to Model I in ethanol–water and acetone–water solvent mixtures. In 5% cholate, the rates appear to be somewhat slower than the predictions of Model I; in bile salt–lecithin solutions, the rates appear to be more than a factor of 10 slower. In Fig. 10, the data of Small (2) are compared to some theoretical calculations. As can be seen, rates based upon Model I appear to be significantly greater than Small's *in vitro* results. These preliminary comparisons of experimental results with the theoretical relationships show that under physiologically important conditions, the simple diffusion-controlled model may or may not be applicable and that careful scrutiny of the various factors is necessary.

From the clinical standpoint, the idea that a 2.5-mm. gallstone might be dissolved in a few days is very appealing. Therefore, the question of why the observed rates might be significantly slower than diffusion controlled is important clinically.

REFERENCES

(1) W. H. Admirand and D. M. Small, *J. Clin. Invest.*, **47**, 1043 (1968).

(2) D. M. Small, *Advan. Intern. Med.*, **16**, 243(1970).

(3) Z. R. Vlahcevic, L. C. Bell, and L. Swell, *Gastroenterology*, **59**, 62(1970).

(4) D. M. Small and S. Rapo, *N. Engl. J. Med.*, **238**, 53(1970).

(5) J. L. Thistle and L. J. Schoenfield, *J. Lab. Clin. Med.*, **74**, 1020(1969).

(6) A. B. Bikhazi and W. I. Higuchi, *J. Pharm. Sci.*, **59**, 744 (1970).

(7) A. H. Ghanem, W. I. Higuchi, and A. P. Simonelli, *ibid.*, **58**, 165(1969).

(8) *Ibid.*, **59**, 232(1970).

(9) S. J. Desai, A. P. Simonelli, and W. I. Higuchi, *J. Pharm. Sci.*, **54**, 1459(1965).

(10) S. J. Desai, P. Singh, A. P. Simonelli, and W. I. Higuchi, *ibid.*, **55**, 1224(1966).

(11) *Ibid.*, **55**, 1230(1966).

(12) *Ibid.*, **55**, 1235(1966).

(13) A. W. Hixson and J. H. Crowell, *Ind. Eng. Chem.*, **23**, 923 (1931).

(14) P. Singh, S. J. Desai, D. R. Flanagan, A. P. Simonelli, and W. I. Higuchi, *J. Pharm. Sci.*, **57**, 959(1968).

(15) D. H. Neiderhiser and H. P. Roth, *Proc. Soc. Exp. Biol. Med.*, **128**, 221(1968).

(16) F. P. Woodford, *J. Lipid Res.*, **10**, 539(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 5, 1972, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication January 19, 1973.

▲ To whom inquiries should be directed.

Mechanisms of Dissolution of Human Cholesterol Gallstones

WILLIAM I. HIGUCHI▲, SOMPOL PRAKONGPAN, and FUDAH YOUNG

Abstract □ Experiments were designed for investigating and comparing the *in vitro* dissolution kinetics of human cholesterol gallstones and cholesterol monohydrate compressed pellets. The dissolution rates were determined in 66% acetone–water, in 63% ethanol–water, in bile acids, and in bile acid–lecithin solutions. It was found that the rates of dissolution of the stones compared well with the dissolution rates obtained with the cholesterol monohydrate pellets in all solvents investigated. The dissolution rates for both stones and pellets in the organic–aqueous solvents were extremely rapid and of the order of magnitude expected for a bulk diffusion-controlled process. In sodium cholate solutions, the dissolution rates were about 2–3 times slower than rates predicted by diffusion theory and the data suggested a modest interfacial resistance to dissolution. The rates obtained in 2% bile acid–1% lecithin solutions were about 17 times slower than diffusion-controlled processes, and these results point to an interfacial barrier to dissolution that may be very important clinically.

Keyphrases □ Gallstones, human—mechanisms of dissolution, dissolution rates in different media, compared to prepared cholesterol pellets, existence of interfacial barriers □ Cholesterol gallstones, human—mechanisms of dissolution, dissolution rates in different media, compared to prepared cholesterol pellets, existence of interfacial barriers □ Dissolution, human cholesterol gallstones—mechanisms, dissolution rates in different media, compared to prepared cholesterol pellets, existence of interfacial barriers □ Diffusion—role in dissolution of human cholesterol gallstones, existence of interfacial barriers □ Interfacial barriers—as a factor in the dissolution of human cholesterol gallstones, mechanisms of dissolution

During recent years much research has been done to evaluate the thermodynamic equilibria existing in the bile acid–lecithin–cholesterol–water systems and their relation to cholelithiasis (1, 2). It has been shown that the degree of cholesterol saturation or supersaturation in gallbladder bile is a critical factor in the formation or dissolution of cholesterol gallstones *in vivo*. While it is now clear that thermodynamic factors can play important roles, relatively little is known about the kinetic factors involved. Questions concerning the *kinetics* of gallstone dissolution have become especially important since the finding of Thistle and Schoenfield (3) and Danzinger *et al.* (4) that oral administration of chenodeoxycholic acid in patients with gallstones can result in the simultaneous "normalization" of bile and the dissolution of stones.

The present article reports results of initial physicochemical investigations on the mechanisms of dissolution of cholesterol gallstones. An attempt was made to determine: (*a*) whether or not the rate of cholesterol gallstone dissolution *in vitro* compares closely to the dissolution rate of "synthetic" gallstones of compressed cholesterol monohydrate pellets, and (*b*) whether or not the dissolution rates of gallstones in various solvent media are governed by diffusion in the bulk or by interfacial factors (5).

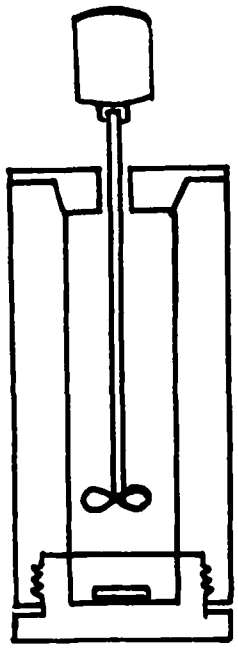


Figure 1—*The diffusion cell.*

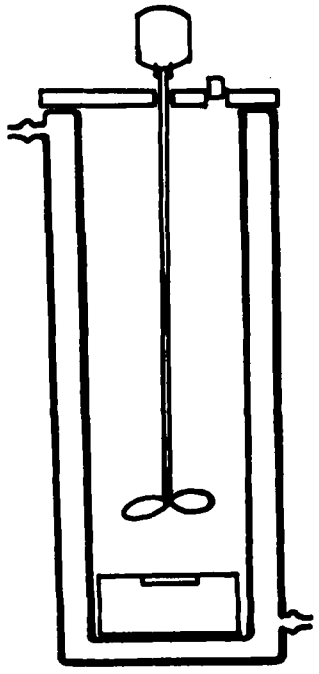


Figure 2—*The dissolution apparatus.*

Table I—Dissolution Rates (mg. cm.$^{-2}$ sec.$^{-1}$ × 10^4) of Human Gallstones[a] and Cholesterol Monohydrate Pellets in Solvent Media at 37°

Gallstones	63% Ethanol	66% Acetone	5% Cholate[b]	5% Glycocholate[b]	2% Cholate[b] and 1% Lecithin
Black					
o[c]	1.10	3.01	1.64	0.51	—
i[d]	2.70	5.28	2.62	0.88	—
Dark brown					
o	2.40	—	1.36	—	—
i	2.57	—	2.90	—	—
Light brown					
o	3.41	5.90	1.20	—	0.24
i	2.58	3.89	2.33	—	0.25
Gray					
o	1.87	3.90	1.94	—	0.17
i	2.33	5.66	2.21	—	0.21
White					
o	2.12	5.28	2.09	—	—
i	2.76	6.66	3.46	—	—
Cholesterol monohydrate pellets	2.60	4.45	1.95	0.34	0.18

[a] Spherical gallstones of diameters ranging from 0.60 to 1.10 cm. were used. [b] Containing 0.1 *M* phosphate buffer. [c] o = dissolution from external surface of gallstone. [d] i = dissolution from cross-sectional cut surface of gallstone.

EXPERIMENTAL

Materials—Radioactive cholesterol monohydrate was prepared by mixing 5 g. of a three-times recrystallized cholesterol[1] with 50 μc. of benzene solution of ^{14}C-cholesterol[2] and drying *in vacuo*. The mixture was dissolved in 400 ml. of 95% ethanol at 60°. It was then seeded with a few crystals at room temperature. After 48 hr., the mixture was filtered and dried *in vacuo* overnight.

Various aqueous compositions of the following materials were used as solvent media. Sodium cholate and glycocholate[3] were used as received. Sodium taurocholate[4] was prepared by the method of Pope (6). Egg lecithin was prepared by the method of Small *et al.* (7), except that commercial egg lecithin of purified grade[5] was used as starting material. Human gallstones were obtained from Hofmann and his group[4].

Solubility Determination—The solubilities of cholesterol monohydrate in various media were determined by introducing an excess amount of radioactive cholesterol monohydrate of about 100 mg. into 20 ml. of a solvent in a 50-ml. volumetric flask. The flask was simultaneously flushed with nitrogen gas and shaken by a wrist-action shaker[6] in a water bath at 37°. An aliquot of 5 ml. was taken and filtered through a 0.22-μ filter[6] in a controlled atmosphere at 37°. Ten milliliters of scintillation fluid (8) was added to 1.0 ml. of the filtrate, and the radioactivity was determined in a scintillation system[7]. Aliquots were taken and assayed for cholesterol daily. Solubility of cholesterol monohydrate in the medium was obtained when the concentration in the filtrate reached a constant level.

Diffusion Coefficient Determination—Measurements were made at 37° in a diaphragm diffusion cell made of Lucite (Fig. 1) and by a method similar to that described by Keller *et al.* (9), except that the two reservoirs were separated by double silver filter membranes[8] of 1.2-μ pore size. The lower reservoir had a capacity of 3.4 ml. and was completely filled with a solution of radioactive cholesterol during operation. The upper reservoir was filled with the same amount of solvent medium. The stirring speed in both reservoirs was maintained at 150 r.p.m. A solution of 0.1 *N* KCl with a known diffusion coefficient[9] of 2.41 × 10^{-5} cm.^{2} sec.$^{-1}$ at 37° was used as standard in the measurements.

Dissolution Rate Determination—*Cholesterol Monohydrate or Benzoic Acid Pellets*—Pellets of radioactive cholesterol monohydrate or benzoic acid were prepared by directly compressing 300 mg. of the material in a die, 1.3-cm. (0.5-in.) i.d., under a force of 3000 lb. using a laboratory press[10]. The surface area of the resulting pellets was 1.267 cm.^{2}. The pellet was held firmly in a die by covering the inside surface with melted paraffin. This die was then mounted at the bottom of a water-jacketed cylinder, with the pellet facing a

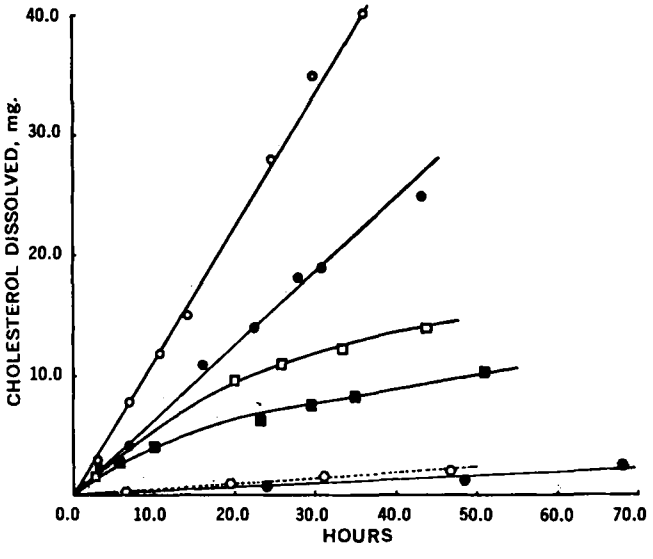


Figure 3—*Dissolution of human cholesterol gallstones at 37° in various solvent media. Key: ○, from external surface; ●, from cross-sectional inner surface of stone in 5% cholate and 0.1 M phosphate, pH 7.4; □, from external surface; ■, from cross-sectional surface of stone in 63% ethanol; ○, from external surface; and ●, from cross-sectional surface in 2% cholate + 1% lecithin + 0.1 M phosphate buffer, pH 7.4.*

[1] Eastman Kodak Co., Rochester, N. Y.
[2] New England Nuclear Corp., Boston, Mass.
[3] Schwarz/Mann, Orangeburg, N. Y.
[4] Supplied by A. F. Hofmann, Gastroenterology Unit, Mayo Clinic and Mayo Foundation, Rochester, Minn.
[5] Burrel Corp., Pittsburgh, Pa.
[6] Millipore Filter Corp., Bedford, Mass.
[7] Beckman Instruments Inc., Fullerton, Calif.
[8] Selas Flotronics, Spring House, Pa.
[9] This value was determined by the method of Saraf *et al.* (10).
[10] Model B, Fred Carver, Inc., Summit, N. J.

Table II—Solubilities (C_s), Diffusion Coefficients (D), Theoretical Dissolution Rates (J_{Th}/A), and Measured Dissolution Rates (J_{obs}/A) of Solutes in Solvent Media at 37°

Solute	Solvent Medium	C_s, mg. cm.$^{-3}$	$D \times 10^6$, cm.^{2} sec.$^{-1}$	$J_{Th}/A \times 10^4$, mg. cm.$^{-2}$ sec.$^{-1}$	$J_{obs}/A \times 10^4$, mg. cm.$^{-2}$ sec.$^{-1}$	J_{Th}/J_{obs}
Cholesterol monohydrate pellet	63% Ethanol	0.26	3.10	1.61	2.60	0.62
	66% Acetone	0.28	—	—	—	—
	2% Cholate[a]	0.53	2.17	2.30	0.67	3.43
	5% Cholate[a]	1.34	1.90	5.09	1.95	2.61
	2% Taurocholate[a]	0.12	2.15	0.52	0.17	3.03
	2% Cholate[a] + 1% lecithin	1.05	1.49	3.13	0.18	17.4
	2% Taurocholate[a] + 1% lecithin	0.51	1.24	1.26	0.075	16.9
Benzoic acid pellet	0.01 N HCl	4.70	14.0	131.0	131.0	1.00

[a] Containing 0.1 M phosphate buffer at pH 7.4.

stirring paddle inserted at the top of the cylinder (Fig. 2). The stirring speed was maintained at 150 r.p.m. during dissolution. Seventy milliliters of solvent preequilibrated at 37° was used. To measure the dissolution rates, the amount of cholesterol or benzoic acid dissolved in the solvent was analyzed against time.

Human Cholesterol Gallstones—Gallstones of various colors and sizes, ranging from 0.6 to 1.1 cm. in diameter, were used. Dissolution rates were measured from both the intact outer surface and the cross-sectioned inner surface by embedding a gallstone in a die with melted paraffin exposing half of the stone to the bulk solvent medium. The inner surface was obtained by cutting a spherical stone into halves cross sectionally. The surface areas for dissolution were estimated according to the specific geometry of the surfaces. The same apparatus and procedures as already described were then used. Samples of 5 ml. were taken and replaced with fresh solvent. Cholesterol in these samples was analyzed by the method of Dam *et al.* (11) or of Sperry and Webb (12) when lecithin was present in the medium.

RESULTS AND DISCUSSION

Figure 3 shows typical raw data obtained for the dissolution of human cholesterol gallstones in several solvent media. As can be seen, the dissolution in some solvents gave nearly linear plots; in these cases, the solvents remained much undersaturated with cholesterol during the dissolution period. In other solvents, apparent bending of the dissolution curves occurred as a result of the more rapid increase in cholesterol saturation with time.

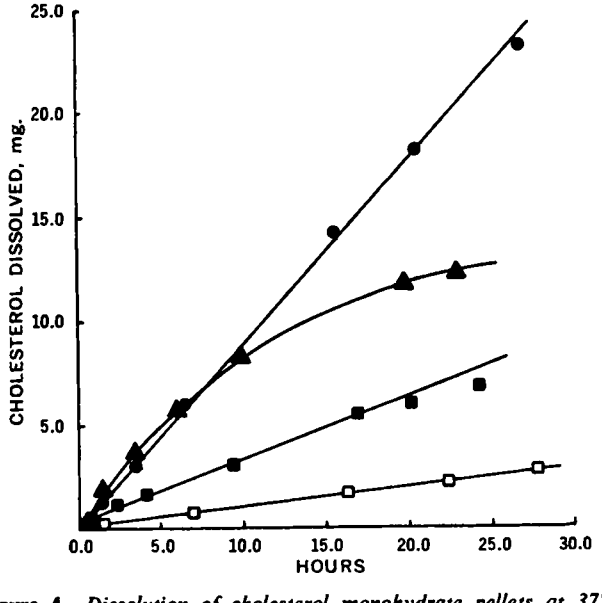


Figure 4—*Dissolution of cholesterol monohydrate pellets at 37°. Key: ●, in 5% cholate and 0.1 M phosphate buffer, pH 7.4; ■, in 2% cholate + 0.1 M phosphate buffer, pH 8.0; □, in 2% cholate + 1% lecithin + 0.1 M phosphate buffer, pH 8.0; and ▲, in 63% ethanol.*

The dissolution rate data of the compressed pellets of cholesterol monohydrate crystals are shown in Fig. 4. The dissolution patterns in Fig. 4 are similar to those in Fig. 3 for the gallstones. The dissolution rates (in mg. cm.$^{-2}$ sec.$^{-1}$) were obtained by taking the initial slopes of the dissolution curves such as those in Figs. 3 and 4 and correcting for the surface area.

Correlation between Gallstone and Cholesterol Monohydrate Dissolution Rates—The dissolution rates of various human gallstones and cholesterol monohydrate pellets in the organic-aqueous solvents and the bile media are shown in Table I. In each solvent studied, the dissolution rates of gallstones were found to compare closely with those for cholesterol monohydrate pellets. The variations in the gallstone dissolution rates, with a few exceptions, are well within a factor of two of the dissolution rates for the cholesterol monohydrate pellets in all solvents.

The dissolution rates determined from the inner cross-sectional surfaces were, in general, greater than those determined from the external surfaces. These differences and the variations found among the stones may be due to deposited film and matrix-forming substances derived from the biliary environment.

In view of the reported cholesterol content in human gallstones running as high as 98% (13), the consistent agreement between the dissolution rates of gallstones and cholesterol monohydrate pellets is not surprising. The data in Table I show that cholesterol monohydrate appears to be a good experimental model for the dissolution of the gallstones in these experiments. The presence of insoluble residues, bile pigments, mucus, and proteins may create physical situations where the rate of dissolution can be affected (5).

Diffusion-Controlled Model—In Table II, the experimental dissolution rate data for the cholesterol monohydrate pellets are compared to the dissolution rates predicted by the Nernst equation (14):

$$J = \frac{ADC_s}{h} \qquad \text{(Eq. 1)}$$

where A is the surface area of the pellet, D is the diffusivity of cholesterol in the solvent, C_s is the solubility of cholesterol monohydrate in the solvent, and h is the effective diffusion layer thickness. The theoretical rate calculations for J/A involved the use of Eq. 1 and the experimental D and C_s values, with an h value of 50 μ determined from a benzoic acid pellet dissolution experiment. This analysis points out several interesting aspects of the dissolution kinetics. First, the dissolution rates in the organic-aqueous solvents are maximal, *i.e.*, essentially diffusion controlled. Second, in the bile acid media, the rates are about 2–3 times slower than diffusion-controlled processes for the hydrodynamic situation prevailing in those experiments. Finally, when the solvent is 2% bile acid–1% lecithin, the rates are about 17 times slower than diffusion-controlled values and a very large interfacial barrier clearly dominates the kinetics.

REFERENCES

(1) A. F. Hofmann and D. M. Small, *Ann. Rev. Med.*, **18**, 333 (1967).

(2) D. M. Small, *Advan. Intern. Med.*, **16**, 243(1970).

(3) J. L. Thistle and L. J. Schoenfield, *J. Lab. Clin. Med.*, **74**, 1020(1969).

(4) R. G. Danzinger, A. F. Hofmann, L. J. Schoenfield, and J. L. Thistle, *N. Engl. J. Med.*, **286**, 1(1972).

(5) W. I. Higuchi, F. Sjuib, D. Mufson, A. P. Simonelli, and A. F. Hofmann, *J. Pharm. Sci.*, **62**, 942(1973).

(6) J. L. Pope, *J. Lipid Res.*, **8**, 146(1967).

(7) D. M. Small, M. C. Bourges, and D. G. Dervichian, *Biochim. Biophys. Acta*, **125**, 563(1966).

(8) G. A. Bray, *Anal. Biochem.*, **1**, 279(1960).

(9) K. H. Keller, E. R. Canales, and S. I. Yun, *J. Phys. Chem.*, **75**, 379(1971).

(10) D. N. Saraf, P. A. Witherspoon, and L. H. Cohen, *Science*, **142**, 955(1963).

(11) H. Dam, I. Kruse, H. E. Kallehauge, O. E. Hartkopp, and M. K. Jensen, *Scand. J. Clin. Lab. Invest.*, **18**, 385(1966).

(12) W. M. Sperry and M. Webb, *J. Biol. Chem.*, **187**, 97(1950).

(13) K. Juniper, Jr., *Mod. Treatment*, **5**, 480(1968).

(14) W. Nernst, *Z. Physik. Chem.*, **47**, 52(1904).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 5, 1972, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication January 26, 1973.

▲ To whom inquiries should be directed.

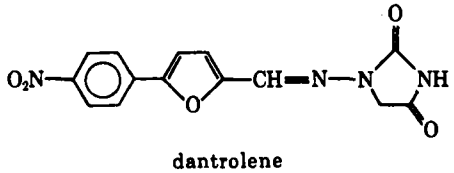
Dantrolene, a Direct Acting Skeletal Muscle Relaxant

K. O. ELLIS▲, A. W. CASTELLION, L. J. HONKOMP, F. L. WESSELS, J. F. CARPENTER, and R. P. HALLIDAY

Abstract □ Dantrolene causes skeletal muscle relaxation in animals without prominent CNS actions, and it has little or no measurable effect on smooth or cardiac muscle. Dantrolene reduces rigidity in decerebrate cats. Unlike centrally acting muscle relaxants, it has no preferential effect on polysynaptic flexor reflexes and still produces its maximum effect on the muscle twitch in an isolated, neurally intact, perfused hindlimb. Dantrolene blocks the twitch response when stimulated through the motor nerve in a way different from the action of tubocurarine or decamethonium. It inhibits direct twitch responses in denervated muscle and is equally effective in attenuating the direct and indirect twitch responses. It is hypothesized that dantrolene causes skeletal muscle relaxation by a direct action on muscle at a site beyond the neuromuscular junction.

Keyphrases □ Dantrolene—effects on skeletal muscle, CNS, and smooth or cardiac muscle □ Skeletal muscle relaxants, potential—dantrolene, proposed site of action

The synthesis of a series of 1-[(5-arylfurfurylidene)-amino]hydantoins and their identification as skeletal muscle relaxants were reported by Snyder *et al.* (1). One member of this series, dantrolene, 1-{[5-(*p*-nitrophenyl)furfurylidene]amino}hydantoin, subsequently received further evaluation (2, 3). This report details the general pharmacology of dantrolene and the proposed site of action as a skeletal muscle relaxant[1].



dantrolene

[1] Sodium dantrolene (Dantrium, Eaton Laboratories, Division of Norwich Pharmacal Co., Norwich, N. Y.) is now undergoing clinical trials as a skeletal muscle relaxant.

EXPERIMENTAL

Methods—*Gross Observational Evaluation*—Male albino mice[2], weighing 20–27 g., were used. The rating scale was similar to that described by Irwin (4). Dantrolene, suspended in 1% carboxymethylcellulose, was administered intraperitoneally and orally in logarithmically spaced doses.

Muscle Incoordination—A rotarod test similar to that reported by Dunham and Miya (5), except that the rod rotated at 20 r.p.m., was employed. The test drugs were administered intraperitoneally in groups of six previously trained mice (maximum of three training trials per animal), and the rotarod tests were conducted 15, 30, 60, and 180 min. following drug administration. The end-point for muscle incoordination was the animal's inability to stay on the rotarod for 30 sec. An ED_{50} (the dose of drug that caused 50% of the animals to fall off before 30 sec.) was estimated by probit analysis (6).

Flexor-Reflex Studies—Cats of either sex, weighing 2–4 kg., were anesthetized with an α-chloralose–urethan solution (50 and 500 mg./kg., respectively) in 50% propylene glycol with water administered intraperitoneally. Flexor-reflex preparations were prepared by a method similar to that of Berger (7), using the tibialis anticus muscle under a resting tension of 50 g. Sensory reflex muscle contractions were elicited by electrical stimulation of the central end of the cut tibial nerve. Motor nerve-induced contractions of the same muscle were obtained by electrical stimulation of the ipsilateral peroneal nerve. A pool of warm mineral oil bathed the nerves to prevent drying. Stimulus train durations of 50 msec. with a frequency of 100 Hz., a duration of 0.5 msec., and a voltage of 7–12 v. were applied to the tibial nerve. Motor nerve stimulation parameters were a duration of 1 msec., a frequency of 0.1 Hz., and a voltage adjusted so that the contraction response was comparable in magnitude to the tibial nerve-induced contraction. Nerve excitation was accomplished with bipolar platinum electrodes connected through a stimulus isolation unit[3] to a stimulator[4]. Contractions were recorded with a force-displacement transducer[5] on a polygraph[6].

[2] Taconic Farms.
[3] Grass SIU-5.
[4] Grass S88.
[5] Grass FT 10.
[6] Grass 7B.