N-methylcarbamoxypropane, Table I, No. 13, m.p. 55–57°, using the previously given procedure.

BIOLOGICAL RESULTS

All of the above carbamates except Compound 13, for which no suitable vehicle could be found, were screened for muscle relaxant activity in mice. A substance was said to possess muscle relaxant activity if a given dose caused the hind portion of the test animal to go limp while it was still able to walk on its front legs, dragging the back ones behind. All known muscle relaxants gave this test. Using this criterion, none of the above compounds showed significant muscle relaxant activity up to 1000 mg./Kg. However, the ability to delay the onset of pentylenetetrazol induced convulsions appeared to be general, particularly in Compounds 9 and 10.

No deaths to mice resulted from oral intubation of doses up to 1 Gm. per kilogram. Ataxia, paralysis, convulsions, and decreased respiration were notably absent in all tests at these levels.

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Toxicity of Plastics Used in Medical Practice I

Investigation of Tissue Response in Animals by Certain Unit Packaged Polyvinyl Chloride Administration Devices

By W. H. LAWRENCE†, J. L. MITCHELL‡, W. L. GUESS, and J. AUTIAN

In the past workers in our laboratory and other investigators have noted that polyvinyl chloride tubings used in medical practice, as administration or collection devices, will release one or more constituents to several types of solvent systems used in pharmacy. Since a great many formulations may be employed in manufacturing these plastic tubings, it was thought that a toxicity study might reveal if one or more of the currently used plastic administration devices might contain an ingredient which could produce a tissue response when implanted in animals. The results of the study revealed that under the experimental conditions used in this study a number of the tubings will produce tissue response while others will not.

For the past number of years, workers in our laboratory have attempted to focus attention on certain problems which might develop in the improper use of plastics, while at the same time encouraging research to develop products which would give the advantages of plastics without introducing potential hazards (1–5). Several approaches in research to the plastic problem have since been undertaken by the laboratory from an academic viewpoint and as a public health service. The work reported in this paper is the first of a series devoted to the exploration of the acute and toxic properties of plastics and the various ingredients which might be incorporated within the polymer to achieve a desired plastic which may be used in medical practice. Specifically, this paper will be devoted to ascertaining if certain plastic tubings (primarily of the polyvinyl chloride type) which are parts of administration devices might contain an ingredient or ingredients which could be considered toxic if released into animal tissue.

EXPERIMENTAL

Selection of Samples for Investigation.—Various types of administration devices having a polyvinyl chloride tubing as a component were obtained in their original package. A number of these packages indicated that the contents were sterile and nonpyrogenic. Each sample was assigned a code number with the manufacturer's name designated by a specific letter. Forty-eight different samples of administration devices from 17 manufacturers or distributors were employed in the investigation (see Table I). Several polyethylene tubings and one unidentified tubing used in a hospital were also included in the total number of samples.

Implantation Studies.[1]—In all the studies reported here only the tubings were evaluated, the other portions of the administration devices being stored for

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[1] This technique was recommended to our laboratory by Dr. J. H. Brewer and Dr. H. H. Bryant, Hynson, Westcott and Dunning Laboratory, Baltimore, Md.

TABLE I.—INTRAMUSCULAR IMPLANTATION OF PLASTIC SAMPLES IN RABBITS FOR A PERIOD OF 7 DAYS[a]

Company (in code)	Sample (in code)	Result[b]	Remarks[c]
A	X-1	−	S. P.
B	X-19	+	S. P.
B	X-24	−	S. P.
B	X-25	+	S. P.
B	X-41	+	S. P.
B	X-44	−	S. P.
B	X-45	+	S. P.
B	X-49	+	S. P.
B	X-67	+	S. P.
B	X-74	+	S. P.
C	X-2	−	C. R.
C	X-17	+	C. R.
C	X-39	−	S. P. (B. T.)
C	X-54	+	S. (A. T.)
D	X-3	−	S. P.
D	X-50	−	S. P.
E	X-4	+	S. P.
E	X-48	+	S. P.
F	X-5	−	S. P.
F	X-69	−	S. P.
F	X-70	−	S. P.
F	X-71	−	S. P.
F	X-72	−	S. P.
F	X-73	−	S. P.
G	X-0	−	In roll
G	X-6	+	In roll
H	X-7	−	From opened package
H	X-66	−	S.
I	X-8	+	From opened package
I	X-61	+	S.
J	X-18	+	C. R.
J	X-21	+	S.
J	X-36	+	C. R.
J	X-37	+	S. (B. T.)
J	X-53	+	C. R.
J	X-63	+	C. R.
J	X-64	+	S.
J	X-65	+	S. (B. T.)
K	X-20	−	S. P. (S. T.)
L	X-23	−	S. (A. T.) polyethylene
L	X-52	−	S. (A. T.) polyethylene
L	X-68	−	(A. T.) polyethylene
M	X-40	+	No information concerning quality on package
N	X-47	−	S. P.
O	X-35	+	S. P.
P	X-60	−	S.
Q	X-43	−	S. P.
Q	X-55	+	Supplied to laboratory for evaluation

[a] If not designated, all tubings are of the vinyl type and are unit-packaged ready for use. [b] −, no reaction; + tissue reactions. [c] Information on package: S—sterile; P—nonpyrogenic; C. R.—clean, ready to use; (B. T.)—biologically tested; (A. T.)—animal tested; (S. T.)—safety tested.

future investigation. Sections of each sample were taken and cut into strips measuring approximately 1 mm. × 1.5 cm. and were implanted into rabbits in the following manner. For each sample two healthy, female, albino rabbits (1.6 to 2.0 Kg.) were used. Prior to an implantation, the rabbit's back

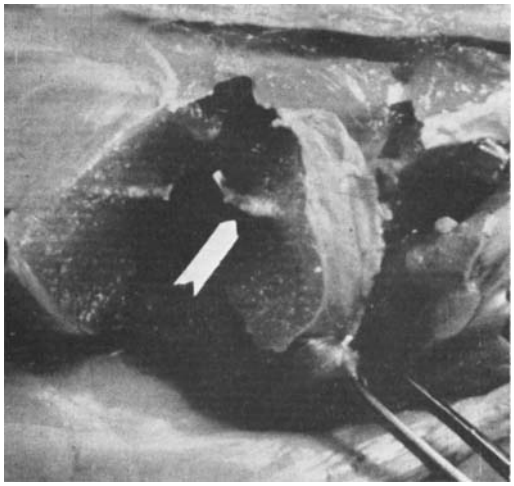

Fig. 1.—Photograph of an implantation site in rabbit muscle showing a sample of tubing X-74 (in center) after 1 week of implant. White-like zone around sample indicates tissue response.

was clipped and the excess hair removed by careful sponging (50% water-alcohol) with a lint-free towel. The rabbits were then anesthetized by an injection into the marginal ear vein of 0.6 ml./Kg. of a solution of pentobarbital sodium (50 mg./ml.) and stretched out on a table. A strip of the plastic was placed into the bevelled point of a 15 G needle having a length of 1.5 in., and the needle was introduced into the paravertebral muscle. Four strips of each sample were introduced (approximately 1 in. apart) into each animal. Two strips of a previously studied polyvinyl chloride tubing were also implanted in an identical manner as controls in the same rabbit.[2] The introduction of these plastic strips was done in a "clean" manner but not under aseptic conditions. Thiomersal solution was applied to all the sites of injection.

After implantation, each rabbit was returned to his cage and observed for a period of 1 week, at which time the animal was sacrificed and the paravertebral muscle exposed. Each implant site was isolated and examined macroscopically for tissue damage compared to the control samples. Tissue reaction or toxic reaction was indicated if an opaque or white-like zone was seen around the implant. Often the "toxic" strip showed an encapsulation which extended into the tissue for several mm. All the controls produced no apparent reaction other than the accepted mild trauma from the introduction of a foreign body. Any strip showing the tissue manifestation described above was regarded as toxic and was so recorded. If a question arose as to the accuracy of the observation, one or two more rabbits were included in the test. Invariably, if one strip showed a toxic effect, the other three strips showed exactly the same picture. The results of this 7-day study are included in Table I. Figure 1 shows a photograph of a section of muscle tissue (in rabbit) with an implanted strip (X-74). A white-like zone around the strip indicates the toxic response. Figure 2 is a subcutaneous implant (in rabbit) of sample

[2] Previous history of this sample has indicated that it is nonreactive to tissue.

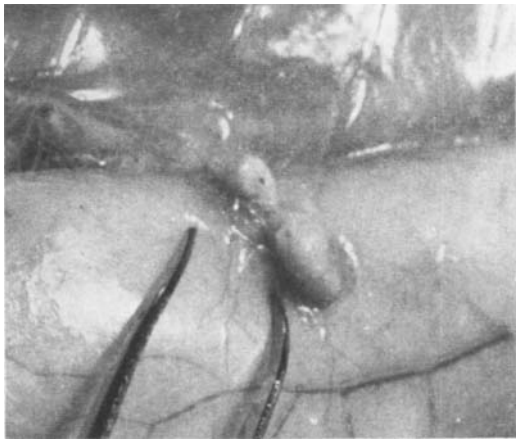

Fig. 2.—Photograph of an implantation site (subcutaneous) in rabbits of sample X-55 showing the toxic response (capsule formation around tubing).

X-55 and clearly demonstrates the formation of a capsule around the implant.

It will be noted that 25 of the 48 samples produced a toxic response under the conditions of the experiment.

Further Implantation Studies of Samples X-37 and X-74 in Rabbits.—It seemed highly desirable to investigate several of the toxic tubings in more detail, and for this reason samples X-37 and X-74 were selected, since adequate quantities of each were on hand with all the packages making up each sample having the same control number. It was felt that the samples should be of the same lot number throughout the investigations because it is known that at times a company may change its formulation for a tubing without revealing that this has been done.

In one series of experiments, several groups of rabbits were implanted with X-37 and X-74 by the intramuscular route as described. Both samples were also implanted by the subcutaneous route in several groups of rabbits. All animals were observed for a period of 28 days, a representative animal from each group being sacrificed at the end of each week for evaluation of toxicity.

Table II.—Reaction Induced in Rabbit Tissue by Samples X-37 and X-74 (Intramuscular Implantation)

Sample	Days 7	14	21	28
X-37[a]	*e*	*e*	*e*	*e*
X-74[a]	*d*	*b*	*b*	*b*
Control	*b*	*b*	*b*	*b*

[a] Four strips of each sample were used in each animal. Two control strips were also employed in the same animal. [b] Nonreactive. [c] Slightly reactive. [d] Moderately reactive. [e] Very reactive.

Table III.—Reaction Induced in Rabbit Tissue by Samples X-37 and X-74 (Subcutaneous Implantation)

Sample	Days 6	14	21	28
X-37[a]	*f*	*f*	*e*	*e*
X-74[a]	*e*	*c*	*c*	*d*
Control	*b*	*b*	*b*	*b*

[a] Four strips of each sample were used in each animal. Two control strips were also employed in the same animal. [b] Nonreactive. [c] Questionable. [d] Slightly reactive. [e] Moderately reactive. [f] Very reactive.

Table IV.—Reaction Induced in Rat Tissue by Samples X-37, X-55, and X-74 (Implantation in Thigh Muscle)

Sample[a]	Days 7	14	21	28	35
X-37	*g*	*d*	*e*	*e*	*e*
X-55	*f*	*f*	*f*	*f*	*f*
X-74	*d*	*g*	*c*	*c*	*b*

[a] One strip was placed into right thigh of the animal, and a control strip placed into the left thigh. [b] Nonreactive. [c] Questionable. [d] Slightly reactive. [e] Moderately reactive. [f] Very reactive. [g] Sample lost.

Tables II and III show the results and reveal that while X-37 persists to induce a toxic response, X-74 reaches a critical time period after which the toxic response appears to regress to a point where the sites are identical to the control samples.

Surgical implants of both X-37 and X-74 were performed in the brain tissue of the rabbits (five to each sample), and these animals were observed over a period of time up to 1 month. Within a period of 3 to 7 days, several of the rabbits were sacrificed and the brain tissue examined. Both samples produced tissue alteration but X-37 to the greatest degree. Within a 10-day period, several of the rabbits were observed to have a swelling immediate to the implant. Gross examination of the swollen area revealed that the cause of the swelling was because of the accumulation of fluid. It was first thought that an infection had caused the accumulation of fluid, but bacteriological examination of both the tissue and fluid proved this assumption to be false. Daily observation of these rabbits indicated that no general

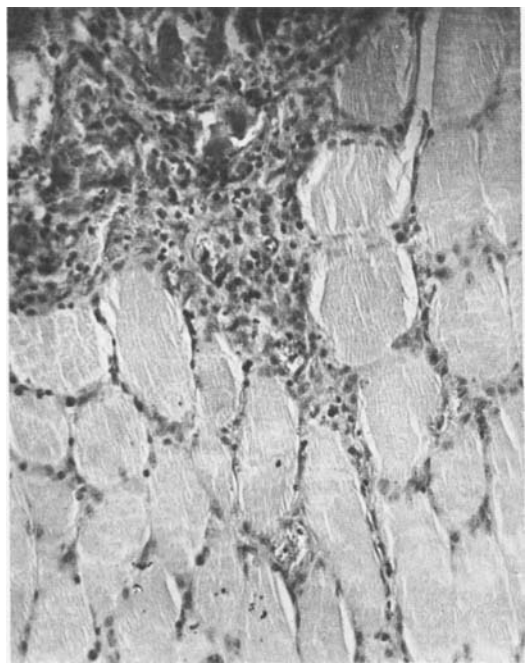

Fig. 3.—Photomicrograph of muscle tissue reaction in rabbit after implantation with sample X-37. Multinucleated giant cells and scattered polymorphonuclear leucocytes are in evidence (1 × 256).

pattern of behavior could be resolved to indicate the effect the implants had. Several of the rabbits died between the third and fourth week. Extensive brain damage could be seen in these rabbits after autopsy.

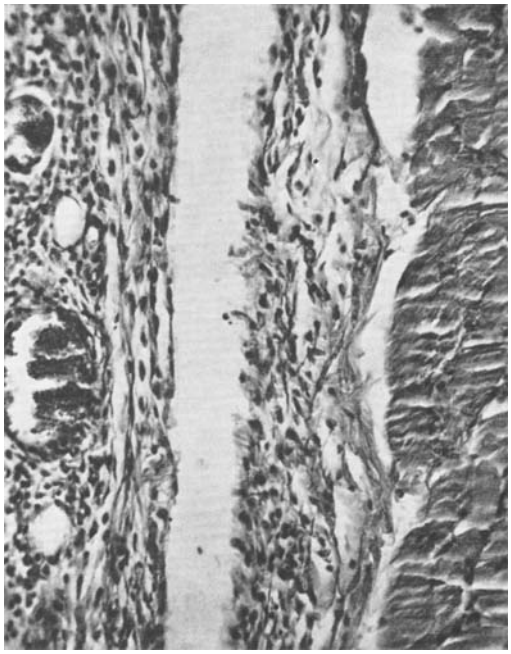

Fig. 4.—Photomicrograph of muscle tissue in rat after implantation with sample X-55. Severe damage to tissue may be noted by observing the formation of fiberous connective tissue (1 × 256).

Fig. 5.—Photomicrograph of muscle tissue in rabbit where a control plastic (nontoxic) was implanted. Mild inflammation may be noted at interface of plastic and tissue because of expected foreign body reaction (1 × 256).

Further Implantation Studies on Samples X-37, X-55, and X-74 in Rats and Mice.—To validate that the reactions seen in the rabbits were not specific to that animal, rats and mice were employed. The rats were of the Holtzman albino type, female, with an average weight of 300 Gm. Uniform samples of the three plastics were implanted by surgical procedure into a pocket formed between two layers of the thigh muscle in three groups of five rats. The implantation sites were closed by sutures. A nonreactive plastic strip (as employed with the rabbits) was implanted in the other leg of each rat as a control. Each week one rat from each group was sacrificed and the sites examined. Table IV summarizes the results.

Samples of the above tubings were surgically implanted into the nape of the neck of three groups of five mice (male, albino, average weight of 30 Gm.). The incisions were sutured, and the mice observed for a period of 1 week at which time they were sacrificed. Toxic reactions were noted for the three in the same order as the rats.

Histopathological Studies.—To rule out that the toxicity was not because of bacterial contamination, sterile transfer was made from a representative number of toxic implants and cultured on agar plates. The absence of growth after the 7-day and 14-day incubation period indicated that the tissue response was due to the plastic or a component in the plastic.

Representative implantation sites were carefully excised from the several animals employed in this study and placed into 10% formalin solution for histological examination. Sections were prepared and stained by the standard hematoxylin-eosin method and examined by a pathologist to confirm the toxic response noted by the macroscopic evidence. Figure 3 is a photomicrograph of tissue reaction (muscle of rabbit) due to X-37. Multinucleated giant cells and scattered polymorphonuclear leucocytes are in evidence indicating the destructive effect of the implant. Severe tissue damage in rats by X-55 is shown in a photomicrograph in Fig. 4. Damage to the tissue has reached a point where fiberous connective tissue may be observed around sections of the plant. Figure 5 is included to show the effect of the control sample of polyvinyl chloride implanted in rabbit muscle. Slight foreign body reaction may be noted along the interface of the plastic and the tissue.

Extraction of Toxic Substance from Plastic.—Ten grams of each sample (X-37 and X-74) were extracted with 75 ml. of 95% ethyl alcohol in a soxhlet extractor for a period of 12 hours. Both samples were removed from the apparatus and rinsed with distilled water, after which they were dried with lint-free towel. Four strips of each sample were implanted by the intramuscular route, and, likewise, the same number of samples were implanted by the subcutaneous route into separate rabbits. These series of animals were sacrificed after 1 week and the sites of implants carefully examined for tissue alteration. None of these strips produced a reaction which differed from the control implants.

The alcoholic extracts were evaporated until a syrupy liquid remained. Approximately 2 to 3 Gm. of the total weight of the plastic sample was extracted by the alcohol. In general the physical ap-

pearance of the syrupy liquid resembled a plasticizer. Strips of nonreactive plastic were placed in intimate contact with the extracts of both samples for a period of 24 hours at which time they were removed and blotted with a lint-free towel. These strips were then intramuscularly implanted in rabbits and the sites examined after 1 week. Each of the strips produced a tissue response indicating that the toxic substance or substances were being released to the alcoholic solvent.

Chromatographic Separation of Components from Extracts.—Alcoholic extracts were prepared, as described before, from samples X-37 and X-74. The solvent was slowly evaporated in a steam bath until the odor of ethanol was no longer apparent. Each sample was then chromatographed on an F & M scientific model 500 gas chromatograph.[3] The operating conditions of the chromatograph were as follows: injection port, 275°; oven, 250°; detector, 250°; helium flow 50 ml./minute.

A 1-M. 1/2-in. aluminum tube was filled with 20% DC silicone grease on 60–80 ASTM mesh chromosorb W[4] and served as the chromatographic column.

Chromatograms of each sample revealed a number of components had been extracted from both of the samples X-37 and X-74. The representative chromatograms of X-37 and X-74 are shown in Figs. 6 and 7. An analysis of both chromatograms indicated that approximately 95 to 99% of the extract consisted of a heavy component or components which were later ascertained to be the plasticizers.

The light components (in Figs. 6 and 7 these would constitute the components resolved on the column in a 16-minute period) were captured by condensing them in a U-tube packed with glass wool and chilled with a dry ice–acetone bath. The heavy fraction or fractions were likewise captured after 16 minutes. Both the light and heavy components were then eluted from the tube with isopentane and the solvent evaporated. Two fractions were then isolated for each sample. These consisted of a fraction of light components and a fraction of heavy components.

Nonreactive plastic strips were kept in contact with each fraction for a period of 24 hours at which time they were intramuscularly implanted into the rabbits. Two strips from each fraction were used since the quantity of the fractions was very small. After 7 days, the animals were sacrificed and the sites examined. The strips containing the light components for both X-37 and X-74 caused a tissue response, while the strips soaked in the heavy components showed no tissue response. No response was seen for the fractions from the control sample. Results of this particular experiment indicated that one or more components in X-37 and X-74 were the causitive agents in eliciting tissue response and that no apparent response was induced by the plasticizers.

It was hoped that various components from the fraction designated as the "light component" could be isolated for implantation studies to pinpoint the substance or substances causing the tissue response; but, unfortunately, the supply of the same lot number of X-37 and X-74 was depleted. This phase of the investigation, therefore, had to be terminated.

[3] F & M Scientific Corporation, New Castle, Del.
[4] From F & M Scientific Corporation, New Castle, Del.

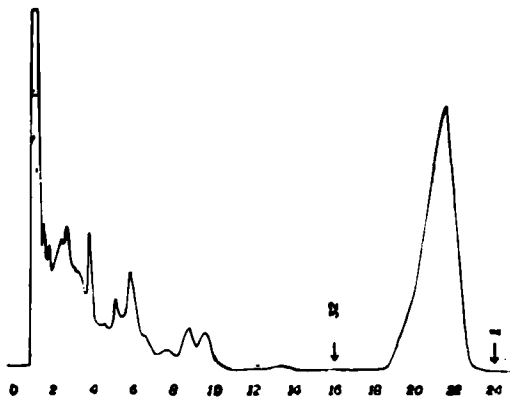

Fig. 6.—Gas chromatogram of extract from sample X-37 showing the number of components in the light fraction (up to 16 minutes) and the one component in the heavy fraction (plasticizer).

DISCUSSION

The results of the study reported in this paper indicate that there are now on the market tubings such as administration devices which contain one or more ingredients in microquantities which will migrate from the tubing and cause a toxic response in tissue when small samples are implanted. Forty-five of the 48 samples were of the vinyl type. Often for these particular tubings, approximately 40 to 60% of the total weight of the material will be the additives such as the stabilizers, antioxidants, colorants, and other components. The implantation studies revealed that 25 of these tubings caused a toxic response in a tissue. It is interesting to note that several manufacturers distributed a number of tubings which showed no tissue response, while other manufacturers produced tubings which showed a tissue response. One should also keep in mind that most of the tubings used in the study were packaged and ready for use. A number of these packages were labeled to indicate that safety tests had been performed on the device; others stated only that the package was sterile and nonpyrogenic.

In recent years several investigators have reported that certain polyvinyl chloride tubings contained one or more toxic ingredients (6). Generally, the causative agent was not the plasticizer but one of the other ingredients. This observation has been confirmed in the study reported in this paper, but it should not indicate that a particular plasticizer might not cause a toxic response under other experimental conditions.

Prior to and during the study, attempts were made to find the formulas used for the various tubings, but it soon became clear that the manufacturer would not, for obvious proprietary reasons, disclose his formula. Certain previous experiments also indicated to us that companies might change their formula for the tubing without indicating to the user that this in fact had been done.

Even though sterile conditions were not used in the implantation experiments, infections were ruled out as the causative agents in producing the toxic responses. If infection was indeed the causative agent, it would certainly have been noted with one or more of the control samples since these were implanted under identical experimental conditions. Confirma-

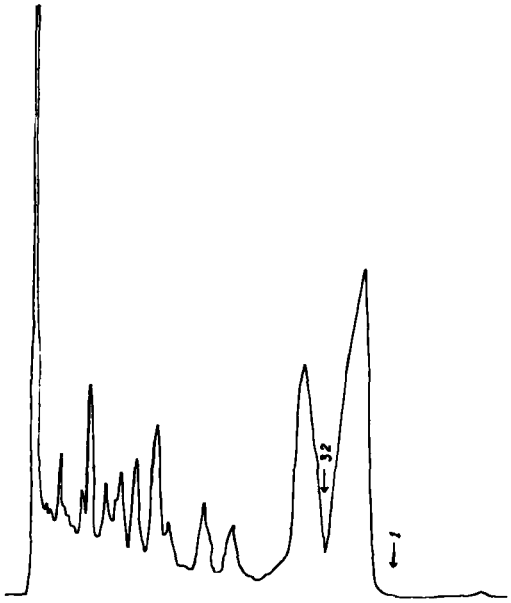

Fig. 7.—Gas chromatogram of extract from sample X-74 showing the number of components in the light fraction (up to 16 minutes) and the two components in the heavy fraction (plasticizers). Note: Same time scale as in Fig. 6.

tion of this fact was made by the bacteriological tests on the "toxic" tissue.

Further investigations with X-37 and X-74 revealed that the toxic ingredient was not because of the component making up the largest bulk of the ethanol extract—the plasticizer or plasticizers—but rather because of one of the number of components referred to as the "light fractions." There was no basis for assuming that the toxic ingredient was a contaminant picked up by the plastic during the manufacturing or packaging process since other tubings from the same manufacturer also showed a toxic effect. Certain investigators have concluded that toxic substances in polyvinyl chloride tubings are probably due to one or more organo-metallic compounds used as stabilizers (6, 7).

The implantation results for X-37, X-55, and X-74 were rather interesting since they did show that the degree of toxicity varied from sample to sample, X-55 eliciting the most severe response while X-74 the least. Sample X-74 lost its toxic activity with time, indicating that the animals were able to nullify or detoxify the offending agent in the plastic.

The implantation method for testing the toxicity of plastics was first developed by Brewer and Bryant (8), who found that certain disposable plastic items could cause a tissue response. This laboratory has since confirmed the value of the implantation technique because it can reveal in a very short period of time (from 3 to 7 days) that the particular plastic item has a component which may be considered undesirable. Cruickshank, *et al.* (9), has suggested the use of tissue cultures as a testing or screening method for plastics.

The question, of course, may now be raised that the tubings which are reported in this paper were never intended to be implanted either in animals or humans; consequently, for their intended use, they may be quite safe. Questions of this sort can best be answered by stating that good public health practice would seem to dictate that no plastic item should contain an ingredient which has a potential harmful ingredient that might leach into a solution to be administered to a patient. Furthermore, since there are tubings which have not shown toxic effects under the experimental conditions employed in this paper, it would seem prudent for manufacturers to re-examine their tubing formulations to remove those offending agents which may become potential sources of danger to patients.

SUMMARY

Forty-eight plastic administration devices were obtained from various sources. For the most part these devices were unit-packaged in sealed or closed packets or cartons. In general the tubings were of the vinyl type. Sections of the tubings from the various devices were investigated by implantation techniques into rabbits, rats, and mice. Morphological and histopathological examinations were used to detect toxic responses in the implanted tissue. The results revealed that 25 of the 48 samples used in the study produced a toxic response. Gas chromatographic techniques with implantation tests suggested that the toxic ingredient or ingredients in several of the tubings were a direct consequence of one or more of the additives, exclusive of the plasticizers.

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