Lysophosphatidylcholine-Induced Vascular Relaxation and Production of cGMP are Mediated by Endothelium-Derived Relaxing Factor (43625)

R. DUDEK, A. CONFORTO, AND R. J. BING[1]
Huntington Medical Research Institutes, Pasadena, California 91109-7013

Abstract. **Endothelial cells produce powerful vasorelaxant substances, among them an endothelium-derived relaxing factor that is believed to be nitric oxide. It relaxes vascular smooth muscle via activation of guanylate cyclase and a subsequent rise in cyclic GMP level. Lysophosphatidylcholine is a potent endothelium-dependent vascular smooth muscle relaxant. Its action, similar to that of endothelium-derived relaxing factor, mediates an increase of cGMP in smooth muscle cells. The experiments reported here demonstrate that inhibitors of nitric oxide formation, such as *N*-ω-nitro-L-arginine and its methyl ester, inhibit relaxation and cyclic GMP formation by lysophosphatidylcholine in bovine pulmonary artery strips with intact endothelium in a dose-dependent manner. *N*-ω-Nitro-D-arginine methyl ester does not inhibit relaxation; L-arginine, but not D-arginine, reverses the effect of *N*-ω-nitro-L-arginine and its methyl ester. It is concluded that lysophosphatidylcholine-induced endothelium-dependent vasorelaxation is endothelium-derived relaxing factor-mediated.** [P.S.E.B.M. 1993, Vol 203]

Previous experiments have shown that a phospholipid, lysophosphatidylcholine (LPC), relaxes endothelium-intact rabbit aortic and bovine pulmonary artery strips (1–3). The possibility was discussed that relaxation might be independent of nitric oxide, which is believed to be identical to endothelium-derived relaxing factor (4, 5). This view was based on the observation that 10^{-6} *M* of an inhibitor of nitric oxide (NO) synthesis, *N*-omega-nitro-L-arginine (NNA), failed to prevent LPC-induced relaxation of bovine pulmonary artery strips and cGMP production (6). On the other hand, Saito *et al.* (1) reported that the effect of LPC was inhibited by oxyhemoglobin and methylene blue, suggesting involvement of NO. The question, therefore, remains of whether LPC acts through the formation of NO. To answer this question, the effect of different concentrations of inhibitors of NO production on LPC-evoked relaxation of bovine pulmonary artery strips was investigated. Results indicate that concentrations of from 10^{-6} *M* to 10^{-4} *M* of NNA and its more soluble methyl ester (NNAME) inhibit both LPC-induced relaxation and cGMP production in a dose-dependent manner.

Material and Methods

The method of Menon (6) was followed. In brief, bovine lungs were obtained from a local abattoir (Shamrock Co., Vernon, CA). The third branch of the pulmonary artery was removed, the perivascular tissues were dissected, and the artery was cut spirally. The strips, approximately 3 mm wide, were cut into pieces about 1.5 cm long. They were each mounted in a 20-ml organ bath, with a resting tension of 2 g. The organ bath was filled with oxygenated Krebs-Henseleit solution of the following composition: 143 m*M* Na^+, 5.9 m*M* K^+, 2.5 m*M* Ca^{2+}, 1.2 m*M* Mg^{2+}, 127 m*M* Cl^-, 25 m*M*, HCO_3^-, 1.2 m*M* SO_4^{2-}, and 10 m*M* glucose; the temperature was 37°C. Indomethacin at a concentration 10^{-5} *M* was added to Krebs-Henseleit solution to prevent prostaglandin formation.

Tension was measured by means of isometric trans-

[1] To whom requests for reprints should be addressed at Huntington Memorial Hospital, 100 West California Boulevard, P.O. Box 7013, Pasadena, CA 91109-7013.

Received November 19, 1992. [P.S.E.B.M. 1993, Vol 203]
Accepted April 13, 1993.

0037-9727/93/2034-0474$3.00/0

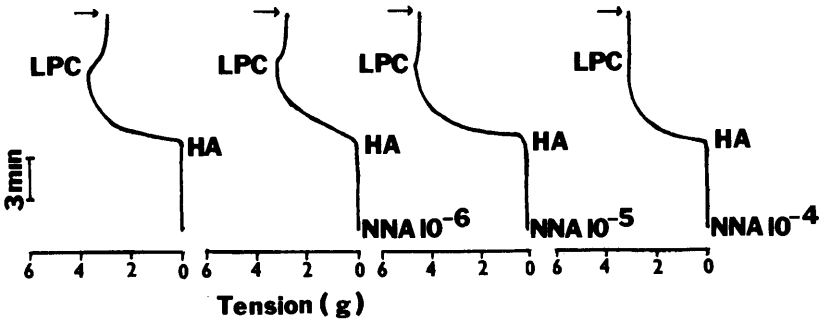

Figure 1. Histamine (HA, 10^{-5} *M*)-precontracted arterial strips with endothelium intact relaxed after LPC (10^{-5} *M*). Relaxation was inhibited by preincubation with NNA in a concentration-dependent manner (range, 10^{-6}-10^{-4} *M*). Arrows indicate the collection of tissue for determination of cGMP.

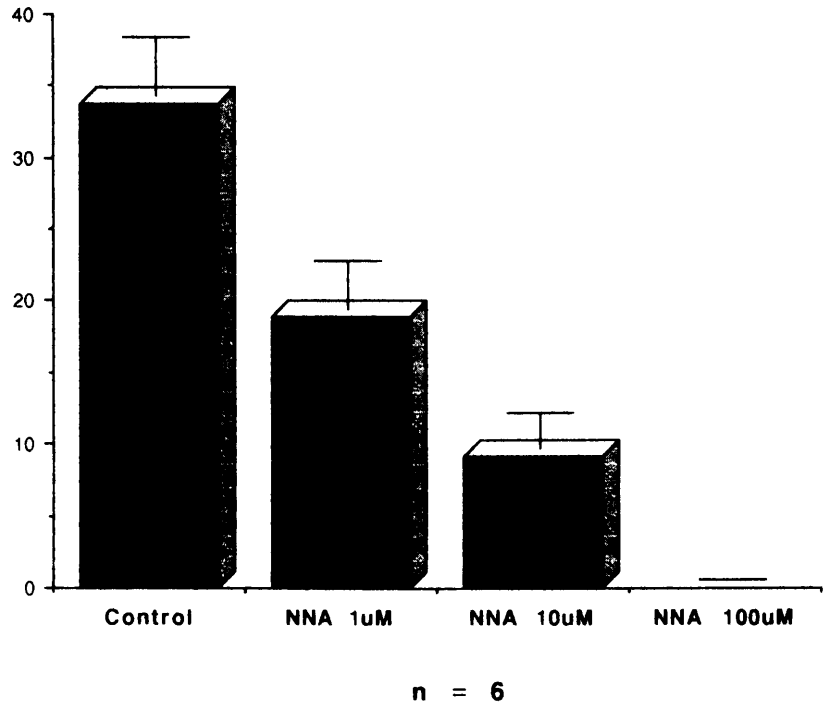

Figure 2. Inhibition of relaxation of arterial strips preincubated with NNA produced by LPC 10^{-5} *M*. "Control" shows the percentage of relaxation produced by LPC without NNA added ($P < 0.01$; see also Table I).

ducers (U-20-Gr, Shinkoh, Minnebea Co., Ltd., Tokyo, Japan) and recorded on Gould 2600 multichannel recorder (Gould Inc. Instruments Division, Cleveland, OH). The strips were allowed to equilibrate for 45 min. They were then incubated with different concentrations of inhibitors for 20 min and were precontracted with 10^{-5} *M* of histamine. After 4 min, when contraction had reached a steady state, LPC was added to Krebs-Henseleit solution for a final concentration of 10^{-5} *M*. To study the effect of inhibitors on the effect of histamine, NNA or NNAME was added to the organ chamber after steady state had been reached. The additional contractions were compared with the effect of histamine alone. The effects of L- and D-isomers of arginine were studied as follows: After the addition of histamine and proof of the integrity of the endothelium by acetylcholine 10^{-7} *M*, the inhibitors (10^{-6}, 10^{-5}, and 10^{-4} *M*) were added to the organ chambers for 30 min. The preparation was then washed and D- or L-arginine (3×10^{-6}, 3×10^{-5}, or 3×10^{-4} *M*) was added for 30 min. After addition of LPC (10^{-5} *M*), changes in tension were observed.

At maximum relaxation, the strips were rapidly frozen and stored in liquid nitrogen for cGMP determination, according to the method of Steiner *et al.* (7). Cyclic GMP level was determined in fmol/1 mg of wet tissue. The detection limit was 8.0 fmol/mg. Percentage of relaxation was calculated from the decrease in tension after administration of LPC.

L-Lysophosphatidylcholine (from egg yolk), *N*-ω-nitro-L-arginine, *N*-ω-nitro-L-arginine methyl ester hydrochloride, *N*-ω-nitro-D-arginine methyl ester hydro-

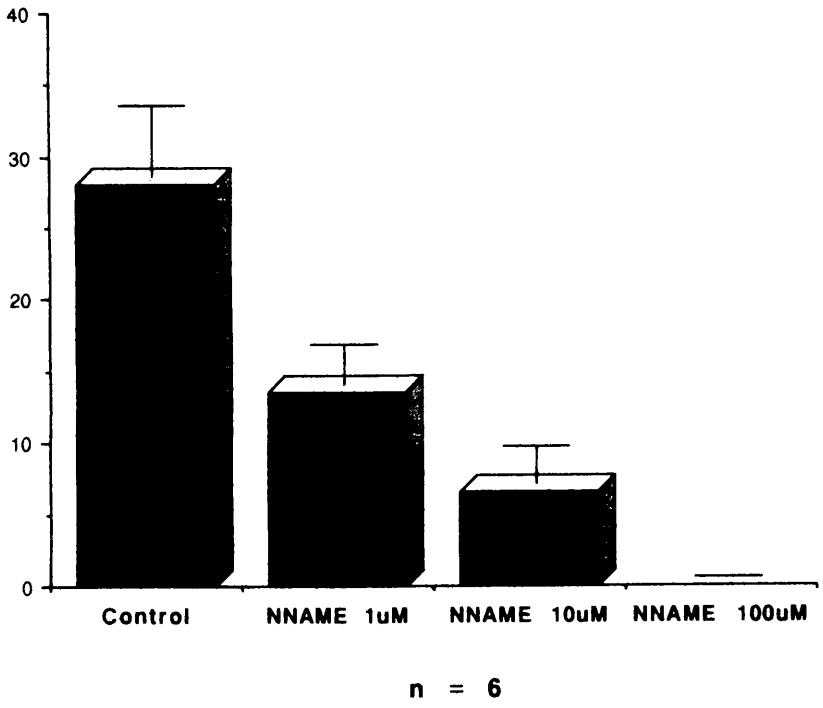

Figure 3. Inhibition of relaxation of arterial strips preincubated with NNAME produced by LPC 10^{-5} *M*. "Control" shows the percentage of relaxation produced by LPC without NNAME added ($P < 0.01$; see also Table II).

Table I. Percentage of Relaxation Produced by LPC 10^{-5} *M* Inhibition by NNA in Different Concentrations

Experiment	LPC only	NNA		
		10^{-6} *M*	10^{-5} *M*	10^{-4} *M*
1	31.0	20.0	15.0	0
2	41.0	26.0	16.0	0
3	47.0	20.0	8.0	0
4	37.5	28.0	10.0	0
5	22.0	6.0	0	0
6	23.5	13.5	5.5	0
Mean ± SE	33.7 ± 4.0	18.9 ± 3.3	9.1 ± 2.5	0
	$P < 0.01$	$P < 0.01$		$P < 0.01$

Table II. Percentage of Relaxation Produced by LPC 10^{-5} *M* Inhibition by NNAME in Different Concentrations

Experiment	LPC Only	NNAME		
		10^{-6} *M*	10^{-5} *M*	10^{-4} *M*
1	16.5	9.0	6.0	0
2	24.5	11.0	7.0	0
3	50.0	25.0	18.0	0
4	30.5	6.5	0	0
5	28.0	15.0	4.0	0
6	19.0	15.0	4.5	0
Mean ± SE	28.1 ± 4.9	13.6 ± 2.7	6.6 ± 2.2	0
	$P < 0.01$	$P < 0.01$	$P < 0.05$	

Table III. Influence of NNA and LPC on Level of cGMP[a]

Experiment	LPC Only	NNA		
		10^{-6} *M*	10^{-5} *M*	10^{-4} *M*
1	53.3	13.6	9.2	*
2	20.1	27.6	*	*
3	26.1	*	*	*
4	17.0	*	*	*
5	17.9	14.0	*	*
6	40.0	*	*	*
Mean ± SE	29.0 ± 6.0			

[a] cGMP is expressed as fmol/mg wet wt of tissue. Asterisk indicates that values are below detection range (8.0 fmol/mg).

chloride, D-arginine, L-arginine, acetylcholine chloride, histamine dihydrochloride, and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO. For cyclic GMP assay, the Amersham radioimmunoassay kit (Amersham Corp., Arlington Heights, IL) was used. Statistical analysis was done by Student's *t* test. $P < 0.05$ was considered significant.

Results

Lysophosphatidylcholine (LPC) administered at a concentration of 10^{-5} *M* produced 30.9 ± 3.1% relaxation in preconstricted bovine pulmonary artery strips (Figs. 1–3 and Tables I and II). After incubation of the strips with 10^{-6} *M* of either of the two inhibitors, LPC-induced relaxation was reduced to 18.9 ± 3.3% in the

Table IV. Influence of NNAME and LPC on Level of cGMP[a]

Experiment	LPC Only	NNAME		
		10^{-6} *M*	10^{-5} *M*	10^{-4} *M*
1	18.2	13.2	*	*
2	38.3	15.2	14.7	*
3	37.8	15.3	*	*
4	21.4	10.2	14.7	*
5	35.0	22.6	*	*
6	29.8	21.8	*	*
7	66.4	*	*	*
8	79.3	26.5	24.6	*
9	28.8	31.5	33.3	*
Mean ± SE	39.3 ± 6.8			

[a] cGMP is expressed as fmol/mg of wt of tissue. Asterisk indicates that values are below detection range (8.0 fmol/mg).

case of NNA and to 13.6 ± 2.7% for NNAME (Figs. 1–3 and Tables I and II). The effect was even more pronounced after incubation with larger concentrations of inhibitors. In comparison to the control, 10^{-5} *M* of NNA and 10^{-5} *M* of NNAME reduced LPC-induced relaxation to 9.1 ± 2.5% and 6.6 ± 2.5%, respectively. Relaxation was completely abolished after 10^{-4} *M* of both inhibitors. When the inhibitors (10^{-6}, 10^{-5}, and 10^{-4} *M*) were added after histamine precontraction, a further dose-dependent increase of tension was noticed (5.8 ± 2.6%, 35.8 ± 10.8%, and 69.1 ± 7.6%, respectively). This additional contraction was the result of inhibition of basal release of nitric oxide. In eight experiments, the D-isomer of the inhibitor (D-NNAME) had no effect on either histamine-induced contraction or LPC-induced relaxation: 35.5 ± 2.8% as compared with 30.9 ± 3.1% with LPC alone (Tables I and II). In concentrations that were three times higher than those of the inhibitors, L- but not D-arginine reversed the inhibition induced by NNA and NNAME ($n = 6$).L-Arginine relaxation induced by LPC was 28.7 ± 7.3% as compared with 30.9 ± 3.1% by LPC alone.

Cyclic GMP content of bovine pulmonary artery strips also decreased as a result of the inhibitors (Tables III and IV and Figs. 4 and 5). After administration of LPC alone, the average cGMP level was 35.3 ± 4.8 fmol/mg wet wt of tissue. Incubation of the strips with

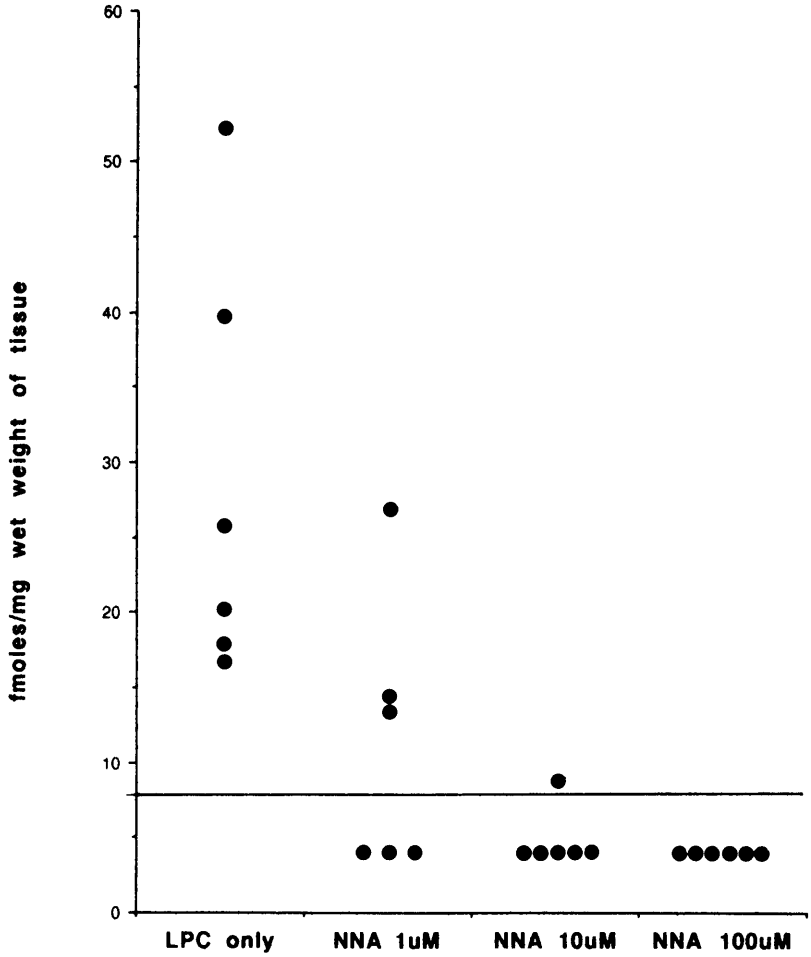

Figure 4. Levels of cGMP in arterial tissue after preincubation with NNA and stimulation by LPC. The horizontal line shows the radioimmunoassay detection limit (8 fmol; see also Table III).

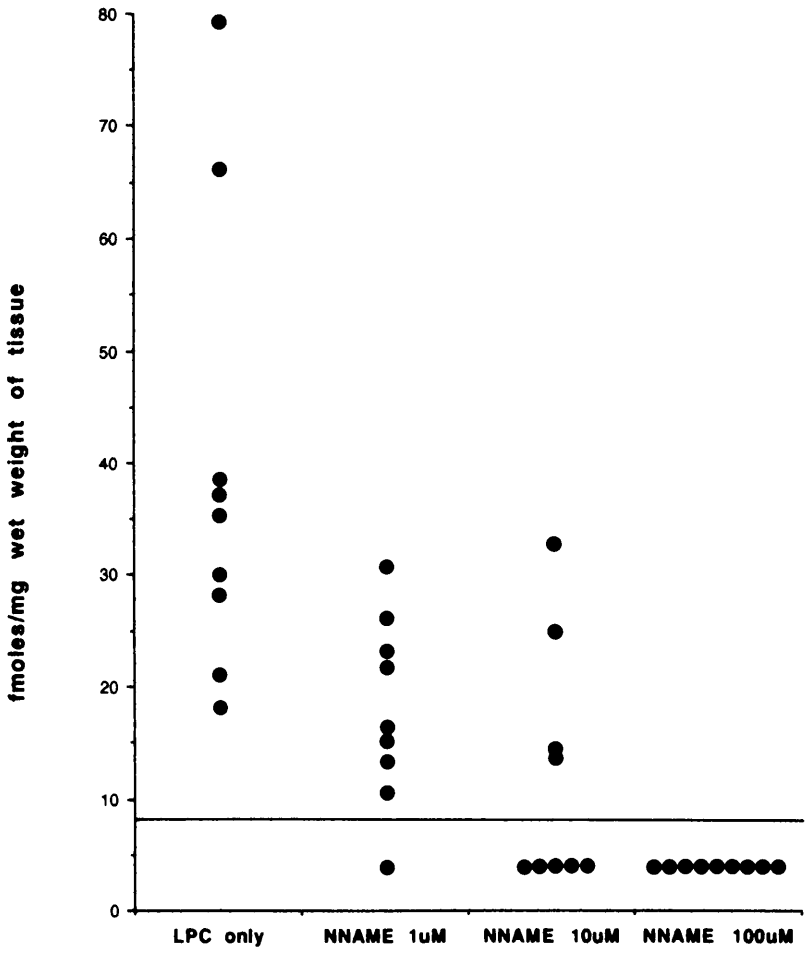

Figure 5. Levels of cGMP in arterial tissue after preincubation with NNAME and stimulation by LPC. The horizontal line shows radioimmunoassay detection limit (8 fmol; see also Table IV).

10^{-6} *M* of both inhibitors significantly reduced cGMP content. The decrease in cGMP levels was more significant with increasing concentrations of the inhibitors. With 10^{-5} *M* of NNA or NNAME, most of the values of cGMP were below the detectable range (Tables III and IV and Figs. 3 and 4). After administration of 10^{-4} *M* of the inhibitors, none of the values was in the range of the standard curve.

Discussion

Lysophosphatidylcholine (LPC) is a vascular relaxant of rabbit thoracic aorta and bovine intrapulmonary arteries and veins (1–3, 6). Previous experiments suggested that endothelium-derived relaxing factor may not be involved in relaxation of vascular smooth muscle by LPC (6). This was supported by the finding of Menon and colleagues (1–3, 6) that NNA, an inhibitor of formation of nitric oxide from arginine, at a concentration of 10^{-6} *M* fails to influence LPC-induced relaxation. On the other hand, Saito *et al.* (1) found that both oxyhemoglobin, which abolishes the action of endothelium-derived relaxing factor (8), and methylene blue, which prevents the formation of cGMP (9), inhibit LPC-induced relaxation. The results reported here suggest that NO is involved in the relaxation induced by LPC. This conclusion is based on the finding that inhibitors of NO production from L-arginine, such as *N*-ω-nitro-L-arginine (NNA) and its methyl ester (NNAME), inhibit both relaxation and cGMP formation (10, 11). The concept of formation of NO from L-arginine was first proposed by Schmidt *et al.* (12). Based on their findings, Ishi *et al.* (10), as well as Kobayashi and Hattori (11) and others (13, 14), used arginine derivatives to inhibit the formation of endothelium-derived relaxing factor. Inhibition by these compounds, therefore, suggests the participation of NO in the relaxation as proposed by Palmer *et al.* (4) and Ignarro *et al.* (5).

In previous work, Menon and Bing (6) showed no significant inhibition of LPC-induced relaxation or cGMP production in the endothelium intact strips by 10^{-6} *M* of NNA. The present data demonstrate that at a higher concentration (10^{-4} *M*) of either NNA or its methyl ester (NNAME), inhibition is complete (Tables I-IV). Some relaxation is also present at concentrations of 10^{-5} and 10^{-6} *M*. These results differ from a previous

publication (6) in which only a concentration of 10^{-6} M of the inhibitor was used. Possibly at this small a concentration, the response of the pulmonary artery is inconsistent. Supporting the involvement of nitric oxide in LPC-induced relaxation is the finding that N-ω-nitro-D-arginine methyl ester does not inhibit relaxation. Equally, L-arginine, but not D-arginine, reverses the inhibition of LPC-induced relaxation by NNA and NNAME.

Several publications have suggested a role of membrane phospholipids in vascular relaxation. For example, Förstermann and colleagues (15, 16) found that mellittin, an activator of phospholipase A_2, and thimerosal, which inhibits lysolecithin acyltransferase, induce endothelium-dependent relaxation. Equally, Huang and Lee (17) and Bing and Saeed (18) discovered that phospholipase A_2 is a vasorelaxant. Lysolecithins function as membrane transducers and are known to activate nucleotide cyclases (19). In addition, LPC is a surfactant that, similar to Triton X-100, stimulates production of cGMP (20). Fujimoto and Okabayashi (21) also found that Triton X-100, similar to phospholipase A_2, acts primarily on particular guanylate cyclase as a result of perturbation of membrane architecture.

It is, therefore, likely that LPC as a detergent causes changes in membrane fluidity that result in formation of an NO-like product which, through activation of guanylate cyclase, leads to relaxation of vascular smooth muscle.

This work was supported by grants from the Council for Tobacco Research-USA, Inc., New York, NY; the Margaret W. and Herbert Hoover, Jr. Foundation, Pasadena, CA; and Miles Inc., New Haven, CT. Dr. Dudek was supported in part by the Polish American Congress, Woodland Hills, CA.

We would like to thank D. Tang, C. Kim, S. Holdsworth, and A. Csjetey for technical assistance, as well as L. DeChaine for secretarial assistance.

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