Alcohol & Alcoholism Vol. 36, No. 1, pp. 44–47, 2001

LONG-TERM VOLUNTARY ETHANOL CONSUMPTION INDUCES IMPAIRMENT OF THE MECHANICAL PERFORMANCE IN THE PAPILLARY MUSCLE OF SARDINIAN ALCOHOL-PREFERRING RATS

D. LICHERI, R. VARGIU, F. FADDA*, A. FABRIZI[1] and R. MANCINELLI

Department of Applied Sciences for Biosystems, Division of Human Physiology and Nutrition, University of Cagliari, Via Porcell 4, I–09124 Cagliari and [1]Institute of Human Physiology, Catholic University, Largo F. Vito 1, I–00168 Rome, Italy

(*Received* 25 *April* 2000; *in revised form* 26 *July* 2000; *accepted* 28 *July* 2000)

Abstract — The effects of chronic (26 weeks) ethanol consumption on cardiac muscle contractility in Sardinian alcohol-preferring drinkers (sP-D) and Sardinian alcohol-preferring naive (sP-N) rats were investigated. Experiments were carried out 1 week after ethanol administration ceased. Length–tension and force–frequency responses in left ventricular papillary muscles from both sP-D and sP-N rats were recorded *in vitro*. Papillary muscles were gradually stretched in steps of 5% of the reference (initial) length (L_r) from 100 to 130% L_r. In sP-D rats, length–tension relationships showed a significant reduction in active tension: at optimal length for maximum active tension (130% L_r), the developed force value (1.38 ± 0.36 mN/mg, dry tissue) was 54% lower than that found in sP-N rats (3.16 ± 0.6 mN/mg, dry tissue). In sP-D papillary muscles, a decrease in contractile behaviour was also observed in force–frequency responses (0.03–120 pulse/min), when compared with sP-N rats; developed force was found to be reduced by about 2.5 times. These results indicate that long-term ethanol consumption impairs the mechanical performance of sP papillary muscle, inducing a negative inotropic effect.

INTRODUCTION

It is well known that chronic ethanol consumption induces structural and functional alterations in the heart of various animal species including humans (for review, see Thomas *et al.*, 1994). The most prevalent syndrome of alcoholic heart disease is cardiomyopathy, which is associated with depressed cardiac output, reduced myocardial contractility, and dilatation of heart chambers (Wynn and Braunwald, 1987).

In studies on animal models, the effects of chronic ethanol treatment on the heart are similar to those found in man. Indeed, chronic ethanol treatment produces an impaired ventricular function in dogs (Pachinger *et al.*, 1973; Regan *et al.*, 1974; Thomas *et al.*, 1980). Moreover, chronic ethanol treatment induces a decrease in cardiac function in isolated rat heart (Weishaar *et al.*, 1977; Segel *et al.*, 1979; Chan and Sutter, 1982; Capasso *et al.*, 1991, 1992). Ethanol also produces altered contractile activity associated with a decrease in action potential duration in the left ventricular papillary muscle of rats (Tepper *et al.*, 1986). However, at variance with humans, rodents do not drink ethanol to the point of intoxication voluntarily. In fact, animals consume intoxicating amounts of ethanol only when forced to (Fadda and Rossetti, 1998). Thus, in all the above studies chronic ethanol was forcibly administered.

The selectively bred alcohol-preferring animals willingly and freely drink alcohol under free choice with water. Like other selected rat lines, alcohol-preferring Sardinian (sP) rats display a clear preference for ethanol over water and drink 6 g/kg of ethanol or more in a day under the two-bottle, free choice paradigm (Gessa *et al.*, 1991).

The present study was undertaken to assess whether voluntary ethanol consumption for long periods of time also produces an impairment of heart motor performance in sP rats. We investigated the mechanical properties of isolated papillary muscles in terms of length–tension and force–frequency relationships. The isolated papillary muscle was chosen for this experiment, because it is an exceptional *in vitro* preparation for studying the mechanical properties of the rat myocardium without neuronal and hormonal influences. Mechanical properties were determined in isolated papillary muscle of sP rats which consumed ethanol (12% w/v) for 26 weeks in a free choice paradigm with water.

METHODS

Animals

Twelve sP male rats from the 38th generation were used for this study. Animals, weighing approximately 220–250 g at the start of the experiment, were maintained at 22°C and 60% relative humidity, on a 12 h light–12 h dark cycle (lights on at 07:00). Animals were divided into two groups: (1) six naive rats (sP-N) had food and water freely available throughout the entire experimental period (26 weeks); (2) six drinking rats (sP-D), maintained under the same conditions as the first group, but with free choice between tap water and 12% (w/v) ethanol for the same period of time. After the treatment period, ethanol was withdrawn and there was a 1-week wash-out period before the start of the experiments. The measured mean amount of alcohol consumed by the sP-D rats during the 26-week period was 6.94 ± 1.08 g/kg/day.

Experimental procedure

Animals were killed with a lethal dose of ether anaesthesia and their hearts were excised rapidly and placed initially in modified Krebs–Henseleit solution pre-equilibrated with 95% O_2–5% CO_2 at room temperature. Hearts were massaged to encourage them to beat and expel residual blood from the ventricles. Left ventricular papillary muscle was carefully dissected and mounted vertically in a water-jacketed bath

*Author to whom correspondence should be addressed.

(10 ml) containing modified Krebs–Henseleit solution of the following composition (mM): NaCl (123), $NaHCO_3$ (20), $MgSO_4$ (0.8), KCl (6), $CaCl_2$ (2.52), KH_2PO_4 (1.16), glucose (11.98). The solution was kept at 32°C and bubbled continuously with a mixture of 95% O_2–5% CO_2 maintaining a pH of 7.4. The base of the muscle was fixed firmly to the bottom of the organ bath while its free upper end was tied with a silk thread to a Grass FT03 force-displacement transducer mounted on a moving support allowing minimum length increments of 5 μm.

Stimulation of the muscle was accomplished with platinum mass electrodes (20 × 5 mm) placed along the parallel aspect of the muscle. The muscle was stimulated with a Digit 3T stimulator connected to a multiplexing pulse booster (Basile). Supramaximal square-wave pulses (80–150 mA, 4 ms) were used.

A frequency of stimulation of 30 pulses/min was generally employed, except when the response to rate of stimulation was assessed.

Length–tension responses

The papillary muscle was allowed to equilibrate for a period of 1 h under a preload of 3 mN. After equilibration and before stretching, the length of the muscle was measured using a stereomicroscope with an ocular micrometer. This initial length was called the reference length and indicated as L_r. Recorded tension without stimulation was called *resting* (or *passive*) *tension*. With stimulation, an active contraction was superimposed on the resting tension. This developed tension was termed *active tension*.

The papillary muscle was gradually stretched in steps of 5% L_r from 100% to 130% L_r, allowing force to stabilize at each new length. Resting and active isometric tensions that developed at each increase in length were recorded.

Frequency response recordings

The papillary muscle was elongated up to 130% L_r and stimulated at the following rates: 0.33, 1, 3, 6, 12, 18, 24, 36, 48, 60, 120, 180 pulses/min for as long as necessary to obtain steady-state amplitude of contraction waves. Outputs from the force-transducer were amplified and displayed continuously on a Grass polygraph. They were collected by a data acquisition board and fed into a personal computer for subsequent analysis.

Hearts and papillary muscles were blotted to remove excess fluid and dried in an oven set at 80°C until they reached a constant weight (dry weight).

Data analysis

At each increment in length of the papillary muscle, the resting tension was measured in the rest period between contractions, from the baseline tension determined at 100% L_r. The developed tensions recorded at each increase in length were calculated from the mean amplitude of the last 10 contractions before the next stretch. Active tensions developed at 130% L_r in response to different electrical stimulation rates were obtained from the mean amplitude of the contractions on the last 10 waves of the steady-state periods at each value of stimulation frequency.

All tension values were normalized to dry weight (mg) of papillary muscle and are presented as means ± SE. Data were analysed using one-way ANOVA. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 gives the weights of body, heart, and papillary muscle of both sP-N and sP-D rats. sP-D animals had significantly ($P < 0.05$) lower body weight values, compared with sP-N rats. Although no significant differences between sP-D and sP-N rats' heart dry weights were observed, the dry heart to body weight ratio was significantly ($P < 0.05$) higher in sP-D animals. This could indicate either a state of cardiac hypertrophy or that the lower weight gain affected most tissues in sP-D rats. This aspect will be clarified in our continuing experiments, in which the weight of organs will be considered.

Typical experimental traces of length–tension responses of papillary muscles from sP-N and sP-D rats are shown in Fig. 1. With each increase in length (1.00–1.30 L_r), there was a corresponding increase in resting and developed tensions in all preparations. However, in sP-D papillary muscles, the active tension was depressed at each increase in length in comparison to sP-N animals.

The length–tension relationships of six sP-N and six sP-D rats are shown in Fig. 2. In sP-D papillary muscles, chronic

Table 1. Body weight (BW), dry heart weight (DHW), dry papillary weight (DPW) and dry heart weight to body weight ratio (DHW/BW) for Sardinian alcohol-preferring naive (sP-N) and drinking (sP-D) rats

Experimental groups	BW (g)	DHW (mg)	DPW (mg)	DHW (mg)/ BW (g) ratio
sP-N ($n = 6$)	590 ± 23*	321 ± 11	2.10 ± 0.10	0.546 ± 0.012
sP-D ($n = 6$)	510 ± 23	302 ± 11	1.96 ± 0.39	0.593 ± 0.012*

Values are means ± SEM for six animals, *n* indicates the number of rats in each group.
*$P < 0.05$.

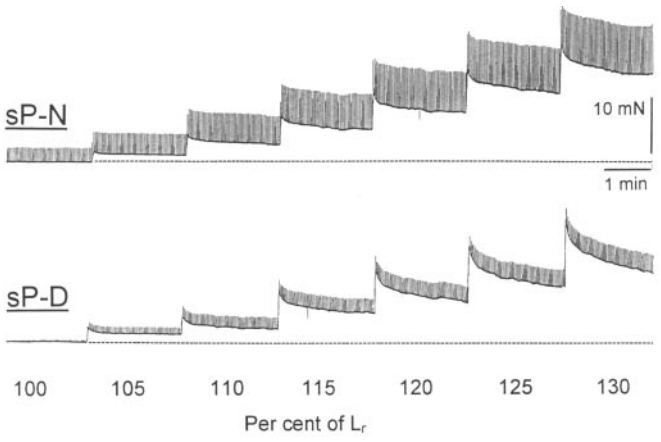

Fig. 1. Typical experimental traces of the effect of increasing length on resting and developed tensions in two papillary muscles from Sardinian alcohol-preferring non-drinking (sP-N) and Sardinian alcohol-preferring drinking (sP-D) rats.

The broken line represents the resting tension recorded at 100% L_r. Developed tension was obtained by electrical stimulation (80–150 mA, 4 ms, 30 pulses/min).

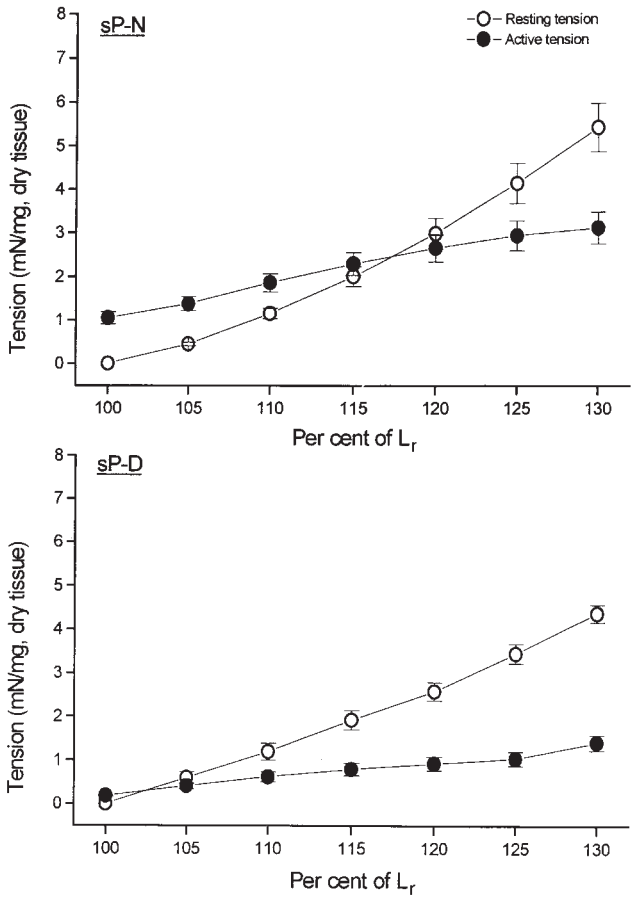

Fig. 2. Length–tension relationship for Sardinian alcohol-preferring non-drinking (sP-N) and Sardinian alcohol-preferring drinking (sP-D) rat papillary muscle.
Muscle length is expressed as a percentage of the reference length (L_r).
Points are means ± SEM for six experiments.

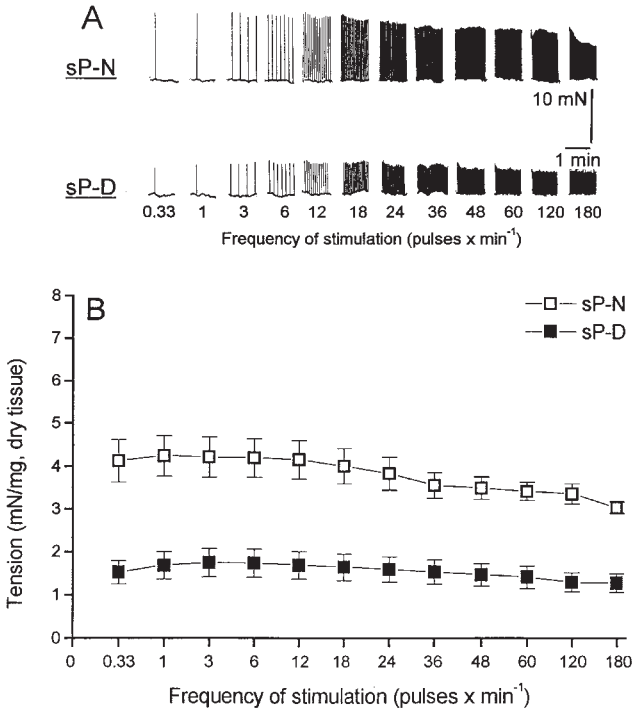

Fig. 3(A). Typical experimental traces showing the effect of increasing rate of electrical stimulation on developed tension of papillary muscles from sP-N and sP-D rats.
Papillary length: 130 L_r.
(B). Force–frequency relationships obtained in sP-N and sP-D papillary muscle.
Points are means ± SEM for six experiments.

ethanol intake induced a significant ($P < 0.05$) negative inotropic effect, as indicated by a decrease in developed tension under the levels observed in papillary muscles from naive rats. In fact, active tension was lower for sP-D rats over the entire range of muscle lengths studied. In these animals, at final length (1.30 L_r), the value of maximum active tension of 1.40 ± 0.17 mN for left ventricular papillary muscles was significantly lower ($P < 0.05$) than the value obtained in naive rats (3.11 ± 0.36 mN). At initial (1.00 L_r) and final (1.30 L_r) lengths, the mean amplitude values of active tension were on average 3.27-fold lower in comparison to naive rats.

Chronic ethanol consumption did not significantly alter the resting length tension of papillary muscle. As shown in the experimental traces in Fig. 3A and in the diagrams in Fig. 3B, altered mechanical properties of the papillary muscles from sP-D rats were also noted in response to electrical field stimulation at graded impulse rates. In both sP-N and sP-D papillary muscles, the maximum and quite constant response to electrical stimulation was obtained at frequencies between 0.33 and 12 pulses/min; with increasing frequencies (18–180 pulses/min), the amplitude of responses decreased (Fig. 3A).

A significant difference ($P < 0.05$) was found between SP-D and sP-N papillary muscles in force–frequency relationships (Fig. 3B). In sP-D preparations, the response to different pulse rates was reduced by about 2.42 fold, as compared to sP-N rats.

DISCUSSION

The present work demonstrates that voluntary ethanol consumption for a period of 26 weeks induces changes in motor performance in the heart of sP-D rats. In fact, in isolated papillary muscle, a negative inotropic effect, following long-term ethanol intake, was evident in the length–tension curves. In sP-D rats, the entire active tension curve was significantly depressed in comparison to that obtained in sP-N rats. On the contrary, few or no significant changes in the resting tension between sP-N and sP-D rats were observed.

Several studies have shown that rats chronically treated with ethanol have impaired cardiac muscle contractility (Segel *et al.*, 1975; Kino *et al.*, 1981; Preedy and Peters, 1989; Capasso *et al.*, 1992). Measurement of developed isometric tension in papillary muscles dissected from the hearts of rats fed ethanol for periods of 5 weeks to several months, generally demonstrates significant reductions in one or more indices of mechanical performance (Segel *et al.*, 1975; Kino *et al.*, 1981; Tepper *et al.*, 1986). By contrast, Capasso *et al.* (1992) reported that, in the papillary muscle, no difference in isometric developed tension was found between controls and ethanol-treated rats when

either the left or right side was compared. However, these researchers found alterations in cardiac mechanical performance, alterations in myofibrillar Mg^{2+}-ATPase activity and a significant decrease in the total number of myocitic nuclei in the left ventricular myocardium. However, our results are in agreement with those of Tepper *et al.* (1986), who reported that forced long-term ethanol administration (30 weeks) in unselected Wistar rats produced mechanical alterations (depressed force–velocity relation) in the left papillary muscle. This altered contractile activity is associated with a decrease in action potential duration. However, in the study by Tepper *et al.*, (1986), rats received a very highly concentrated ethanol solution (40% v/v) as the sole source of fluid, with consumption at the end of the treatment period of a very high amount of ethanol (10.1±1.8 g). In addition, the rats did not have a washout period before being tested. Thus under such experimental conditions, besides the chronic effect, there was also an acute effect of ethanol.

In the present experiments, sP rats drank ethanol solutions voluntarily in a free choice with water and did not consume intoxicating amounts of ethanol (see Fadda and Rossetti, 1998). Nonetheless, the results obtained showed that the quantity of ethanol ingested by sP rats was sufficient to produce alterations in contractile performance in the papillary muscle, and that this manifestation was not reversible after abstinence from alcohol for 1 week. Alterations of contractile performance were also observed in the isolated portal vein motility in sP rats (Licheri *et al.*, 1999), thus indicating that chronic ethanol consumption in a free choice paradigm induces a general depression of motor events in the cardiovascular system.

The mechanism of action of ethanol in the papillary muscular cells of sP rats is at present unknown. Evidence from various laboratories suggests that two major mechanisms appear to be important. One consists of a reduction in the capacity of the sarcoplasmic reticulum to accumulate Ca^{2+} (Swartz *et al.*, 1974; Retig *et al.*, 1977; Hara and Kasai, 1977) together with a decrease in the activity of specific sarcolemmal ion channels (Guarnieri and Lakatta, 1990). The other consists of an inhibitory effect of ethanol on the contractile proteins (Segel *et al.*, 1975; Sarma *et al.*, 1976; Weishaar *et al.*, 1977; Preedy and Peters, 1989; Capasso *et al.*, 1992). Further investigations of these forms of alteration of contractile behaviour in our animal model should not only provide insight into more basic cellular mechanisms responsible for their appearance, but should also lead to possible methods for their prevention or amelioration.

Acknowledgement — The authors thank Sandro Capra for the arrangement of the experimental set-up and for his able technical assistance during the experiments.

REFERENCES

Capasso, J. M., Giancarlo, P. L., Guideri, G. and Anversa, P. (1991) Left ventricular dysfunction induced by chronic alcohol ingestion in rats. *American Journal of Physiology* **261**, H212–H219.

Capasso, J. M., Li, P., Guideri, G., Malhorta, A., Cortese, R. and Anversa, P. (1992) Myocardial mechanical, biochemical and structural alterations induced by chronic ethanol ingestion in rats. *Circulation Research* **71**, 346–356.

Chan, T. C. K. and Sutter, M. C. (1982) The effects of chronic ethanol consumption on cardiac functions in rats. *Canadian Journal of Physiology and Pharmacology* **60**, 777–782.

Fadda, F. and Rossetti, Z. L. (1998) Chronic ethanol consumption: from neuroadaptation to neurodegeneration. *Progress in Neurobiology* **56**, 385–431.

Gessa, G. L., Colombo, G. and Fadda, F. (1991) Rat lines genetically selected for differences in voluntary ethanol consumption. In *Current Practices and Future Developments in the Pharmacotherapy of Mental Disorders*, Meitzer, H. Y. and Nerozzi, D. eds, pp. 192–200. Elsevier, Amsterdam.

Guarnieri, T. and Lakatta, E. G. (1990) Mechanism of myocardial contractile depression by clinical concentrations of ethanol. *Journal of Clinical Investigation* **85**, 1462–1467.

Hara, K. and Kasai, M. (1977) The mechanism of increase in the ATPase activity of sarcoplasmic reticulum vesicles treated with n-alcohols. *Journal of Biochemistry* **82**, 1005–1017.

Kino, M., Thorp, A., Bing, O. H. L. and Abelmann, W. H. (1981) Impaired myocardial performance and response to calcium in experimental alcoholic cardiomyopathy. *Journal of Molecular and Cellular Cardiology* **13**, 981–989.

Licheri, D., Vargiu, R., Del Monaco, S., Fadda, F. and Mancinelli, R. (1999) Chronic ethanol consumption induces hypomotility in the portal vein of Sardinian alcohol-preferring rats. *Alcohol and Alcoholism* **34**, 169–174.

Pachinger, O. M., Tillmanns, H., Mao, J. C., Fauvel, J. M. and Bing, R. J. (1973) The effect of prolonged administration of ethanol on cardiac metabolism and performance in the dog. *Journal of Clinical Investigation* **523**, 2690–2696.

Preedy, V. R. and Peters, T. J. (1989) Synthesis of subcellular protein fractions in the rat heart *in vivo* response to chronic ethanol feeding. *Cardiovascular Research* **23**, 730–736.

Regan, T. J., Kahan, M. I., Ettinger, P. O., Haider, B., Lyons, M. M. and Oldewurtel, H. A. (1974) Myocardial function and lipid metabolism in the chronic alcoholic animal. *Journal of Clinical Investigation* **54**, 740–752.

Retig, J. N., Kirchberger, M. A., Rubin, E. and Katz, A. M. (1977) Effects of ethanol on calcium transport by microsomes phosphorylated by cyclic AMP-dependent protein kinase. *Biochemical Pharmacology* **26**, 393–396.

Sarma, J. S. M., Ikeda, S., Fischer, R., Maruyama, Y., Weishaar, R. and Bing, R. J. (1976) Biochemical and contractile properties of heart muscle after prolonged alcohol administration. *Journal of Molecular and Cellular Cardiology* **8**, 951–972.

Segel, L. D., Rendig, S. V., Choquet, Y., Chacko, K., Amsterdam, E. A. and Mason, D. T. (1975) Effects of chronic graded ethanol consumption on the metabolism, ultrastructure, and mechanical function of the rat's heart. *Cardiovascular Research* **9**, 649–663.

Segel, L. D., Rendig, S. V. and Mason, D. T. (1979) Left ventricular dysfunction of isolated working rat hearts after chronic alcohol consumption. *Cardiovascular Research* **13**, 136–146.

Swartz, M. H., Repke, D. I., Katz, A. M. and Rubin, E. (1974) Effects of ethanol on calcium binding and calcium uptake by cardiac microsomes. *Biochemical Pharmacology* **23**, 2369–2376.

Tepper, D., Capasso, J. M., and Sonnenblick, E. H. (1986) Excitation–contraction coupling in rat myocardium: alterations with long term ethanol consumption. *Cardiovascular Research* **20**, 369–374.

Thomas, A. P., Rozanski, D. J., Renard, D. C. and Rubin, E. (1994) Effects of ethanol on the contractile function of the heart: a review. *Alcoholism: Clinical and Experimental Research* **18**, 121–131.

Thomas, G., Haider, B., Oldewurtel, H. A., Lyons, M. A., Yeh, C. K. and Regan, T. J. (1980) Progression of myocardial abnormalities in experimental alcoholism. *American Journal of Cardiology* **46**, 233–241.

Weishaar, R., Sarma, J. S., Maruyama, Y., Fischer, R., Bertuglia, S. and Bing R. J. (1977) Reversibility of mitochondrial and contractile changes in the myocardium after cessation of prolonged ethanol intake. *American Journal of Cardiology* **40**, 556–562.

Wynn, J. and Braunwald, E. (1987) The cardiomyopathies and myocarditis. In *Heart Disease: A Textbook of Cardiovascular Medicine*, Braunwald, E. ed., pp. 1437–1500. W. B. Saunders, Philadelphia, PA.