Toxicological and Pharmacological Actions of Methacrylate Monomers II: Effects on Isolated Guinea Pig Ileum

G. N. MIR, W. H. LAWRENCE, and J. AUTIAN▲

Abstract □ Methacrylic acid and 12 methacrylate esters were tested for their effects upon contraction of the isolated guinea pig ileum. Each compound was employed at three concentration levels, ranging from 1:500 to 1:100,000 (v/v), depending upon activity and solubility of the compounds. Most of the compounds, except dimethylaminoethyl methacrylate, produced an inhibition of spontaneous contractions of the isolated ileum which antagonized the stimulant actions of both acetylcholine and barium chloride. Although most of the methacrylate effects were readily washed out, 1,3-butylene dimethacrylate produced a relatively irreversible response. Dimethylaminoethyl methacrylate stimulated the isolated ileum, an effect that was not blocked by 0.1–1 mcg. of atropine/ml. of tissue bath fluid. Those compounds producing inhibition were ranked in order of their relative activities in antagonizing acetylcholine- or barium-induced contraction by determining the approximate molar ratio (*i.e.*, molar concentration of methacrylate per molar concentration of acetylcholine or barium chloride) required to produce 50% inhibition of the stimulant effect of acetylcholine or barium chloride.

Keyphrases □ Methacrylate monomers—effects on isolated guinea pig ileum, acetylcholine- and barium-induced contractions □ Smooth muscle relaxation—effects of methacrylate monomers on isolated guinea pig ileum, acetylcholine- and barium-induced contractions

The polymerized acrylates, particularly methyl methacrylate, find a number of applications as biomedical materials, especially in dentistry and orthopedic surgery. Acute lethality of the various monomers shows considerable variability. The LD_{50} values in mice for the specific samples employed in this study were presented in a previous paper (1). Deichmann (2) and Spealman *et al.* (3) reported that the acute lethal effect in laboratory animals was due to respiratory depression for monomeric methyl methacrylate and some related esters. Various aspects relating to other potential toxicity problems with methacrylates were presented by a number of workers (4–9). Previous work on the methacrylate monomers conducted in these laboratories included investigations into the use of mathematical models to predict acute toxicity (10), alteration of drug biological half-life by methacrylate inhalation (11), embryo–fetal toxicity and teratogenic effects in rats (12), and effects upon the isolated, perfused rabbit heart (1).

MATERIALS AND METHODS

Materials—Methacrylic acid and 12 methacrylate esters were tested on the isolated guinea pig ileum. The following compounds were included: methacrylic acid[1], 1,3-butylene dimethacrylate[1], and the methyl[1], ethyl[1], *n*-butyl[1], isobutyl[1], isodecyl[1], lauryl[1] (dodecyl), hydroxyethyl[1], *tert*-butylaminoethyl[1], dimethylaminoethyl[1], *n*-propyl[2], and 2-ethylhexyl[2] methacrylates.

Methods—Guinea pigs (300–500 g.) of either sex were sacrificed and exsanguinated, and the intestine was promptly exposed *via* a midline incision. An actively contracting loop of ileum, 4–6 cm. in length, was removed and placed in oxygenated Tyrode's solution. A 1.5–2-cm. section of ileum was suspended in oxygenated (95% oxygen + 5% carbon dioxide) Tyrode's solution in a 25-ml. chamber of an isolated organ-tissue bath[3], which was maintained at a constant temperature of 37 ± 0.3°. One end of the intestine was attached to a glass holder and oxygenator; the other end was attached to a force-displacement transducer[4], which was electrically connected to a polygraph[5] to produce a permanent record of the response. To maintain uniform sensitivity, the polygraph was calibrated to produce a 1-cm. pen deflection/g. of tension applied to the transducer.

In all cases the spontaneous activity of the intestine in Tyrode's solution was recorded; the methacrylate was then added to the bath and thise response was recorded. The methacrylate was administered by forceably dispensing the measured quantity of test compound from a syringe with a small bore needle, the tip of which was placed below the liquid surface of the chamber. Preliminary experiments were conducted to determine the concentration of methacrylate that would produce a measurable effect upon the isolated ileum; the other concentrations employed were two and four times as great as the initially determined one.

RESULTS

The responses of the guinea pig ileum to treatment with nine of these 13 compounds are presented in Table I. Three of the other four compounds, isodecyl, 2-ethylhexyl, and lauryl methacrylates, were rather insoluble in Tyrode's solution; it was, therefore, not possible to obtain solutions sufficiently concentrated to produce an observable response. The other compound, 1,3-butylene dimethacrylate, tended to produce relaxation of the smooth muscle but, unlike the majority of these compounds, the effect was very slow in onset. This slow, relatively mild effect did not lend itself well to quantitative measurement.

Concentrations of 1:1000 and 1:500 (v/v) of 1,3-butylene dimethacrylate exhibited a latent period of about 5 min., which was followed during the next 5 min. or so by a gradual decrease in pendular movements and a slow relaxation of the muscle. After the maximum effect developed, it appeared to be irreversible since the muscle would not return to control levels of activity even after repeated washing with Tyrode's solution. The activity of 1,3-butylene dimethacrylate partially antagonized neurogenic stimulation by acetylcholine (0.1 mcg./ml.) and myogenic stimulation by barium chloride (30 mcg./ml.).

Eight of the other nine compounds produced prompt (within 15–30 sec.) and qualitatively rather uniform effects, consisting of inhibition of pendular movements and relaxation of the muscle. The effect of each compound could be terminated by promptly washing the ileum with fresh Tyrode's solution. All of these compounds produced a concentration-dependent inhibition of the usual effects of acetylcholine and barium chloride upon the isolated ileum.

Dimethylaminoethyl methacrylate acted differently, both qualitatively and quantitatively, from the other compounds tested. While the other compounds either produced an inhibition of smooth muscle activity or had no effect on its activity, dimethylaminoethyl methacrylate produced contraction of the ileum. This smooth

[1] Rohm & Haas, Philadelphia, Pa.
[2] K&K Laboratories, Inc., Plainview, N.Y.
[3] Phipps & Bird, Richmond, Va.
[4] FT03C, Grass Instrument Co., Quincy, Mass.
[5] Grass model 7.

Table I—Effects of Methacrylates on the Isolated Guinea Pig Ileum and upon Acetylcholine- and Barium Chloride-Induced Contractions

Concentration in Tissue Bath (v/v)	Response to Methacrylate, mm.[a]	Response to Acetylcholine[b], mm.[a]	Response to Methacrylate and Acetylcholine, mm.[a]	Inhibiton of Acetylcholine Response, %	Response to Barium Chloride[b], mm.[a]	Response to Methacrylate and Barium Chloride[a], mm.	Inhibition of Barium Chloride Response, %
Methacrylic acid		+31.6 (3)[c]			+30.0 (3)		
1:10,000	−8.2 (5)		+25.5 (2)	19.3		+26.5 (2)	11.6
1:5000	−13.6 (5)		+16.5 (2)	47.7		+16.5 (2)	45.0
1:2500	−22.0 (5)		+2.0 (2)	93.6		+5.5 (2)	81.6
Methyl methacrylate		+35.6 (3)			+31.6 (3)		
1:2000	−9.4 (5)		+29.5 (2)	17.1		+26.5 (2)	16.1
1:1000	−15.4 (5)		+16.0 (2)	55.0		+16.0 (2)	49.3
1:500	−30.2 (5)		+4.5 (2)	87.3		+3.0 (2)	90.5
Ethyl methacrylate		+30.6 (3)			+32.6 (3)		
1:2000	−7.6 (5)		+29.5 (2)	5.2		+29.0 (2)	11.0
1:1000	−14.6 (5)		+15.5 (2)	49.3		+16.5 (2)	49.3
1:500	−23.0 (5)		+2.5 (2)	91.8		+3.5 (2)	89.2
n-Propyl methacrylate		+29.6 (3)			+32.0 (3)		
1:2000	−7.2 (5)		+23.2 (2)	21.6		+27.5 (2)	15.6
1:1000	−16.2 (5)		+15.0 (2)	44.3		+17.5 (2)	45.3
1:500	−23.6 (5)		+4.5 (2)	84.8		+6.0 (2)	81.2
n-Butyl methacrylate		+35.6 (3)			+31.3 (3)		
1:2000	−10.6 (5)		+29.0 (2)	18.5		−29.2 (2)	7.3
1:1000	−18.0 (5)		+19.5 (2)	45.2		+15.5 (2)	50.4
1:500	−24.0 (5)		+4.0 (2)	88.7		+3.5 (2)	88.8
Isobutyl methacrylate		+34.6 (3)			+31.0 (3)		
1:2000	−14.4 (5)		+28.5 (2)	17.6		+25.5 (2)	17.7
1:1000	−18.8 (5)		+17.5 (2)	49.4		+15.5 (2)	50.0
1:500	−25.6 (5)		+2.5 (2)	92.7		+2.0 (2)	93.5
"Substituted" methacrylates							
Hydroxyethyl methacrylate		+38.3 (3)			+33.3 (3)		
1:10,000	−11.2 (5)		+27.5 (2)	28.1		+23.5 (2)	36.4
1:5000	−17.8 (5)		+16.5 (2)	56.9		+17.5 (2)	52.7
1:2500	−26.0 (5)		+0.0 (2)	100.0		+0.0 (2)	100.0
tert-Butylaminoethyl methacrylate		+37.6 (3)			+33.3 (3)		
1:10,000	−11.8 (5)		+31.0 (2)	17.5		+27.5 (2)	17.4
1:5000	−19.2 (5)		+16.5 (2)	56.1		+15.0 (2)	54.9
1:2500	−26.4 (5)		+0.0 (2)	100.0		+0.0 (2)	100.0
Dimethylaminoethyl methacrylate		n/a[d]			n/a		
1:100,000	+23.5 (5)		n/a	n/a		n/a	n/a
1:50,000	+32.4 (5)		n/a	n/a		n/a	n/a
1:25,000	+35.2 (5)		n/a	n/a		n/a	n/a
			(This contraction was not blocked by 1×10^{-7} or 10^{-6} atropine sulfate)				

[a] + = contraction; − = relaxation. 10 mm. ≈ 1 g. tension. [b] Acetylcholine concentration = 1:10,000,000; barium chloride concentration = 3:100,000 (w/v). [c] Number of observations in parentheses. [d] n/a = not applicable.

muscle stimulation was not blocked by 0.1 mcg./ml. of atropine sulfate. Of the other compounds tested, hydroxyethyl and *tert*-butylaminoethyl methacrylates were the most potent, inhibiting ileum contraction by about 11 mm. at a 1:10,000 dilution, while dimethylaminoethyl methacrylate produced about 23 mm. contraction at a dilution of 1:100,000 (v/v).

DISCUSSION

Most of the methacrylates examined produced a depressant effect upon spontaneous motility of the isolated guinea pig ileum, and generally a good dose-response (concentration-dependent) relationship was observed (eight of the nine compounds listed in Table I). These eight compounds also demonstrated a good concentration-dependent antagonism of the neurogenic and myogenic stimulant effects of acetylcholine and barium chloride upon the isolated ileum. With the concentrations used, from 5 to 100% of the stimulant effect of acetylcholine was blocked by prior administration of the methacrylate, and from 7 to 100% of the stimulant effect of barium chloride was blocked. These data suggest that the inhibitory effects of these methacrylates upon the isolated ileum are myogenic in origin. The effects of these eight methacrylates may be terminated by promptly washing the intestine with fresh Tyrode's solution.

1,3-Butylene dimethacrylate [1:1000 and 1:500 (v/v)] also produced a reduction in pendular movements and a relaxation of the intestine. Unlike those already discussed, where the effect occurred within 15–30 sec., this compound showed a latent period of about 5 min. before any effect could be observed, and then the effect developed very slowly over the next 5–10 min. When the effect had developed, it antagonized the stimulant effect of acetylcholine (0.1 mcg./ml.) and barium chloride (30 mcg./ml.) upon the muscle. Unlike the previously discussed compounds, the depressant effects of 1,3-butylene dimethacrylate were not dissipated by repeated washings with Tyrode's solution. This may be due to either a stronger binding of this compound to the receptor tissue of the muscle or a greater physical penetration (solubility related) of the compound into the tissue, since the onset of action was much slower with greater difficulty in "washing out" the effect.

The three least soluble members of the series, 2-ethylhexyl, isodecyl, and lauryl methacrylates, did not elicit a demonstrable effect by this procedure. Since the effects produced by a compound of limited solubility are dependent upon the quantity that will go into solution to reach its target site and the inherent activity of the compound at its target site, the combination of solubility and inherent activity must not have reached critical values in these experiments for the isolated ileum; thus, no effect was observed. Use of a more sensitive system for detection of the action of the methacrylates may lead to quantitative results for these compounds;

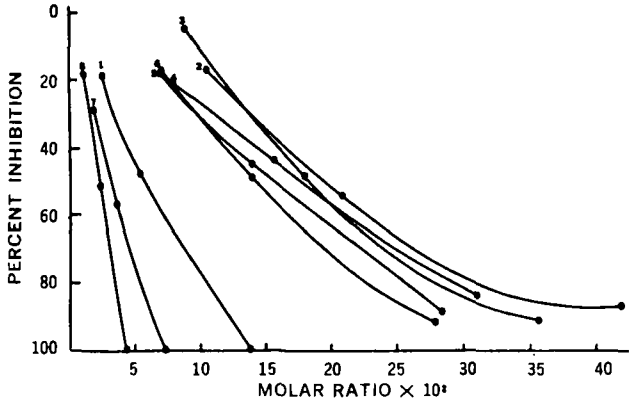

Figure 1—*Methacrylate inhibition of acetylcholine-induced contraction. Key: 1, methacrylic acid; 2, methyl methacrylate; 3, ethyl methacrylate; 4,* n*-propyl methacrylate; 5,* n*-butyl methacrylate; 6, isobutyl methacrylate; 7, hydroxyethyl methacrylate; and 8,* tert*-butylaminoethyl methacrylate.*

in the isolated heart, these compounds did demonstrate a measurable response (1).

These eight methacrylates may be grouped into two general orders of activity: (*a*) those in which the highest dilution used was 1:2000 (methyl, ethyl, *n*-propyl, *n*-butyl, and isobutyl methacrylates), and (*b*) those in which the highest dilution used was 1:10,000 (methacrylic acid and the "substituted" methacrylates, hydroxyethyl and *tert*-butylaminoethyl).

Figure 1 shows the antagonistic effect of the methacrylates upon acetylcholine-induced contraction of the isolated ileum. In this figure the molar ratio (molar concentration of the methacrylate per molar concentration of acetylcholine) is plotted against percent inhibition of the acetylcholine response. The standard concentration of acetylcholine bromide (0.1 mcg./ml.) employed in these studies represents 4.422×10^{-7} mole/l.

Figure 2 presents a similar graph showing the antagonistic effect of the methacrylates upon barium chloride-induced contraction of the isolated ileum. Again, the molar ratio (molar concentration of methacrylate per molar concentration of barium chloride) is plotted against percent inhibition of the barium chloride response. The standard concentration of barium chloride (30 mcg./ml.) used in these studies represented 1.228×10^{-4} mole/l.

The approximate molar ratios required for the compounds to produce 50% inhibition of the acetylcholine and barium chloride responses were estimated from Figs. 1 and 2, arranged in order of decreasing potency, and listed in Table II. The lower the molar ratio required to provide 50% inhibition, the more active is the compound in inhibiting contractions produced by the stimulant agent. Since these curves are constructed from only three data points, they must be considered as estimates; however, they do clearly represent the order of magnitude involved in the antagonism.

The activity of dimethylaminoethyl methacrylate was atypical of the series of compounds investigated by differing both in terms of

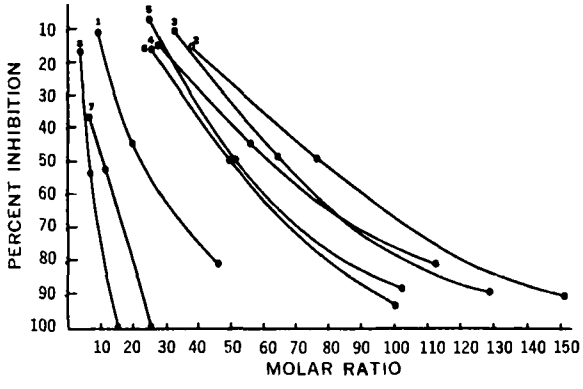

Figure 2—*Methacrylate inhibition of barium chloride-induced contraction. For key, see Fig. 1.*

Table II—Molar Ratio[a] to Produce 50% Inhibition of Induced Contraction

Compound	Acetylcholine	Barium Chloride
tert-Butylaminoethyl methacrylate	2,250	6.5
Hydroxyethyl methacrylate	3,250	10.0
Methacrylic acid	5,750	22.0
Isobutyl methacrylate	14,125	50.0
n-Butyl methacrylate	15,500	51.0
n-Propyl methacrylate	17,375	61.0
Ethyl methacrylate	18,125	65.0
Methyl methacrylate	19,375	76.5

[a] Molar ratio = molar concentration of methacrylate per molar concentration of stimulating agent at point where contraction is 50% of control contraction.

relative potency and type of action. Unlike the others, this compound produced contraction of the isolated ileum, and it was active in more dilute solutions than the others. At a concentration of 1:100,000 (v/v) (0.000059 mole/l.), dimethylaminoethyl methacrylate produced a significant contraction of the ileum. Pretreatment of the guinea pig ileum with atropine sulfate [1×10^{-7} and 10^{-6} (w/v) in the tissue bath] did not block or inhibit the contraction produced by this dimethylaminoethyl derivative. This would imply a myogenic effect, as suggested for the other homologs; but unlike the others, dimethylaminoethyl methacrylate stimulated the ileum to contract, while the other methacrylates inhibited such contractions.

SUMMARY AND CONCLUSIONS

The effects observed in this study, in which methyl methacrylate and most of the other monomers tested produced relaxation of the smooth muscle of the guinea pig ileum, are not inconsistent with the reports of Homsy *et al.* (9), who reported a fall in blood pressure of the dog by various concentrations of methyl methacrylate, or of Mir *et al.* (1), who observed a relative increase in coronary flow in the isolated rabbit heart following perfusion with methyl methacrylate and most of the other homologs included in this study. This latter response would suggest a direct vasodilator effect of the compounds upon the coronary circulation, although cardiac slowing *per se* may contribute to this effect by allowing more complete filling of coronary vessels prior to contraction. The work of Mir *et al.* (1) also showed that the pacemaker of the isolated heart was slowed and that the force of myocardial contraction was reduced when the heart was perfused with a solution containing the methacrylate monomer. While the hypotensive effect of methyl methacrylate on blood pressure in the dog (9) and possibly some hypotensive responses reported from the clinical use of self-curing acrylic cement (methyl methacrylate) in orthopedic surgery (8, 13, 14) may be due to a combination of effects, the reduction in tone of the smooth muscle of the arterioles, and hence vasodilation, must be considered as a possible primary or contributory factor in such hypotensive responses.

REFERENCES

(1) G. N. Mir, W. H. Lawrence, and J. Autian, *J. Pharm. Sci.*, **62,** 778(1973).

(2) W. Deichmann, *J. Ind. Hyg. Toxicol.*, **23,** 343(1941).

(3) C. R. Spealman, R. J. Main, H. B. Haag, and P. S. Larson, *Ind. Med.*, **14,** 292(1945).

(4) J. F. Borzelleca, P. S. Larson, C. R. Henningar, Jr., E. C. Huf, E. M. Crawford, and R. B. Smith, Jr., *Toxicol. Appl. Pharmacol.* **6,** 29(1964).

(5) J. C. Strain, *J. Prost. Dent.*, **18,** 465(1967).

(6) N. Castellino and G. Colicchio, *Folia Med.*, **52,** 337(1969); through *Chem. Abstr.*, **74,** 97257c(1971).

(7) S. Krumbholz, B. Schyra, and K. Winnfeld, *Wiss. Chir.-Tag. Deut. Demokrat. Repub., 6th,* **2,** 1776(1967); through *Chem. Abstr.*, **70,** 76094(1969).

(8) C. A. Cohen and T. C. Smith, *Anesthesiology*, **35,** 547(1971).

(9) C. A. Homsy, H. S. Tullos, and J. W. King, *Clin. Orthoped.*

Rel. Res., 67, 169(1969).
(10) W. H. Lawrence, G. E. Bass, W. P. Purcell, and J. Autian, *J. Dent. Res.*, **51**, 526(1972).
(11) W. H. Lawrence and J. Autian, *ibid.*, **51**, 878(1972).
(12) A. R. Singh, W. H. Lawrence, and J. Autian, *ibid.*, **51**, 1632 (1972).
(13) P. N. Frost, *Brit. Med. J.*, **3**, 524(1970).
(14) J. Charnley, "Acrylic Cement in Orthopedic Surgery," Williams & Wilkins, Baltimore, Md., 1970, p. 73.

ACKNOWLEDGMENTS AND ADDRESSES

Received January 24, 1973, from the *Materials Science Toxicology Laboratories, College of Dentistry and College of Pharmacy, University of Tennessee Medical Units, Memphis, TN 38103*
Accepted for publication March 12, 1973.
Supported in part by Research Contract DE02944-03, National Institute of Dental Research, Bethesda, MD 20014
▲ To whom inquiries should be directed.

Tetracycline Binding to Bovine Serum Albumin Studied by Fluorescent Techniques

JOSEPH K. H. MA, H. W. JUN, and LOUIS A. LUZZI▲

Abstract □ Binding of demeclocycline and oxytetracycline to bovine serum albumin was studied using fluorescent methods. The mechanism of binding for tetracyclines is shown to be hydrophobic. The number of binding sites and the equilibrium constants were calculated over a range of protein concentrations. Two strong binding sites, at or near the tryptophan residues of bovine serum albumin, were found for both tetracyclines. The equilibrium constants for tetracyclines increased with increasing protein concentration, and the number of binding sites on the protein decreased with increasing protein concentration. These findings suggest the possibility of a sharing of one tetracycline molecule by more than one protein molecule at relatively high protein concentrations.

Keyphrases □ Demeclocycline—mechanism of binding to bovine serum albumin studied using fluorescent techniques □ Oxytetracycline—mechanism of binding to bovine serum albumin studied using fluorescent techniques □ Tetracyclines, demeclocycline and oxytetracycline—binding to bovine serum albumin, fluorescent techniques □ Serum protein binding—demeclocycline and oxytetracycline, fluorescent techniques □ Fluorescent techniques, probe and quenching titration—used to study mechanism of binding of demeclocycline and oxytetracycline to bovine serum albumin

The binding of drugs by plasma proteins has been recognized as an important factor in drug availability, drug efficacy, and drug transport for many years (1). Many experimental techniques have been employed to study drug–protein interactions (2–4). The fluorescence probe technique (5, 6) has recently been employed to study the mechanism of drug–protein binding and the nature of binding sites. This technique has also been very useful in biochemical research related to binding (7–9). The number of probes available at this time is limited; to explore the use of probes in binding studies more fully, it is necessary to search for new probe compounds. Therefore, the fluorescence studies of tetracyclines and bovine serum albumin were carried out in this laboratory.

Tetracyclines are fluorescent compounds with the excitation and emission maxima in the range of 390 and 520 nm., respectively. Ibsen and Urist (10) reported that the excitation maximum of tetracyclines varies with the presence of metal ions. They found that increasing metal concentration produced a bathochromic shift, indicating the formation of metal complexes. The quantum yields of several tetracyclines in various solvents and of their protein mixtures were reported by Popov *et al.* (11, 12). In a recent paper (13), they also studied the interaction of tetracyclines with bovine serum albumin using a fluorescence technique. The increase of the fluorescence of tetracyclines accompanied by an auxochromic shift of the fluorescence maxima in the presence of protein and in nonpolar solvents suggests that the tetracyclines exhibit the main features of fluorescence probes.

Fluorescence quenching titration is another sensitive method for the study of drug–protein molecular interactions. The procedure has been applied to many systems (14–16) and was best described by Chignell (17). It was found that the native fluorescence of bovine serum albumin was quenched by most of the tetracyclines. To examine the possible use of tetracyclines as probe compounds for drug–protein binding studies, the nature of binding of demeclocycline and oxytetracycline to bovine serum albumin was studied by both methods.

EXPERIMENTAL

Materials—Demeclocycline hydrochloride[1], oxytetracycline hydrochloride[2], and crystalline bovine serum albumin[3] were obtained from commercial sources. Methanol[4] was spectroscopic grade, and all other chemicals were reagent grade or of special purity. All chemicals were used without further purification.

Apparatus—Fluorescence measurements were made with a spectrophotofluorometer[5] equipped with a 150-w. xenon lamp and 1P21 photomultiplier tube. The relative fluorescence intensities were recorded directly from fluorometer readings.

Fluorescent Titration—The fluorescence and quenching titrations were performed manually with microsyringes[6]. Two milliliters of

[1] Lot 48151-915, American Cyanamid Co.
[2] Lot 48175-939, American Cyanamid Co.
[3] Control No. 1322, Nutritional Biochemicals Corp.
[4] Matheson, Coleman & Bell, Norwood, Ohio.
[5] Aminco-Bowman, American Instrument Co., Silver Spring, Md.
[6] Hamilton.