Negative Inotropic Effects of Phenol on Isolated Cardiac Muscle

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Phenol appears in high concentrations in renal failure with uremia. The effects of this material on contractile activity of isolated cardiac muscle were studied in right ventricular moderator band (MB) of piglets and papillary muscle (PM) of cats and kittens. The muscles were bathed in modified Krebs solution containing 5.6 mM glucose at 30 C and gassed with 95% O_2 and 5% CO_2. They were paced at 24 contractions per minute, isometrically at L_{max}. Over the range 2.5–119.0 mg%, phenol produced dose-related decreases in both developed tension (DT) and maximal rate of tension development (max dT/dt) in MB of piglets. In contrast, the dose-dependent negative inotropic effect of phenol was not detected in feline PM until concentrations in excess of 12.5 mg% were used. Increasing extracellular Ca^{2+} from 2.5 to 5.0 mM as well as the addition of norepinephrine (3.94×10^{-7} M) attenuated the phenol-induced cardiac depression in porcine MB. There were no further changes in either DT or max dT/dt when the extracellular Ca^{2+} was increased to 10 mM. These findings demonstrate that phenol elicits a direct negative inotropic effect on mammalian cardiac muscle that is modified by calcium and norepinephrine. Phenol may participate in the biochemical alterations leading to cardiac failure and death in uremia. (Am J Pathol 1981, 102:367–372)

UREMIA is frequently accompanied by cardiac failure,[1-4] although the basis for reduced myocardial function is not fully understood. Often the clinical syndrome of uremic cardiomyopathy improves substantially following hemodialysis,[2,5] suggesting the presence of a myocardial depressant substance.[1,4,5-7] Phenol is among the several metabolites that are retained during renal failure. The effect of this substance on cardiac function has received little attention, although early studies demonstrated a cardioinhibitory action on isolated frog heart.[4,6] No data are available from mammalian hearts, however. Accordingly, we undertook the present investigation to explore the effects of various concentrations of phenol on contractile performance of isolated feline and porcine cardiac muscle. Interactions between phenol, calcium, and norepinephrine were also examined.

Materials and Methods

All animals were anesthetized with sodium pentobarbital (25 mg/kg). Following thoracotomy, the heart was quickly removed and a papillary muscle (cat or kitten) or moderator band (piglet) was rapidly excised from the right ventricle.

Methods detailing the muscle preparation and instrumentation employed in this study have been described elsewhere.[8] In brief, the muscle strip was mounted in a constant-temperature chamber at 30 C, bathed with a modified Krebs solution (mM: Na^+, 146; K^+, 3.6; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 126; H_2PO_4, 1.2; SO_2^{2-}, 1.2; HCO_2^-, 25; glucose, 5.6), and gassed with 95% O_2 and 5% CO_2.

The muscle was paced at 24 contractions per minute isometrically with a Grass stimulator (S44) at the peak of the length-active tension curve (L_{max}) using two parallel platinum electrodes, with voltages 10% above threshold and stimulus durations of 5 msec. Changes in contractile force (F) were measured with a Statham (UC-4) force transducer and recorded on a Sanborn Oscillograph at chart speeds of 0.25 or 50 mm/sec. The maximum rate of force development was measured from the contractile force curve. F and dF/dt were normalized from muscle cross-sectional area and expressed as tension (g/sq mm) and maximum rate of

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tension development (g/sq mm/sec). The papillary muscles were assumed to be cylindrical, and cross-sectional area was calculated by dividing muscle mass (wet weight) by specific gravity (1.05) and by muscle length at the apex of the length-tension curve. The mean cross-sectional areas of feline (5 kittens, aged 2–4 weeks, and 4 adult cats) and porcine (N = 20, aged 10–35 days) isolated muscles were 0.79 ± 0.23 sq mm and 0.91 ± 0.17 sq mm, respectively.

Each muscle was allowed at least 60 minutes for stabilization before the initiation of any tests. The drugs used were phenol (carbolic acid, Fisher), and L-norepinephrine (Winthrop). Only one phenol dose-response curve was performed on each muscle using the cumulative technique. Cumulative concentration-response relationships were achieved by consecutive additions of phenol to the tissue bath. A 3-minute period during which the measured contractile force did not exhibit further change was employed before the next dose was administered.

All data were processed and analyzed by standard statistical methods,[9] and significance was determined by the unpaired Student *t* test. Differences were considered significant when the *P* value was less than 5%.

Results

Contractile Response of Isolated Cardiac Muscle to Phenol

Seven porcine right ventricular moderator bands (MB) and 6 feline right ventricular papillary muscles (PM) were employed to obtain phenol dose-response curves over the concentration range of 2.5 mg% to 11.8 mg%. The original traces shown in Figure 1 illustrate typical changes in the isometric developed force following a dose of 2.5 mg% of phenol in an MB from a 2-week-old piglet (upper tracing), and in an PM from a 4-week-old kitten (lower tracing). Shortly after the addition of phenol, the isometric developed force fell substantially in the piglet MB. In contrast, phenol elicited a small positive inotropic response in the kitten PM. This latter response pattern was also obtained from adult cat PM.

Figure 2 compares phenol dose-response curves (expressed as a percentage of the control) from MB of piglets and PM of kittens and cats. There were no differences in the responses of the latter, and the data have been pooled. Phenol produced significant dose-dependent decreases in both peak developed tension (DT) and maximal rate of tension development (max dT/dt) in porcine MB. In contrast, a slight but significant increase in DT was observed in feline PM at doses of 2.5 and 4.9 mg% ($P < 0.05$). This positive effect gradually diminished toward the control level as the phenol concentration reached 11.8 mg%. The initial changes in max dT/dt were not significant.

With higher concentrations of phenol, feline PM also exhibited progressive negative inotropic responses. Typical contractile changes over the range 12.4–119 mg% are shown in Figure 3. Mean values from three papillary muscles and seven moderator bands are compared in Figure 4.

A dose-dependent decrease in both DT and max

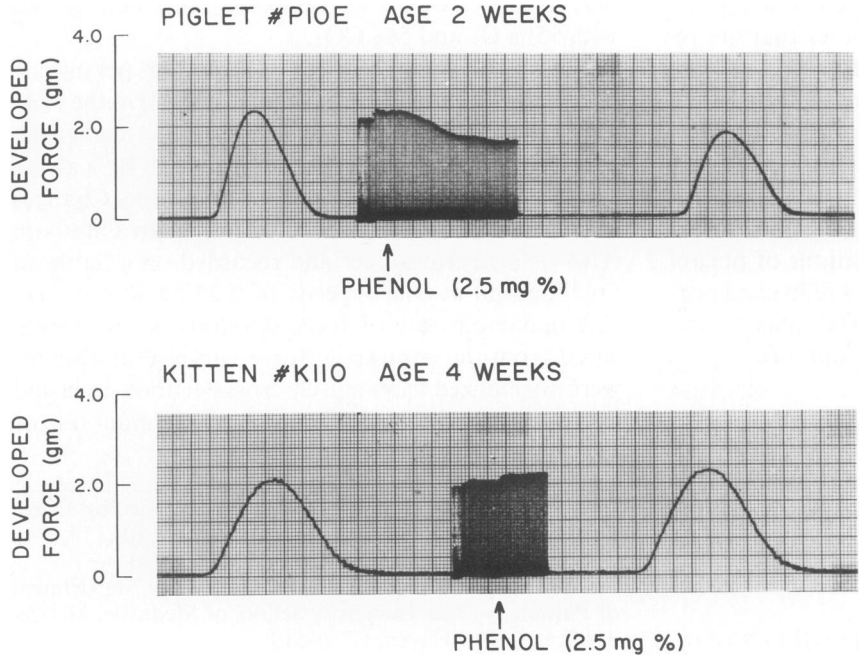


Figure 1—Original traces showing isometric contractions of an isolated right ventricular moderator band from a 2-week-old piglet (*upper*) and right ventricular papillary muscle from a 4-week-old kitten (*lower*). Phenol (carbolic acid) added at *arrow* to provide 2.5 mg% concentration.

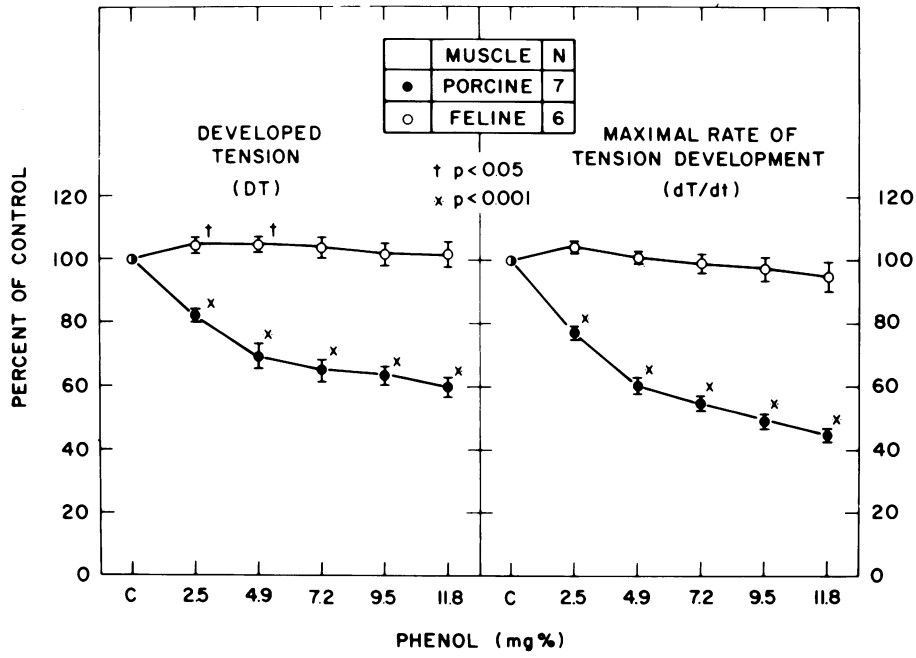


Figure 2—Dose-response curves for DT (*left panel*) and dT/dt (*right panel*) obtained from isolated porcine (*closed circles*) and feline (*open circles*) preparations showing effects of increasing phenol concentrations. Vertical brackets indicate SE. Values for *P* refer to differences from initial control values (*C*).

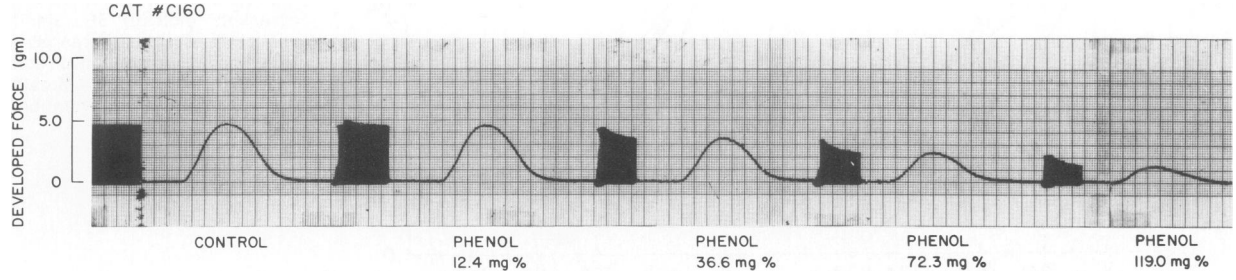
dT/dt was observed in both species. As with the lower doses, piglet cardiac muscle was much more sensitive to phenol depression than that from cats. At the highest concentration (119.0 mg%), phenol reduced feline PM DT to 35%, and max dT/dt to 37% of control. However, isometric contraction of porcine MB was completely abolished. The differences between the two species are significant over the entire dose range ($P < 0.001$, Figure 4). It is noteworthy that resting tension in both species remained constant as the phenol concentration was increased, indicating that neither contracture nor decreased compliance had occurred in these muscles.

Influence of Extracellular Ca^{2+} on the Negative Inotropic Action of Phenol

Ten additional porcine MBs were studied with increased intracellular Ca^{2+} concentrations. In three, 5 mM Ca^{2+} was used; and in seven, 10 mM Ca^{2+}. The results were compared with the previous group using 2.5 mM Ca^{2+}. The baseline DT (g/sq mm) and max dT/dt (g/sq mm/sec) with increased Ca^{2+} concentrations were 2.26 ± 0.25; 12.85 ± 1.78; 3.58 ± 0.41; 14.62 ± 1.87; 4.38 ± 0.37; and 27.19 ± 2.80, respectively. As shown in Figure 5, with 5 mM Ca^{2+}, the phenol-induced cardiac depression was significantly reduced, as compared with the 2.5 mM Ca^{2+}. There was no further improvement as the extracellular Ca^{2+} was increased to 10 mM, however.

Influence of Norepinephrine on the Cardiac Action of Phenol

The negative inotropic effect of phenol was reduced by exposure of the muscle to norepinephrine (3.94×10^{-7} M), as shown in Figure 6. Mean values for DT and dT/dt after norepinephrine were 5.29 ± 0.21 g/sq mm and 16.7 ± 0.87 g/sq mm/sec. At the highest concentrations of phenol (119 mg%) DT and dT/dt were reduced to 33.4 ± 2.8 and 37 ± 7.7% of initial values, respectively. However, in the controls without nor-



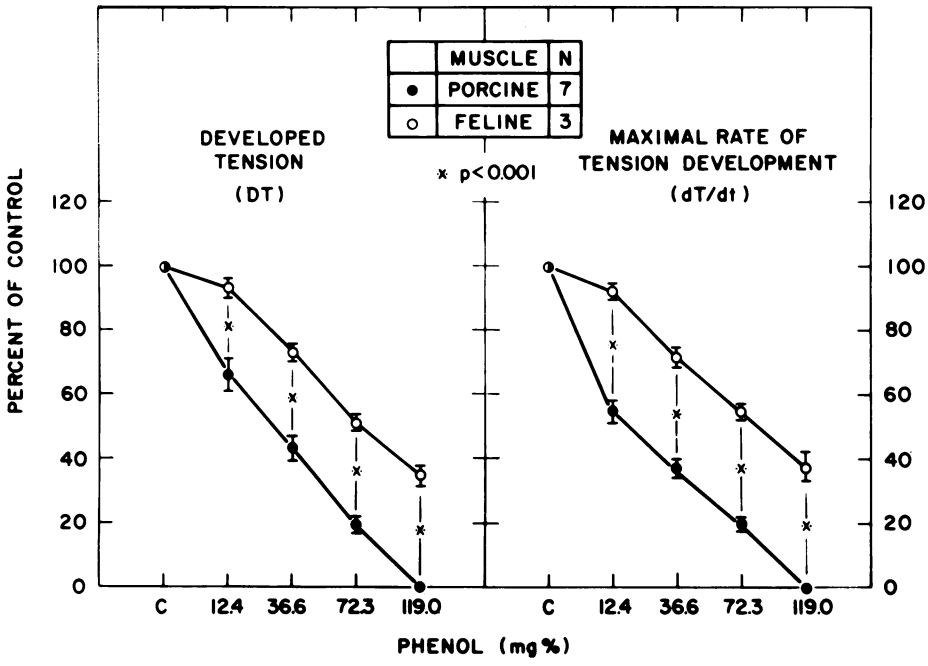


Figure 4—Dose-response curves for DT (*left panel*) and dT/dt (*right panel*) in isolated porcine (*closed circles*) and feline (*open circles*) muscle preparations using higher phenol concentrations range. Vertical brackets indicate SE. Depression was significantly greater at all concentrations in piglet than in cat preparations.

epinephrine, contractions completely ceased (Figure 6, open circles).

Discussion

The results obtained in the present study demonstrate a direct negative inotropic action of phenol on mammalian cardiac muscle. Myocardium from piglets is more sensitive to phenol depression than is that from cats or kittens (Figures 2 and 4). Increasing extracellular Ca^{2+} from 2.5 to 5.0 mM, or the addition of 3.94×10^{-7} M norepinephrine significantly attenuates phenol-induced cardiac depression of porcine PM (Figures 5 and 6). There were no additional attenuation of either DT or max dT/dt as the extracellular Ca^{2+} was increased to 10 mM. This is consistent with previous observations showing that maximal inotropic changes appear when extracellular Ca^{2+} is between 5.0 and 8.0 mM.[10,11] It suggests that intracellular Ca^{2+} storage sites may become functionally saturated at this level, even though the total calcium in the myocardium may continue to increase

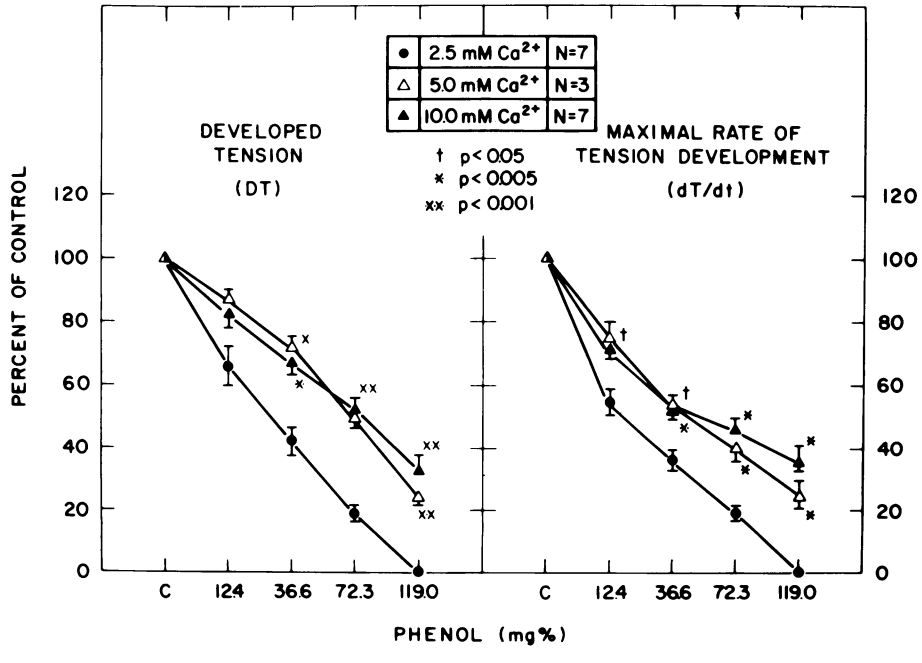


Figure 5—Influence of external calcium concentration on the negative inotropic action of phenol in the isolated moderator band of the piglet. Vertical brackets indicate SE. Significance levels for differences at each phenol concentration compare 5.0 mM and 10.0mM calcium with 2.5 mM concentrations.

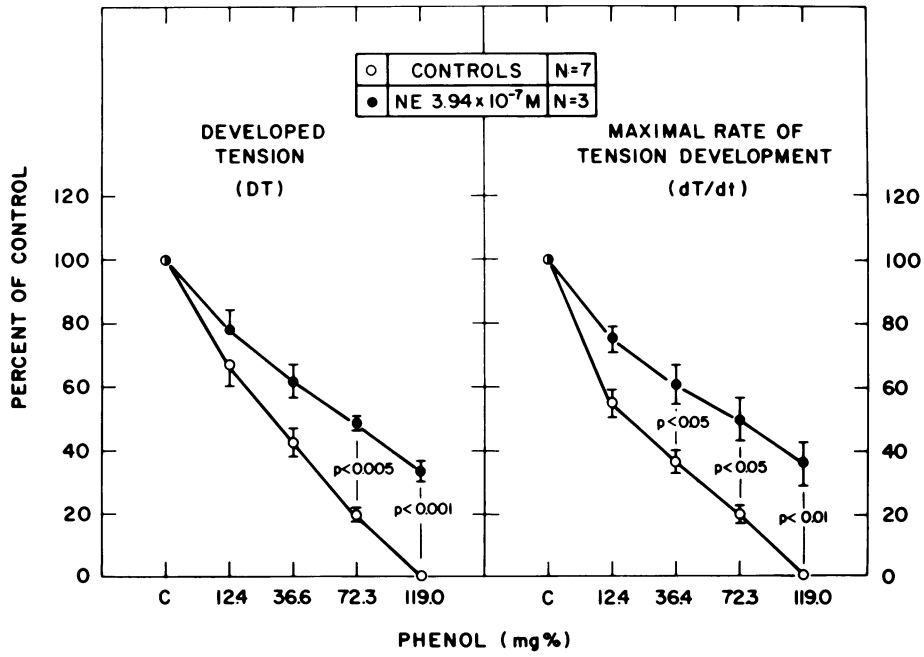


Figure 6—Influence of norepinephrine (NE) on the negative inotropic effects of phenol in the isolated moderator band of the piglet. Vertical brackets indicate SE. Contractile activity ceased at the highest concentration (*open circles*) but persisted in the presence of NE.

with higher extracellular concentrations.[12] There is no clear explanation for the observed species differences in sensitivity of the contractile responses to phenol. Membrane systems and mechanisms for excitation-contraction coupling vary among species and could be responsible.[13] But this remains to be demonstrated.

The pharmacologic actions of phenol are not well understood. It has been reported in cultured cells that 1 mg/ml phenol causes a marked increase in the permeability of lysosomal and mitochondrial membranes[14] and significant reductions in ATP[15] and DNA synthesis.[16] Phenolic compounds are also known to uncouple oxidative phosphorylation in intact mitochondrial membranes.[17] There is evidence indicating that phenol may partially block sodium channels in frog atria and reduce the early sodium inward current.[18] This would of course, be expected to influence muscle contraction.

Phenolic compounds appear in the blood largely by absorption from the intestinal tract of breakdown products derived from bacterial action on proteins. Some free phenols are detoxified by the liver, but most are excreted in the urine. Retention of phenols in the blood is a regular feature of uremia and may cause metabolic defects in red cells and platelet aggregation in uremic patients.[19,20] Dickes[21] has reported average concentrations of total and free phenol in 28 uremic patients of 1.79 and 1.51 mg%, respectively. Geferman et al[22] have reported values uniformly above 3.4 mg%. This concentration in our study caused a decrease of 20% in DT and max dT/dt in porcine MB (Figure 2).

Mason et al,[6] working with isolated perfused frog hearts, was able to show that serum from uremic dogs has a cardioinhibitory action, and that this effect could be mimicked by addition of phenol to the perfusion fluids. Subsequently, Raab[4] reported similar findings. He further demonstrated that a concentration of 1 mg/ml caused complete abolition of cardiac contraction in isolated frog hearts. This observation is consistent with our findings in porcine MB (Figure 4). The beneficial hemodynamic effects of dialysis are well documented,[11,14] and the possibility that this is consequent to removal of one or more cardiodepressant factors has been suggested.[2,5] Several compounds, including urea, creatinine, methyl guanidine, and guanidinosuccinic acid are known to be elevated in uremia. Scheuer and his associates,[7] using isolated working rat heart preparations, showed that cardiac function is depressed when normal hearts are perfused with mixtures of these materials. The degree of depression seems to be related to the number of compounds included in the mixture. While urea is obligatory for the mixture to be depressant, urea itself exhibits no dose-depression relationship. Recently, Kersting et al[23] have shown that urea independently produces some depressant action on isolated guinea pig hearts.

It may be concluded from this study that phenol elicits a substantial dose-dependent negative inotropic effect on isolated mammalian cardiac muscle. Moreover, this occurs in concentrations often found in the circulation of patients with renal failure. Increasing extracellular Ca^{2+} or the addition of norepinephrine significantly attenuates this cardioinhibitory effect. Precise delineation of the cellular mechanisms that

underlie these observations will require more detailed understanding of cardiac muscle chemistry. Nevertheless, this study clearly suggests that the beneficial hemodynamic effects of dialysis in uremic patients is in part related to removal of cardiodepressant factors, such as phenol. It further suggests that phenol, and possibly other metabolites, participates in biochemical derangements that lead to cardiac failure and death in renal failure.

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Acknowledgments

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