Structure–Toxicity Relationships of Iodinated Aromatic Carbonates and Related Compounds

BARRY N. NEWTON

Received June 8, 1977, from the *Research and Development Department, Lafayette Pharmacal Incorporated, Lafayette, IN 47904.* Accepted for publication December 2, 1977.

Abstract □ Structure–toxicity relationships of iodinated aromatic carbonates, carbamates, and esters are presented. The approximate lethal dose of intraperitoneal injections in mice was used for toxicity determinations. Increasing the alkyl portion of the molecules reduced toxicity. *m*-Amino and *m*-acetamido groups also reduced toxicity. Carbonates were preferred X-ray contrast agents because of their low viscosity and more rapid elimination.

Keyphrases □ Iodinated aromatic compounds, various—toxicity in mice, structure–activity relationships □ Structure–activity relationships—various iodinated aromatic compounds, toxicity in mice □ Radiopaque media—various iodinated aromatic compounds, toxicity in mice, structure–activity relationships

The X-ray visualization of the subarachnoid space from the sacral region through the cervical area is referred to as myelography. Three classes of contrast agents are used for this examination: gas, water-soluble compounds, and oily, water-insoluble compounds.

BACKGROUND

Gas myelography is performed by injecting either air or carbon dioxide into the subarachnoid space, thus providing negative contrast. The interpretation of this examination is difficult, and there is a high incidence of patient headaches (1). For these reasons, gas myelography is used infrequently.

Water-soluble compounds for positive contrast myelography have been used for more than 30 years in Europe. Iodomethane sulfonate was the compound of choice for about three decades, but its use was discontinued because of patient reactions, including convulsions and adhesive arachnoiditis. Other ionic water-soluble salts have been used, but they are not widely accepted because of their side effects. A dimeric compound is available in the United States, but, because of its toxicity, it is recommended only for use in the lumbar sacral region[1] (2, 3).

Nonionic water-soluble radiopaques, prepared by attaching a hydrophilic sugar group to a triiodinated moiety, were first proposed by Hebky and coworkers (4, 5). More recently, this approach was used to design metrizamide, a nonionic water-soluble compound receiving attention as a new myelographic medium (6, 7). To date, metrizamide appears to be the least toxic water-soluble myelographic medium. However, Sackett *et al.* (8) reported that 39% of their patients experienced moderate to severe adverse reactions following metrizamide myelography and that three out of 215 patients had seizures. This report, coupled with other factors, confirms that the ideal myelographic medium has yet to be determined (9).

Oil myelography has dominated the U.S. market since the early 1940's. Iophendylate, ethyl 10-(iodophenyl)undecanoate, has been the preferred contrast agent. During this period, millions of examinations have been performed with a very low incidence of side effects (10). However, since iophendylate is only very slowly eliminated by the body, it is withdrawn from the subarachnoid space at the conclusion of the examination.

While there have been numerous reports concerning new water-soluble compounds, there have been very few reports on new oily materials (11–13). The preparation and toxicity of several oily iodinated organic carbonates were reported elsewhere (14, 15). This paper reports initial structure–toxicity relationships encountered with the carbonates and comparisons with related compounds.

[1] This material has been withdrawn from the market since this article was accepted for publication.

EXPERIMENTAL[2]

Details regarding the preparation of iodinated aromatic carbonates from the pertinent alcohols were given previously (14). The approximate lethal dose (ALD) intraperitoneally in mice was determined by injecting six animals with graduated doses, each dose being 50% higher than the preceding one. With this technique, all doses up to a certain level resulted in survival of the animals while all doses above this level were lethal. The ALD previously (16) was found to agree with the LD_{50} value within the limits of approximately ± 30%.

10-(*p*-Iodophenyl)undecanol (VII)—Ethyl 10-(*p*-iodophenyl)-undecanoate was reduced with lithium aluminum hydride by a known procedure (20). Compound VII was recovered; $n_D^{25} = 1.5238$; mass spectrum: *m/e* (measured mass) 374.1114 (calculated for $C_{17}H_{27}IO$, 374.1108).

Anal.—Calc. for $C_{17}H_{27}IO$: C, 54.53; H, 7.27. Found: C, 54.22; H, 7.56.

***p*-Iodobenzyl Hexanoate (XXIX)**—This compound was prepared by a standard esterification procedure (21); $n_D^{25} = 1.5819$; mass spectrum: *m/e* (measured mass) 332.0264 (calculated for $C_{13}H_{15}IO_3$, 332.0275).

Anal.—Calc. for $C_{13}H_{15}IO_3$: C, 46.98; H, 4.55. Found: C, 46.87; H, 4.94.

Similarly prepared from the appropriate iodinated alcohol and aliphatic acid were XXX and XXXII. For XXX, $n_D^{25} = 1.5454$; mass spectrum: *m/e* (measured mass) 360.0554 (calculated for $C_{15}H_{21}IO_2$, 360.0588).

Anal.—Calc. for $C_{15}H_{21}IO_2$: C, 46.98; H, 4.55. Found: C, 46.87; H, 4.94.

For XXXII, $n_D^{25} = 1.5322$; mass spectrum: *m/e* (measured mass) 388.0900 (calculated for $C_{17}H_{25}IO_2$, 388.0901).

Anal.—Calc. for $C_{17}H_{25}IO_2$: C, 52.57; H, 6.49. Found: C, 52.70; H, 6.77.

The following carbonates were prepared as outlined previously (8): Compound XIII: $n_D^{25} = 1.5431$.

Anal.—Calc. for $C_{12}H_{14}IO_5$: C, 43.11; H, 4.53. Found: C, 42.87; H, 4.40.

Compound XXIV: $n_D^{25} = 1.4806$.

Anal.—Calc. for $C_{12}H_{20}IO_3$: C, 71.14; H, 8.54. Found: C, 71.37; H, 8.65.

Compound XXXV: $n_D^{25} = 1.5369$.

Anal.—Calc. for $C_{14}H_{17}IO_5$: C, 42.86; H, 4.37. Found: C, 43.17; H, 4.45.

The following carbamates were prepared using the procedure for carbonate synthesis.

Compound XXXI, mp 78.6–79.2° (ethanol–water); mass spectrum: *m/e* (measured mass) 347.0353 (calculated for $C_{13}H_{18}INO_2$, 347.0384).

Anal.—Calc. for $C_{13}H_{18}INO_2$: C, 44.95; H, 5.23. Found: C, 44.66; H, 5.11.

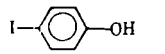
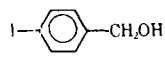
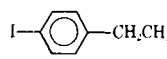
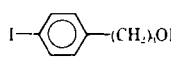
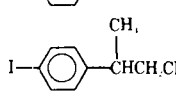
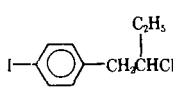
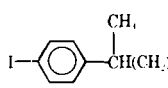
Compound XXXIII, mp 92.2–94.2° (hexane); mass spectrum: *m/e* (measured mass) 389.0863 (calculated for $C_{16}H_{24}INO_2$, 389.0854).

Anal.—Calc. for $C_{16}H_{24}INO_2$: C, 49.35; H, 6.22. Found: C, 49.39; H, 6.17.

[2] Melting points were determined on a Buchi capillary apparatus and are uncorrected. IR spectra were recorded on a Beckman IR 33 spectrometer. NMR spectra were taken on a Varian EM 360 spectrometer with tetramethylsilane as the internal standard. Gas chromatograms were obtained on a Hewlett-Packard 5712A chromatograph equipped with a thermal conductivity detector using a 3% SE-30 column. Refractive indexes were determined on a Bausch & Lomb refractometer. Viscosities were determined using an Ostwalt viscometer at 25 ± 1° and an average of three runs. Mass spectra were obtained on a CEC 21-110B double-focusing mass spectrometer with an accelerating potential of 7.5 kv, nominal; an emission current of 100 μmp, regulated; an ionizing voltage of 70 ev; a reservoir temperature of 200°; a source temperature of 175–200°; and a nominal mass resolution of 1/15,000 (17–19). Throughout the program, emphasis was on pure products rather than on improved yields.

0022-3549/78/0800-1154$01.00/0

Table I—Approximate Lethal Dose of Iodinated Aromatic Alcohols

Compound	Structure	ALD[a]
I		0.7 g/kg
II		1.0 g/kg
III		1.0 ml/kg
IV		1.0 ml/kg
V		0.5 ml/kg
VI		5.5 ml/kg
VII		2.2 ml/kg

[a] Compounds with ALD values in grams per kilogram were administered as suspensions in sesame oil (no toxicity from sesame oil alone). Compounds with ALD values in milliliters per kilogram were administered as a neat oil.

Compound XXXIV, mp 113.2–114.4° ($CHCl_3$); mass spectrum: *m/e* (measured mass) 391.0279 (calculated for $C_{14}H_{18}INO_4$, 391.0282).

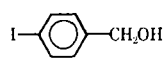
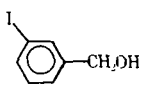
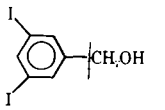
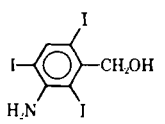
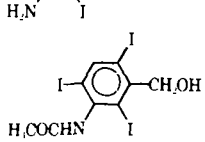
Anal.—Calc. for $C_{14}H_{18}INO_4$: C, 42.96; H, 4.64. Found: C, 42.61; H, 4.64.

RESULTS AND DISCUSSION

Generally, iodine is incorporated into organic compounds to make them opaque to X-rays. Iodine bound to an aliphatic carbon atom is liberated more easily than aromatically bound iodine. Therefore, the aromatic ring was used to incorporate iodine into the carbonates. Various iodinated aromatic alcohols were used to prepare the carbonates. To gain insight into the toxicity of the carbonates, the ALD of the starting alcohols was determined.

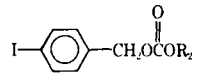
The results of varying the alkyl structure between the iodinated benzene ring and the OH group can be seen in Table I. There was little difference in the ALD values of *p*-iodophenol (I), *p*-iodobenzyl alcohol (II), *p*-iodophenethyl alcohol (III), *p*-iodophenylpropanol (IV), and 3-(*p*-iodophenyl)butanol (V). 2-(*p*-Iodobenzyl)butanol (VI), however, had a disproportionately higher ALD. This difference was not just a result of a higher carbon content in the side chain, because VII has an ALD of only 2.2 ml/kg. The carbon skeleton of VI is the same as that found in iopanoic acid (XL), a relatively nontoxic oral cholecystographic agent (22). The reduced toxicity of IV has been attributed to albumin binding.

Table II—Influence of Ring Substitution on ALD of Iodinated Alcohols

Compound	Structure	ALD, g/kg[a]
II		1
VIII		0.75
IX		<0.5
X		7.5
XI		7.5

[a] Administered as suspensions in sesame oil (no toxicity from sesame oil alone).

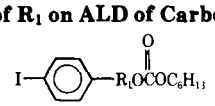
Table III—Influence of R_2 on ALD of Carbonates



Compound	R_2	ALD, g/kg[a]	u_{25}, cps[b]
XII	C_2H_5	2.74	11.76
XIII	C_4H_9	4.5	14.37
XIV	C_5H_{11}	4.38	—[c]
XV	C_6H_{13}	6.78	18.58
XVI	$CH(C_2H_5)C_3H_7$	4.32	21.69
XVII	C_8H_{17}	>27	22.1
XVIII	$CH_2CH(C_2H_5)C_4H_9$	20.25	33.88

[a] Administered as a neat liquid intraperitoneally in mice. [b] Viscosities were determined on samples containing <5% impurities. [c] Not available.

Table IV—Influence of R_1 on ALD of Carbonates



Compound	R_1	ALD, ml/kg[a]
XIX	None	4.5
XV	CH_2	7.0
XX	CH_2CH_2	7.0
XXI	$CH_2CH_2CH_2$	10.2
XXII	$CH(CH_3)CH_2CH_2$	7.0
XXIII	$CH_2CH(C_2H_5)CH_2$	>15

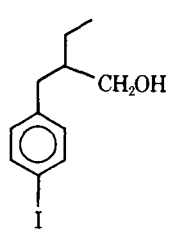
[a] Administered as a neat liquid intraperitoneally to mice.

Substitution on the benzene ring can contribute to reduced toxicity. Thus, the toxicity remained virtually unchanged going from *p*-iodo (II) to *m*-iodo (VIII) to 3,5-diiodobenzyl alcohol (IX) (Table II). However, 3-amino-2,4,6-triiodo- and 3-acetamido-2,4,6-triiodo-substituted benzyl alcohols had much larger ALD values (note X and XI in Table II).

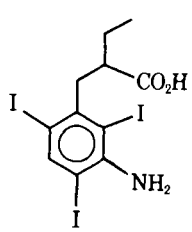
When the iodinated alcohols were converted to carbonates and other related compounds, several interesting structure–activity relationships were found. Generally, an increase in the length of the primary alkyl chain of a carbonate resulted in decreased toxicity. For example, the ethyl carbonate (XII) was more toxic than the butyl carbonate (XIII) (Table III). There was little change in the ALD of the pentyl carbonate (XIV) and 4-substituted hexyl carbonate (XVI). Major toxicity decreases were seen going from *n*-hexyl carbonate (XV) to *n*-octyl carbonate (XVII). Branching of the alkyl portion of the carbonate while holding the carbon content constant reduced the ALD (compare XV with XVI and XVII with XVIII).

The lipophilic character of the carbonates decreases as the chain length decreases. This phenomenon was demonstrated through several examples (23). The decreased lipophilic character results in an increased water solubility and, in turn, may increase the absorption rate. Before a molecule can cross a lipophilic membrane, it must be transported to that membrane. Because of their higher water solubility, the shorter chain members have a greater opportunity to cross the barrier. Thus, the shorter chain compounds may be more toxic because of more rapid transport and absorption.

A good myelographic medium should flow freely within the sub-

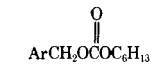


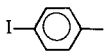
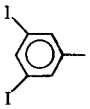
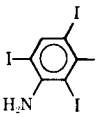
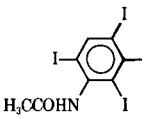
VI



XL

Table V—Influence of Ring Substitution on the Toxicity of Carbonates



Compound	Ar	ALD[a]
XXIV		4.5 ml/kg
XV		4.8 ml/kg
XXV		4.5 ml/kg
XXVI		5.0 ml/kg
XXVII		>22.0 g/kg
XXVIII		>22.0 g/kg

[a] Administered intraperitoneally in mice. Compounds with ALD values in grams per kilogram were administered as suspensions in sesame oil (no toxicity from sesame oil alone). Compounds with ALD values in milliliters per kilogram were administered as a neat oil.

arachnoid space and thus requires a low viscosity. It should also have a high ALD. Increasing the carbon content of the alkyl portion of the carbonates increases the ALD but results in higher viscosities (Table III). Thus, the least toxic compounds are less suitable as myelographic agents because of their higher viscosity[3].

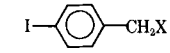
Variation of the alkyl structure between the benzene ring and the carbonate linkage resulted in less dramatic toxicity changes. Compound XIX (Table IV), where the ring is attached directly to the carbonate linkage, had an ALD of 4.5 ml/kg. Interjection of one, two, and three methylene groups gave only slightly less toxic compounds (compare XV, XX, and XXI, respectively). An α-methyl group also only affected the ALD slightly (compare XXI with XXII). The carbonate (XXIII) prepared from alcohol (VII) showed a large increase in the ALD when compared to the other carbonates in Table IV. Again, however, the high viscosity of this compound precludes its use for myelography.

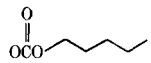
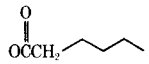
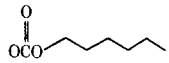
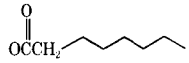
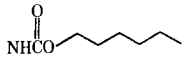
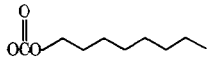
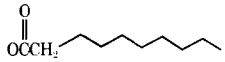
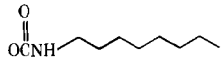
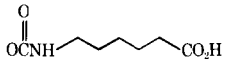
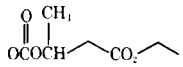
Variation of ring substitution gave results with the carbonates comparable to those seen with the alcohols. There was little change in toxicity due to the addition of *para*-, *meta*-, or 3,5-disubstituted iodine (compare XXIV, XV, XXV, and XXVI, Table V). Hoppe (24) previously demonstrated the reduction in toxicity resulting from *m*-amino and *m*-acetamido groups. Thus, as expected, *n*-hexyl 3-amino-2,4,6-triiodobenzylcarbonate (XXVII) and *n*-hexyl 3-acetamido-2,4,6-triiodobenzylcarbonate (XXVIII) were far less toxic than just iodinated ring compounds.

When the carbonate linkage was compared with ester and carbamido structures, the toxicity did not change significantly (Table VI). The esters and carbamido compounds were of less interest radiographically, since the esters were more viscous and more slowly eliminated while the carbamido compounds were both solid and more slowly eliminated. Comparing carbonate XIV with ester XXIX and carbonate XV with ester XXX illustrates no real difference between them. Carbamide XXXI is of comparable toxicity with XV. The incorporation of a terminal carboxylic acid in a carbamido structure gave a more toxic compound (compare XXXIII with XXXIV). When an ester linkage was included in the alkyl chain of a carbonate, toxicity decreased (compare XV with XXXV).

[3] A direct correlation between the ALD obtained from intraperitoneal injections in mice and neurotoxicity obtained after intrathecal injections in rats was found. For comparative purposes, the viscosity of ethyl 10-(iodophenyl)undecanoate is 51.04 cps at 25°.

Table VI—ALD of Some Iodinated Carbonates and Related Structures



Compound	X	ALD[a], g/kg
XIV		4.38
XXIX		2.94
XV		6.78
XXX		9.66
XXXI		3.4[b]
XVII		>27
XXXII		>19.5
XXXIII		>10.5[b]
XXXIV		<1[b]
XXXV		<4.44

[a] Administered intraperitoneally to mice. [b] Administered as suspensions in sesame oil (no toxicity from sesame oil alone).

Several of these compounds are now undergoing more extensive investigation for myelographic and other radiographic applications.

REFERENCES

(1) P. L. Cook, *Br. J. Radiol.*, **49**, 502 (1976).

(2) B. Hammer and H. Scherrer, *Neuroradiology*, **4**, 144 (1972).

(3) L. Irstam and U. Sellden, *Acta Radiol. Diag.*, **16**, 449 (1975).

(4) J. Hebky and V. Jelinek, Czechoslovakian pat., **106**, 426 (1963).

(5) J. Hebky, B. First, J. Polack, and M. Karasek, *Collect. Czech. Chem. Commun.*, **35**, 867 (1970).

(6) T. Almen, *J. Theor. Biol.*, **24**, 216 (1969).

(7) *Acta Radiol. Suppl.*, **1973**, 335.

(8) J. S. Sackett, C. M. Strother, C. E. Quaglieri, M. J. Javard, A. B. Levin, and T. A. Duff, *Radiology*, **123**, 779 (1977).

(9) H. O. Peterson, *ibid.*, **115**, 237 (1975).

(10) K. Y. Chynn, *ibid.*, **109**, 361 (1973).

(11) J. E. Siggins, J. H. Ackerman, and A. A. Larsen, *J. Med. Chem.*, **8**, 728 (1965).

(12) J. H. Ackerman, V. Akullian, C. Moore, and A. A. Larsen, *ibid.*, **9**, 165 (1966).

(13) J. E. Siggins and J. H. Ackerman, *ibid.*, **9**, 973 (1966).

(14) B. N. Newton, *ibid.*, **19**, 1362 (1976).

(15) B. N. Newton, U.S. pat. 4,022,814 (May 10, 1977).

(16) W. B. Deichmann and T. J. LeBlance, *J. Ind. Hyg. Toxicol.*, **25**, 415 (1943).

(17) R. Venkataraghavan, R. D. Board, R. Klimowski, J. W. Amy, and F. W. McLafferty, *Adv. Mass Spectrom.*, **4**, 65 (1967).

(18) R. J. Klimowski and R. N. McLafferty, *Acc. Chem. Res.*, **3**, 158 (1970).

(19) F. W. McLafferty and G. E. VanLear, *Org. Mass Spectrum.*, **2**, 1 (1969).

(20) R. B. Moffett, "Organic Synthesis," coll. vol. IV, Wiley, New York, N.Y., 1963, p. 834.

(21) A. I. Vogel, "Practical Organic Chemistry," 3rd ed., Wiley, New York, N.Y., 1966, p. 1000.

(22) J. O. Hoppe, and S. Archer, *Am. J. Roentgenol.*, **69**, 630 (1953).
(23) C. Hansch, A. R. Steward, S. M. Anderson, and D. L. Bently, *J. Med. Chem.*, 11, 1 (1968).
(24) J. O. Hoppe, in "Medicinal Chemistry," vol. 6, E. E. Campaigne and W. H. Harting, Eds., Wiley, New York, N.Y., 1963, p. 343.

ACKNOWLEDGMENTS

The author thanks Dr. W. S. Bucke and Dr. A. L. Kunz for valuable discussion and support, Barbara Behrmann and Leonard Klein for technical assistance, and Wilma Lotts for help in manuscript preparation.

Quantitative GLC Analysis of Sterols in Biological Samples

S. N. HOOPER and R. F. CHANDLER ˣ

Received May 23, 1977, from the *College of Pharmacy, Faculty of Health Professions, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5.* Accepted for publication December 6, 1977.

Abstract □ A GLC method for the quantitative analysis of cholesterol, β-sitosterol, stigmasterol, campesterol, 7-dehydrocholesterol, and dihydrocholesterol in biological samples was developed to screen serum and lipid extracts of heart and liver tissue for these sterols precisely. The addition of the internal standard, cholestane, at the beginning of the procedure led to a reduction in the required sample size and the elimination of several steps. The only critical measurements are those of the biological samples and internal standard.

Keyphrases □ Sterols, various—GLC analyses in biological fluids and extracts □ GLC—analyses. various sterols in biological fluids and extracts

A study involving the feeding of cholesterol and other sterols to large numbers of chicks required a simple, precise method for the qualitative and quantitative analysis of the sterol content of serum, heart, and liver samples. The commonly employed colorimetric methods are subject to several limitations, not least of which is the lack of discrimination among two or more sterols in a single sample (1).

Several GLC methods were reported for the determination of serum cholesterol (2–8); however, a procedure was required to determine quantitatively cholesterol and other sterols in a single sample. Additionally, a small sample size and the least number of manipulations consistent with precise and accurate analysis were desirable objectives.

EXPERIMENTAL

Materials and Chemicals—All solvents were reagent grade. Cholestane[1] and the sterols were dissolved in chloroform, and their purity was checked by GLC. Accuracy was monitored by the daily use of commercial controls[2].

Apparatus—Chromatographs A[3] and B[4], equipped with dual hydrogen flame-ionization detectors and glass columns (3 mm × 2 m), were connected to a recorder fitted with an integrator[5] or an automatic programmable integrator[6] Nitrogen was the carrier gas. All gas flows were adjusted according to recommendations of the manufacturer.

The injection port and detector were set at 300°. Columns were packed with 3% OV-17 on 100–120-mesh Gas Chrom Q[7], 3% SE-30 on 100–120-mesh Gas Chrom Q[8], or 3% SP-2401 on 100–120-mesh Supelcoport[9]. Column temperatures (250–280, 280–300, or 240–250°, respectively) were adjusted to give retention times of less than 10 min for cholesterol. All analyses were isothermal, and the attenuation was adjusted to give near full-scale deflection for the internal standard.

Serum Analysis—Fresh serum (0.1 ml), cholestane standard (200 mg/100 ml, 0.1 ml), 33% KOH aqueous solution (0.2 ml), and ethanol (2.0 ml) were measured into a screw-capped test tube (13 × 100 mm). After mixing[10], the tubes were placed in a shaking water bath[11] at 60° for 2 hr. Following hydrolysis, water (0.5 ml) was added and the cholesterol and cholestane were extracted with hexane (2.0 ml). The hexane fraction was subjected directly to GLC analysis.

Tissue Extracts—Total lipid was determined quantitatively by extracting the weighed hearts and livers according to the method of Bligh and Dyer (9). The lipid subsequently was dissolved in chloroform (5 ml), 0.5 ml was immediately transferred to a screw-capped test tube, and the chloroform was evaporated in a stream of dry nitrogen. The analysis was continued as for serum, care being taken that the amount of lipid was small enough (less than 200 mg) to be saponified by the amount of potassium hydroxide being used. Values were then related to the weight of the tissue lipid.

GLC Analysis—GLC standards containing known amounts of cholestane, cholesterol, and/or β-sitosterol, campesterol, stigmasterol, dihydrocholesterol (cholestanol), and 7-dehydrocholesterol were prepared and analyzed[4,5] at the beginning and end of each day. A correction factor was determined by dividing the sterol concentration found by GLC with the known concentration. Sterol concentration of the serum and tissue samples was determined as follows:

Table I—Analysis of Control Serums

Control Serum	Number of Analyses	Mean ± *SE*
1[a]	41	174.7 ± 2.0
2[b]	37	135.8 ± 3.0

[a] Choles-Trol; reported mean value was 196/100 ml when assayed by the Anderson and Keys modification of the Abell method. [b] Moni-Trol I; reported mean value was 151 mg/100 ml when assayed by the Abell method.

[1] Aldrich Chemical Co., Milwaukee, WI 58233.
[2] Choles-Trol and Moni-Trol I, Dade Division, American Hospital Supply Corp., Miami, FL 33152.
[3] Hewlett-Packard model 5710A.
[4] F&M Scientific 700 laboratory chromatograph.
[5] Fisher Recordall series 100 fitted with disk chart integrator.
[6] Hewlett-Packard model 3380A.
[7] Chromatographic Specialties, Brockville, Ontario, Canada.
[8] Applied Science Laboratories, State College, PA 16801.
[9] Supelco, Inc., Supelco Park, Bellefonte, PA 16823.
[10] Vortex-Geni, Fisher Scientific Co.
[11] Magni Whirl model MSB-1122AA-1.

0022-3549/78/0800-1157$01.00/0