtures contained 12.5 mcg. of protein N/ml. instead of 15.

RESULTS

The results of the progressive water extraction of U.V. absorbing material from the type *A* and *B* syringes are presented in Figs. 1 and 2, respectively. The progressive nature of the extraction, particularly in the case of the type *A* plunger, is also demonstrated by the data presented in Fig. 3. Absorbance of the extracts at 200 mμ is plotted as a function of the extraction time. The chart span of our spectrophotometer was one absorbance unit. The higher values were calculated from the absorbance of diluted samples.

The principal substance extracted from the type *A* plunger was identified by its absorption spectrum to be 2-(methylthio)benzothiazole (3, 4). The ultraviolet absorption spectrum for this material, taken from one of the references cited (3), is shown in Fig. 4. The slight difference between the spectrum of the type *A* rubber extractive (Fig. 1) and the spectrum of pure, 2-(methylthio)benzothiazole (Fig. 4), can be attributed to the presence of small amounts of other extractives from the rubber. The chemical structure of this compound is



2-(Methylthio)benzothiazole

The amounts extracted in 1 min. and 1, 24, and 42 hr. were 0.07, 0.41, 1.49, and 2.02 mg., respectively. These values were calculated from the extinction data available for this compound (3). The absorption spectra of the prolonged extractions gave evidence of an additional minor component or components. The extractive from the type *B* syringe has not yet been identified, and the amounts extracted are not known. In terms of absorbance at 200 mμ, however, the type *B* extractive is about one-eighth the amount of *A*. The spectra suggest that the rubber in type *A* and *B* plungers is differently compounded. Syringes made entirely of glass contributed no U.V. absorbing material.

When the rubber plungers were soaked in 0.9% NaCl under the same conditions, the rate of extraction for the type *A* plunger was approximately 90% of the rate with water. The rate of extraction for the type *B* plunger was the same with 0.9% NaCl or water. The qualitative nature of the extractives obtained with both solutions was the same.

The plastic parts of the syringes (plunger stems and barrel) were tested separately for extractables in water and 0.9% NaCl at 25° for up to 70 hr. As much as 5 ml. of solution was used in some tests to allow a substantial amount of contact of the solution with the plastic surfaces. No extractives were detected in any of these tests.

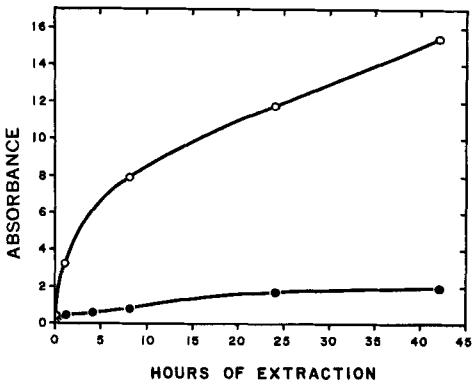


Fig. 3.—Absorbance at 200 mμ of the water extracts of type *A* (○) and type *B* (●) rubber syringe plungers plotted as a function of the extraction time. The plungers were allowed to stand in 2.0 ml. of water for varying periods of time at room temperature. The earliest reading taken for the type *A* plunger was after 1 min. of extraction.

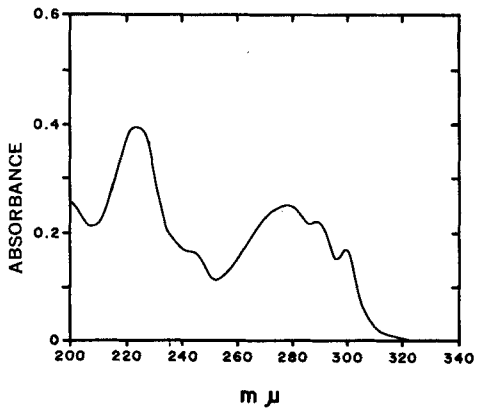


Fig. 4.—Ultraviolet absorption spectrum of 2-(methylthio)benzothiazole (3, 4) calculated for a concentration of 0.033 mg./ml. from the original data (3).

TABLE I.—INHIBITION OF CARDIAC ACTOMYOSIN ATPASE BY WATER EXTRACTS[a] OF TYPE B SYRINGE PLUNGERS

Extraction Time	% Inhibition —Actomyosin Preparations— 1	2	3
2 min.	...	...	18.1
2 hr.	...	21.3	24.8
4 hr.	...	27.5	31.2
20 hr.	...	31.5	47.8
45 hr.	50.8	...	...

[a] All extracts were diluted threefold for the ATPase assays.

The rubber plunger extracts were tested for chemical reactivity in an enzyme system which has been studied extensively in this laboratory. Type *A* and *B* syringe plungers were extracted with water for varying periods of time at room temperature as described above. The volume of water was 2.0 ml., except for the shortest extraction period (2 min.), where the volume of water was 1.0 ml. The syringe extracts represented one-third the final reaction mixture volume of the ATPase assays. Glass plungers of glass syringes were also allowed to stand in the same volumes of water as above. These solutions had no effect on the control ATPase activity of cardiac actomyosin. Extracts of the type *A* syringe also had no effect, but the type *B* extracts showed a distinct inhibition of ATPase activity which increased progressively with the period of extraction. Actomyosin from three animals was tested for sensitivity to these extracts. These results are summarized in Table I.

DISCUSSION

The findings reported above are examples of a continuing problem rather than a new one. The extraction of contaminants from various plastics and the binding of drugs to plastics have been studied and reviewed by Autian (5). Disposable needles have also presented serious contamination problems (6). In regard to rubber items, studies of leaching from rubber closures of injection vials have pointed out the need for quality control in this area (7, 8). In all of the investigations cited (5–8), the authors pointed out the existing need for adequate standards for rubber, plastic, and metal parts of disposable items.

The identification of the type *A* extractive as 2-(methylthio)benzothiazole permitted a survey to be made of its known biological actions. Zsolnai (9) has reported a fungistatic effect of this material on six out of seven organisms which were tested in liquid media containing 10% serum. The material was effective in concentrations of 1 part in 5,000 with three organisms and 1 in 10,000 with the other three organisms. The plunger of the type *A* syringe in 2 ml. of water at room temperature for 1 hr. (see text) yielded a 2-(methylthio)benzothiazole concentration of 1 part in 4,900. After only 1 min. of extraction, the concentration was 1 in 29,000.

In a study of insecticidal actions of a number of organic sulfur compounds, Davies and Sexton (10) reported 2-(methylthio)benzothiazole to be an effective agent. In five out of seven insect species in which it was tested, it was equal to or greater in effectiveness than an equal concentration of lead arsenate. In these experiments, the compound was ingested by the insects. It was also effective as a spray (0.2% solution) against three of four species tested, with mortalities of 32, 73, and 86%. The concentration of the compound in the 42-hr. water extraction of the type *A* plunger (see text) was 0.1%.[1]

The reason for the presence of 2-(methylthio)benzothiazole in the rubber was not explored. Some benzothiazoles have been used for many years as accelerators in the vulcanization of rubber (11).

The inhibition of cardiac actomyosin ATPase by extractives of the type *B* plunger points out the problem presented to the laboratory investigator. The syringe coming in contact with a reaction mixture is not ordinarily considered as an experimental variable. The need for this consideration is also evidenced by the data presented in Figs. 1 and 2. The type *A* extractive was not inhibitory to ATPase; however, its presence would influence the validity of absorbance measurements in the range of 200 to 300 mμ.

The British Standard (12) lists a toxicity test for transfusion rubbers which requires demonstration of a lack of growth-inhibiting activity of rubber segments embedded in culture plates of *Streptococcus pyogenes*. The "United States Pharmacopeia" (13) safety test for transfusion and infusion assemblies consists of a single i.p. injection in 5 mice of a 1-hr. saline extract of the assembly (extraction at 85°). No symptoms of toxicity must be observed at 4, 24, and 48 hr. after the injection. A combination of both tests plus ultraviolet absorbance measurements to determine the presence of rubber extractives would be useful in controlling the quality of rubber plungers for disposable syringes.

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[1] In view of the recent emphasis on the prepackaging of pharmaceuticals in disposable syringes, the significance of the progressive nature of the extractions we observed should be explored further.