Toxicity of Plastics Used in Medical Practice II

Toxicity of Citric Acid Esters Used as Plasticizers

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The acute and chronic toxicity of triethyl citrate, acetyl triethyl citrate, tributyl citrate, and acetyl tributyl citrate was studied in a series of small animals. The behavior response to parenteral administration of the esters was observed in rats, mice, frogs, and rabbits. Mice were used to determine the LD_{50} for each compound. All of the esters exhibited a marked effect on the CNS evidenced by increased respiration rate, convulsions, and cord depression. When applied locally to the nerve, the citrates rapidly blocked nerve conduction. A profound depressor action was observed in cats and rabbits which was attributed to a direct cardiac inhibitory effect. Chronic administration for a period of 2 weeks resulted in a depression of weight gain; in the animals that received acetyl tributyl citrate evidence of a change in the blood picture was observed. Histopathological examinations revealed no damage to the liver, kidney, lungs, and spinal cord in the chronic animals.

MUCH PROGRESS has been made in the use of plastics for various devices used in medicine and pharmacy in the last 5 years. Advancements in plastic technology have made possible many improvements in existing devices which would not have been possible with more conventional materials. However, certain problems have been noted which indicate that greater care should be taken in selecting the plastic device (1–3).

Tubings for the administration of drug and blood products or for the collection of body fluids may be composed of one or more polymeric materials. Usually the polymer is of the vinyl type, if softness, flexibility, and clarity are desired. Plasticizers and other additives must be added to this polymeric material to give the final device the desired physical and chemical properties. Thus, it is possible to have a host of plastic formulas, referred to as polyvinyl chloride, but each differing from the other in composition. In most instances the various ingredients used for polyvinyl chloride tubings which are to be used in medical practice have been approved as food additives by the FDA.

In 1960, Autian and Kapadia (4) reported that certain polyvinyl chloride tubings released a constituent to several hydroalcoholic solvent systems. They did not, however, investigate the possible biological effect of the leached constituent. Meyler, Willebrands, and Durrer (5) in the same year reported that certain polyvinyl chloride tubings released a constituent to blood which in turn caused cardiac arrest and ventricular fibrillation on the isolated perfused heart of rats. Keith, *et al.* (6), reported that one lot of plastic tubings (vinyl type) appeared to enhance the hemolytic effect on perfused blood. This observation was further substantiated with *in vitro* studies by Hirose and associates (7). Lawrence, *et al.* (8), noted that a number of commercially available polyvinyl chloride tubings used as part of administration devices released a constituent into tissue causing a toxic response when implanted for short periods of time in animals. In this particular study the offending agent in the plastic was not the plasticizer but one of the other additives.

Even though the work of Lawrence, *et al.*, revealed that the plasticizers were not causing adverse effects by the experimental method employed, there is no assurance that the same plasticizers or other plasticizers will not produce a biological effect when administered in a different manner to animals. Since present vinyl tubings contain a large proportion of one or more plasticizers and since these tubings will have contact with drug systems or body tissues for varying periods of time, it was thought prudent to investigate the pharmacological properties of one series of plasticizers (citric acid esters) which had previously been reported as quite safe by oral feeding (9).

EXPERIMENTAL

The compounds used in this study included triethyl citrate (TEC), acetyl triethyl citrate (ATEC), tributyl citrate (TBC), and acetyl tributyl citrate (ATBC). The esters were commercial samples containing less than 1% foreign material.[1] Because of the nearly insoluble nature of the butyl

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[1] Samples supplied by Charles Pfizer and Co., Inc., Brooklyn, N. Y.

derivatives, all the esters were administered suspended or dissolved in 3% acacia throughout the experimental work. Ester concentration was varied to be suitable for each particular study.

Behavior Studies.—The general overt effects of the esters were first observed following i.p. administration of graded doses into Swiss albino mice. Both of the ethyl citrates produced a very rapid loss of righting reflex without loss of consciousness in doses slightly exceeding 400 mg./Kg. Usually the animals regained their posture within 15 minutes. Respiration rate was markedly increased and frequently clonic convulsions were observed. The butyl derivatives failed to produce the rapid loss of righting reflex, but did evoke an increase in respiration which was frequently accompanied by clonic convulsions. Some writhing was observed during the first 10 minutes after injection of the butyl compounds. All of tne esters produced the same signs of stimulation in Wistar rats when given i.p. with both TEC and ATEC, again causing a rapid loss of righting reflex of short duration when the dosage exceeded 400 mg./Kg. The stimulating properties of the esters were confirmed by intravenous administration into rabbits. Marked increases in motor activity and respiration were observed following doses of 100 mg./Kg.

Doses of 1000 mg./Kg. placed in the ventral lymph sac of the frog resulted in a short period of clonic activity, followed by the complete abolishment of all reflex activity.

Acute Toxicity.—The i.p. LD_{50}'s for the esters were determined in Swiss albino mice weighing between 16 and 20 Gm. Doses were chosen from a logarithmic scale and the median lethal dose calculated by method of Bliss (10). All deaths occurred in the first hour following administration of TEC and ATEC, but in the case of the butyl compounds a 72-hour observation period was required. The LD_{50} with standard error for TEC was 1750 ± 68 mg./Kg.; for ATEC, 1150 ± 185 mg./Kg.; for TBC, 2900 ± 210 mg./Kg.; and for ATBC, >4000 mg./Kg. Death was attributed to circulatory collapse and postictal depression.

Chronic Toxicity Studies.—To study cumulative effects of the citrates, a 14-day chronic toxicity test was carried out in which mice weighing from 16–20 Gm. were given daily i.p. doses which approximated one-fifth of the acute median lethal dose. Five groups of 20 mice each were selected and the weight, RBC count, WBC count, clotting time, and hemoglobin level of each individual determined. Group *A* received daily injections of TEC 350 mg./Kg.; Group *B*, 230 mg./Kg. of ATEC; Group *C*, 580 mg./Kg. of TBC; and Group *D*, 900 mg./Kg. of ATBC. Group *E* received daily injections of the 3% acacia vehicle and served as the control. The animals were weighed daily and at the end of the 2-week period the RBC count, WBC count, clotting time, and hemoglobin level were redetermined.

Figure 1 shows that all of the compounds inhibited normal weight gain. At the 7-day period this inhibition was significant within 95% confidence limits. It was most striking in the case of ATBC which, although given in the largest doses, had exhibited the lowest acute toxicity.

There were no significant differences in the RBC counts, the WBC counts, the clotting times, and the hemoglobin levels in the mice that received TEC, ATEC, or TBC. However, significant (95% confidence) fall in the RBC count and hemoglobin levels in those animals administered ATBC occurred.

In a further study of the blood picture changes due to ATBC, two albino rabbits were administered 450 mg./Kg. of ATBC daily for 14 days. Two others were given 900 mg./Kg. daily for 7 days. At the end of the period, the RBC count, hemoglobin level, and a differential WBC count were made; red bone marrow smears from the femur bone were examined. In all animals there was a fall in the red blood cell count ranging from 0.5 to 2.5 million and a corresponding fall in hemoglobin levels. However, the basophil count and the bone marrow smears gave no indication of aplastic anemia.

At the end of the chronic toxicity study, two mice from each group were sacrificed; slides were made of liver, lung, and kidney tissues. Examination of these slides did not reveal pathological cellular changes in these organs.

On the day following withdrawal from the chronic test, hexobarbital sleeping time studies were conducted in the remaining mice. There was no significant difference in the sleeping time within the various groups. This confirmed that liver function was not impaired by chronic administration of the esters.

Effects of the Esters on Neural Muscular Transmission.—Behavior studies indicated that the citrate esters produced some neurological effects. Therefore, a series of studies was set up to determine the nature of the neural involvement. In the first experiment, white rats were anesthetized with pentobarbital, and right anterior tibialis muscle freed and arranged for recording contractions, and shielded electrodes placed central to a pledget of cotton around the sciatic nerve. Stimulus was supplied through the electrodes by a stimulator set to deliver a current which would produce a submaximal response every 10 seconds. After obtaining a normal response, 3 drops of the ester were placed on the cotton. All four esters produced a complete sciatic nerve block which was reversible when the cotton was removed and the nerve washed with saline. This activity was confirmed using the isolated gastrocnemius muscle-sciatic nerve preparation from the frog.

To determine central nervous system involvement, pentobarbital anesthetized white rats were set up so that the contralateral reflex could be recorded. After freeing the anterior tibialis muscle in the left leg and arranging it for recording contractions, shielded electrodes were placed around

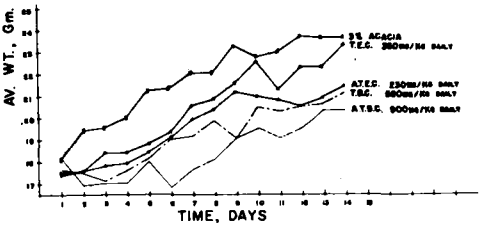

Fig. 1.—The effect of some citric acid esters administered i.p. on weight gain of mice.

the sciatic nerve in the right leg. The stimulator was set to deliver a series of five impulses 10 seconds apart when activated by a switch. After determining a normal submaximal contralateral reflex, each ester was administered through the jugular vein which had previously been cannulated. Figure 2 shows that all of the esters were capable of completely blocking this reflex. The duration of blockage varied according to the ester. Considerably larger doses of ATBC were required to affect the block, but the duration was much longer. In contrast, TBC block occurred in response to the lowest dose but lasted longer than that produced by either of the ethyl esters.

To verify that cord depression and not myoneural junction blocking was the cause of the inhibited contralateral reflex, rats were prepared so that the sciatic nerve stimulation and anterior tibialis muscle contraction occurred on the same side. Doses of the esters which were twice as great as those which abolished the contralateral reflex failed to impair myoneural junction transmission.

Local Anesthesia Action.—All four of the esters temporarily abolished the corneal reflex when 3 drops of a 5% suspension were placed in the conjunctival sac of the rabbit. As expected, the butyl esters were longer in duration than their ethyl counterparts. This local anesthetic effect was confirmed by the intradermal administration of

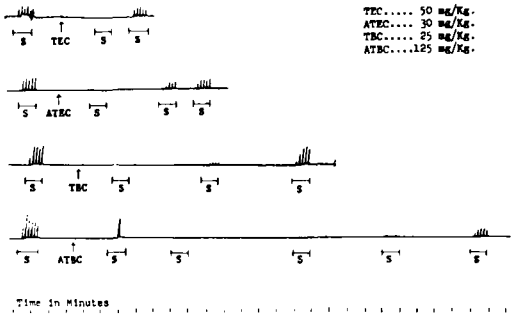

Fig. 2.—Effect of some citric acid esters administered i.v. on the contralateral reflex of the rat.

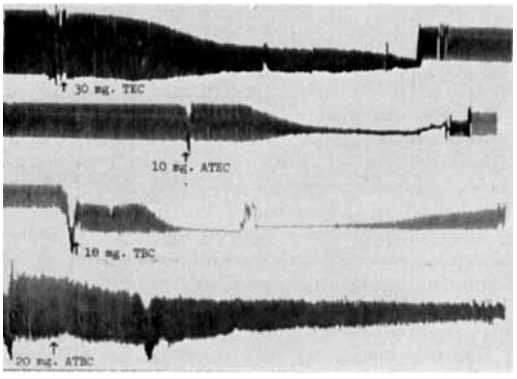

Fig. 4.—Effect of some citric acid esters on rate and amplitude of the isolated rabbit heart.

0.1 ml. of a 2% suspension of the esters into shaved areas on the back of guinea pigs. The duration of insensitivity to pricking of the surrounding area with a pin was 12 to 20 minutes for TEC. However, TBC produced a deadened area for a period of over 2 hours.

Cardiovascular Action of the Citrate Esters.—Acute toxicity studies indicated probable cardiovascular collapse, so a study of the effect of the citrate esters on blood pressure was indicated. Rabbits and cats were used in this study. Blood pressures were recorded from the cannulated carotid artery with a mercury manometer. A tambour connected to the tracheal cannula simultaneously recorded respiration. The esters were administered through the ear vein or through the cannulated jugular. Figure 3 is a tracing showing the typical responses obtained from varying doses of the esters in rabbits. There was an apparent dose-response relationship with all the compounds, and all of them produced complete loss of blood pressure when administered in toxic doses. Interestingly, the comparative doses required to produce a fall in blood pressure for the esters were quite well related to those which were required to produce death. ATBC with a high LD_{50} (greater than 4000 mg./Kg.) did not produce a significant fall in blood pressure in low

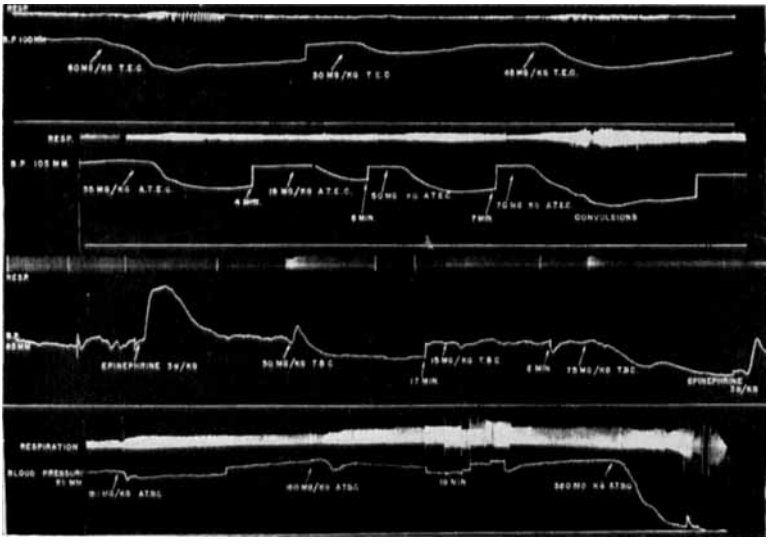

Fig. 3.—Influence of some citric acid esters on blood pressure and respiration of rabbits under pentobarbital anesthesia.

doses. The depressor effect of the esters was similar in cats and rabbits. It could not be blocked by atropine, and it could be reversed by epinephrine. This was indicative that the effect was due to direct smooth muscle relaxation or to the cardiac depression which was quite apparent.

To show that the depressor effect was primarily due to cardiac inhibition, the esters were tested on the isolated rabbit heart after the method of Langendorff (11). As can be seen in Fig. 4, all of the compounds brought about a sharp decline in both rate and amplitude of the heart beat. If given in carefully controlled doses, the heart returned to normal, but higher doses resulted in complete heart arrest. Coronary flow was also markedly diminished, precluding the possibility of significant direct smooth muscle relaxation.

SUMMARY AND CONCLUSIONS

The results of this study indicate that the four citric acid esters used as plasticizers have well defined and marked pharmacological activity when administered parenterally. All four have local anesthetic action and can block neural transmission when they come in direct contact with a nerve trunk. All of them diffuse from the blood stream into the cord in sufficient concentration to depress cord function. At the same time they apparently stimulate the higher centers. Although they vary widely in potency, both the ethyl esters and the butyl esters depress cardiac activity sufficiently to produce cardiovascular collapse. Chronic administration of three of them (TEC, ATEC, and TBC) does not appear to have an effect on the blood picture, but there are indications that ATBC may inhibit production or increase destruction of red blood cells. No species variation was observed throughout the investigation.

One must take care not to extrapolate the results reported in this paper to actual polyvinyl chloride devices which might contain plasticizers as reported here, since the biological activity will be elicited only when the plasticizer is released into the body fluids and, further, that the magnitude of the response shall have a direct relation to the concentration released. The biological responses reported here for a group of plasticizers should, however, alert those manufacturing plastic devices to learn more about the ingredients they employ in their plastic formulation if these items are to be introduced into medical practice.

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Rheology of Gelatin Films

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A procedure has been developed for the evaluation of the viscoelastic properties of gelatin films employed in soft gelatin capsule formulations. It involves the measurement of the tensile relaxation modulus of the film and the fitting of the resultant curve on an analog computer to obtain the appropriate constants. A theory was proposed for the mechanism of stress relaxation in gelatin films. Based upon this theory, an equation for the tensile relaxation modulus was derived. This equation was of the same form as the empirical equation which fit the stress relaxation curves of the gelatin films.

ALTHOUGH MUCH INFORMATION has been gathered concerning changes in the rheological properties of gelatin solutions upon thermal aging, little is known of the characteristics of gelatin films. Since the rheological behavior of gelatin films influences the properties of capsules molded from them, information concerning changes in this behavior might be helpful in evaluating technological problems encountered in the encapsulation process.

The viscoelastic properties of gelatin have been investigated by several authors. Among them, Ferry (1, 2) has studied the concentration effect on gel rigidities, and Tobolsky (3, 4) has studied stress relaxation as a tool in the investigation of gelation mechanisms. In this study the stress relaxation model has been

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