for $C_{10}H_8N_2$; C, 76.95; H, 5.12; N, 17.95. Found: C, 76.71; H, 5.56; N, 17.73).

The mass spectrum (Table) of *m*-cyanohydrocinnamonitrile differed only slightly from that of the *p*-isomer, but was not identical to that of the unknown compound. Its IR-spectrum showed frequencies at 1598, 1582, 1480, 890 and 800 cm^{-1} as expected. There were 2 nitrile bands, 2245 and 2230 cm^{-1}. As with the *p*-isomer they were assigned to aliphatic and aromatic nitrile groups on the basis of intensities. Methylene group frequencies were found at 2950, 2930, 1430 and 765 cm^{-1}. Contrary to expectation the PMR-spectrum showed a single peak at 455 c/sec equivalent to 4 aromatic protons and 4 aliphatic proton peaks at 182, 177, 167 and 161 c/sec. In view of the aromatic proton spectrum shown by the *p*-isomer a minimum of 4 peaks were expected in the 440 c/sec region. The methyl protons showed as a single peak at 141 c/sec. The UV-spectrum of *m*-cyanohydrocinnamonitrile had maxima at 281.5 ($\varepsilon = 978$), 273 ($\varepsilon = 972$), 225 ($\varepsilon = 12{,}500$) and 232 ($\varepsilon = 11{,}000$) nm and shoulders at 267 and 258 nm.

The close agreement in the mass spectra of the 3 isomeric dinitriles (Table) is similar to, but even more striking than that seen in the mass spectra of ethylbenzene and the xylene isomers[6]. Nor did the model compound IR-spectra assist in eliminating hydrocinnamonitrile structures from consideration. The nitrile region of the references clearly suggested that the unknown was an aromatic nitrile. However, an indication of the unknown substance's structure was found by comparison of its PMR-spectrum with those of the models. The references showed 4 aliphatic proton bands, corresponding to each proton in the side chain but the unknown had a single peak equivalent to 4 protons. These data prompted consideration of the xylylenedicyanides, particularly the *p*-isomer[7]. A comparison of synthetic *p*-xylylenedicyanide with the isolated material showed them to be identical.

When tested for antispasmodic activity using the in vitro rat uterus preparation[2] none of the compounds showed activity. However, when assayed for cytotoxicity, using the Eagle K-B cell tube dilution technique[8], a preliminary screen for antineoplastic effects, the following ID_{50} values obtained: *p*-xylylenedicyanide, 8 μg/ml; *p*-cyanohydrocinnamonitrile, 11 μg/ml; *m*-cyanohydrocinnamonitrile, 25 μg/ml. Although these data indicate a modest degree of cytotoxicity, they introduce the interesting prospect of more potent agents based on the benzylcyanide and hydrocinnamonitrile structures. The unusual structures prompt speculation on possible mechanisms for the cytotoxic effect. In each case the compound could act as an effective nucleophile. If so, the dinitriles are new and relatively non-toxic alkylating agents with possible antineoplastic potential.

Zusammenfassung. Extrakte von *V. opulus* von schwacher Zelltoxizität enthalten *p*-Xylylendinitril. Die Isomeren, *m*- und *p*-Cyanohydrozimtsäurenitrile, wurden synthetisiert und auf ihre zelltoxischen Eigenschaften hin untersucht: *p*-Xylylendinitril erweist sich als am stärksten wirksam.

J. A. NICHOLSON, C. H. JARBOE[9], W. HADDON and F. W. MCLAFFERTY[9]

Pharmacology Department, School of Medicine, University of Louisville (Kentucky 40202) and Chemistry Department, Purdue University, West Lafayette (Indiana, USA),
20 November 1967.

[6] American Petroleum Institute, 'Catalog of Mass Spectral Data', Texas A. and M. Univ., College Station, Texas.
[7] A. F. TITLEY, J. chem. Soc. *1926*, 508.
[8] C. G. SMITH, S. L. LUMMIS and J. E. GRADY, Cancer Res. *19*, 843 (1959).
[9] Inquiries on mass spectra should be directed to F.W.M.; all other inquiries should be directed to C.H.J.

A Negative Inotropic Response of Cat Atria to Sympathetic Nerve Stimulation or Norepinephrine

The effects of sympathetic nerve stimulation on cat, rabbit and guinea-pig atria in vitro have been described by many authors[1–8]. None of these reports, however, refer to a negative inotropic response of atria after sympathetic nerve stimulation, an observation we made frequently during recent investigations[9,10] in which innervated cat atria were used. This observation was unexpected in view of the extensive literature describing only positive inotropic and chronotropic effects of sympathetic nerve stimulation or sympathomimetic amines in the mammalian heart. Accordingly, we examined possible causes for this anomalous finding and present here the results of this study.

Isolated atria, with the right cardioaccelerator nerve intact, were prepared by methods described previously[3,9] using cats of either sex. Preparations were maintained under a tension of 1.5–2.0 g in Krebs-bicarbonate solution with the following composition: NaCl, 118.07 m*M*; KCl, 4.75 m*M*; $CaCl_2$, 2.5 m*M*; KH_2PO_4, 0.93 m*M*; $MgSO_4$, 1.19 m*M*; $NaHCO_3$, 25.00 m*M*; glucose, 11.10 m*M*. The fluid was aerated continuously by a mixture of 95% O_2 and 5% CO_2. Rate and force of contraction were monitored by a Grass FT03 force displacement transducer coupled to a Gilson laboratory model polygraph. Changes in force of contraction were considered significant only if they differed from control levels by at least 5%. The nerve was positioned within a double platinum plate shielded electrode located at the surface of the bathing fluid, and

[1] S. HUKOVIC, Br. J. Pharmac. *14*, 372 (1959).
[2] S. HUKOVIC, Br. J. Pharmac. *15*, 117 (1960).
[3] V. CHANG and M. J. RAND, Br. J. Pharmac. *15*, 588 (1960).
[4] M. D. DAY and M. J. RAND, Br. J. Pharmac. *17*, 245 (1961).
[5] K. GREEF, H. KASPERT and W. OSWALD, Arch. exp. Path. Pharmak. *243*, 528 (1962).
[6] F. E. LEADERS and J. P. LONG, J. Pharmac. *137*, 206 (1962).
[7] S. M. KIRPEKAR and R. T. FURCHGOTT, J. Pharmac. *143*, 64 (1964).
[8] U. TRENDELENBURG, J. Pharmac. *147*, 313 (1965).
[9] C. N. GILLIS, F. H. SCHNEIDER, L. S. VAN ORDEN and N. J. GIARMAN, J. Pharmac. *151*, 46 (1966).
[10] F. H. SCHNEIDER and C. N. GILLIS, Am. J. Physiol. *211*, 890 (1966).

was stimulated with a Grass Model 4S stimulator. Trains of square wave impulses were delivered at a variety of voltages, frequencies and impulse durations. Drugs, dissolved in Krebs-bicarbonate medium, were added directly to the bathing fluid.

Stimulation of the right cardioaccelerator nerve (impulse duration, 1–5 msec; frequencies, 0.5–60 impulses/sec; voltages, both sub- and supramaximal) for a short period of time produced positive chronotropic effects in each of 197 isolated atria, although in only 102 preparations was there an increase in the force of contraction. Contraction force was unchanged by nerve stimulation in 26%, and decreased in 22% of the atria (Table). Typical responses are shown in the Figure, which illustrates polygraph recordings from 5 separate experiments.

Both chronotropic and inotropic responses (whether negative or positive) were unaffected by atropine sulfate (2 μg/ml) in the bathing fluid or by varying the tension on the atria between 0.5 and 5 g. Furthermore, the direction of inotropic change was apparently unrelated to the prestimulation contraction rate (Figure). Such lack of relationship was surprising in view of the well-documented relationship between force and rate of beating in the myocardium[11].

Chronotropic responses were maintained throughout the entire stimulation period (10–400 sec) when stimulation of short total duration (400 stimuli or less) was applied to the nerve. Positive inotropic responses, however, frequently decreased in magnitude, despite the maintenance of stimulation (record c, Figure). This decline was more obvious when prolonged stimulation (1–60 impulses/sec for 50 min, submaximal voltage) was used. In only 1 of the 13 preparations in this group that had an initial positive inotropic response was this maintained throughout the entire period of stimulation. In fact, at the end of stimulation, 15 of the 19 preparations were beating with a contraction force less than the prestimulation value (Table).

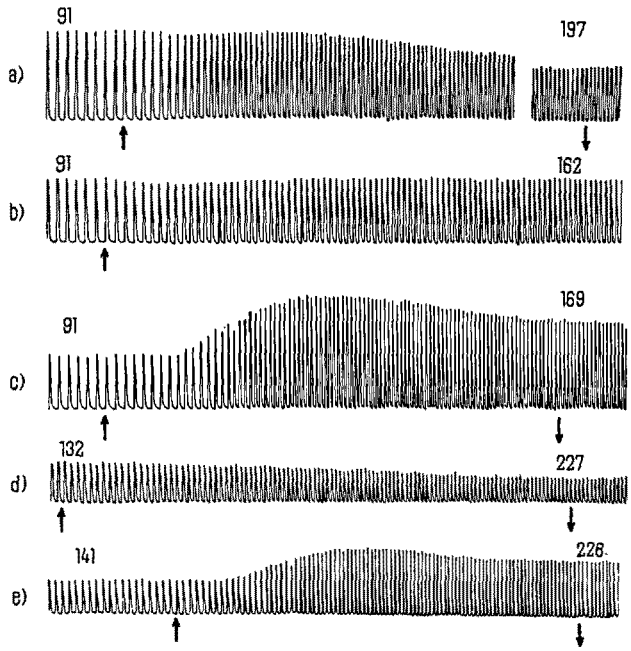
The inability of atria to maintain positive inotropic responses as well as the occurrence of a negative response (or the absence of change in contraction force) in a significant number of preparations suggested that certain metabolic processes may have been unable to meet the increased energy demand imposed by an increased force of contraction. In this regard, the rate at which various nutrients in the bathing fluid diffuse into the tissue may be of considerable importance. Accordingly, the relationship between the qualitative nature of force response and the body weight (which is closely correlated to heart size and presumably, therefore, to thickness of the atrial wall) of the cats used was examined. No correlation between body weight and the qualitative nature of the inotropic response was observed.

The effect of a variety of sympathomimetic amines upon atrial contractility was examined to determine whether a negative inotropic response (or the lack of a response) was associated only with sympathetic nerve stimulation. Forty-five preparations were exposed to noradrenaline hydrochloride, adrenaline bitartrate, isopropylnoradrenaline hydrochloride, dopamine (3-hydroxytyramine) hydrochloride or tyramine hydrochloride in concentrations sufficient to increase the rate of contraction in each preparation. These experiments indicated (Table) that the % occurrence of each type of inotropic response was similar to that seen after nerve stimulation. In 10 cases, responses to both sympathetic nerve stimulation and sympathomimetic amines were examined in the same preparation. In each instance, the change in force of contraction was qualitatively identical with both nerve stimulation or the amines: 5 preparations showed positive, while 5 responded with negative inotropic changes. It is, therefore, apparent that differences in the inotropic responses were not dependent purely on the sympathetic innervation.

Our results show that a significant number of isolated cat atria did not respond to either sympathetic nerve stimulation or to exogenous amines with the expected increase in force of contraction, although all responded

Inotropic response of isolated cat atria to sympathetic nerve stimulation or sympathomimetic amines. Shown are the % of all preparations responding with increased, decreased or unchanged force of contraction

Treatment	No. of preparations	Inotropic response (%) Positive	Negative	Unchanged
Short-term stimulation (400 stimuli or less)	178	52	22	26
Long-term stimulation (total of 50 min)	19			
after 2 min		68	27	5
after 50 min		5	79	16
Sympathomimetic amines	45	62	11	27



Responses of 5 different preparations of atria to sympathetic nerve stimulation. The figures above each record indicate contraction rate (beats/min). The conditions of stimulation were (a) 4/sec, 1 msec, (b) 2/sec, 5 msec, (c) 3/sec, 1 msec, (d) 3/sec, 1 msec and (e) 10/sec, 1 msec. All stimuli were delivered at submaximal voltage. Stimulus on at ↑, off at ↓. Record (b) is part of an experiment in which prolonged (50 min) stimulation was used; accordingly, only the time of starting stimulation is shown.

[11] J. R. Blinks and J. Koch-Weser, Proc. 2nd Int. pharmac. Meet. *5*, 53 (1963).

with a positive chronotropic response. The reason for the lack of consistency in the inotropic responses remains unclear, but apparently is unrelated to simultaneous vagal stimulation, the pre- or post-stimulation contraction rates, or to the size of the myocardium. Nor is it dependant on the sympathetic innervation, since the pattern of responses to exogenous amines was similar to that for nerve stimulation. It is possible that the variation in force responses is related to factors such as the sex of the cats from which the atria were taken, or to variations in the level of some critical metabolic intermediate or energy source within the myocardial tissue. It is clear, however, that the energy required for the increased force of contraction is more directly dependant upon these factors than that required to maintain an increased rate of contraction; the latter observation is in complete agreement with the findings of WEBB in his extensive investigations with isolated rabbit atria[12,13].

Zusammenfassung. Isolierte, sympathisch innervierte Katzenvorhöfe wurden durch den rechten Nervus accelerans mit Impulsen von 1–5 msec Dauer und mit einer Frequenz von 0.5–60 Impulsen/sec stimuliert. In allen Präparaten wurde eine positiv chronotrope Wirkung beobachtet, während nur in 52% der Fälle eine inotrope Wirkung nachweisbar war. Bei 26% war die Kontraktionsstärke nicht beeinflusst, während bei 22% eine negativ inotrope Wirkung festgestellt wurde, für deren Variieren bis jetzt keine Erklärung gefunden werden konnte.

F. H. SCHNEIDER[14], R. CYSYK[15] and C. N. GILLIS[16]

Department of Pharmacology, Yale University School of Medicine, New Haven (Conn. 06510, USA), 12 October 1967.

[12] J. L. WEBB, Br. J. Pharmac. *5*, 87 (1950).

[13] Supported by grants from the U.S. Public Health Service (No. H7249) and the Life Insurance Medical Research Fund (No. G-65-46).

[14] Present address: Department of Pharmacology, University of Colorado, Denver (Colorado, USA). Formerly pre-doctoral Fellow (U.S.P.H.S. Grant No. 5-T4-CA-5012).

[15] Pre-doctoral Fellow (U.S.P.H.S. Grant No. 5-T4-CA-5012).

[16] Established investigator of the American Heart Association.

The Influence of Bee Venom on the Osmotic Fragility of Human Red Blood Cells

The resistance of erythrocytes to hemolysis is clinically measured by the following tests: osmotic fragility test; osmotic fragility after incubation of the blood at 37 °C for 24 h; autohemolysis test and mechanical fragility test[1].

The osmotic fragility curve obtained from these tests is sigmoidal and symmetric and depicts the heterogenity of the osmotic behaviour of the red blood cell (RBC) populations[1]. The frequency distribution curve of the RBC population is a function of the concentration of the hypotonic NaCl solution.

The influence of bee venom on the osmotic fragility of RBC has not yet been studied. This may be attributable to the absence of a suitable method of individual but simultaneous measurement of the 2 factors, i.e. the concentration of the hypotonic NaCl solution and the time period of the presence of the venom, which govern the osmotic behaviour of the red blood cells.

The individual but simultaneous measurement of these 2 factors has now been rendered possible by a new method using the fragiligraph[2–4]. The latter automatically records the degree of hemolysis as a function of time, i.e. of decreasing salt concentration in the RBC suspension.

The changing hemolysis pattern, together with the time period recorded on the fragiligram permit the establishment of the salt concentration at any point by the aid of an established curve[4].

Four men and 2 women, 24–25 years of age, who were clinically healthy and had a negative family anamnesis of hemolytic diseases were studied. Their blood, drawn by finger puncture, was collected in capillary tubes of a type used for microhematocrit.

Normal fragiligrams were obtained by a method based on gradual hemolysis in hypotonic NaCl solutions[2–4]. 0.075 ml of a 1:10 dilution of blood in isotonic buffered NaCl solution were introduced into a container cell with walls of dialyzing membrane. The cell was then placed into a test-tube with distilled water and this again into the fragiligraph, an instrument similar to a colorimeter with a recorder between a source of light and a photoelectric cell. Dialyzing through the membrane resulted in a continuous reduction in the salt concentration of the medium surrounding the erythrocytes. The degree of hemolysis measured, on the basis of the increasing transparency of the erythrocytes suspension in the course of hemolysis. The record of the increasing light transmission in relation to time yielded the fragiligram or its derivative. The salt concentrations at different points of the cumulative curve were found with the aid of a previously established curve.

The influence of the venom was studied by diluting 1 volume of blood in 9 volumes of isotonic buffered NaCl solution which contained 2 γ of bee venom/ml. After 20–30 sec, 0.075 ml of the suspension were introduced into the container cell for recording.

In the fragiligrams, the degree of hemolysis (ordinate-%) was recorded as a function of time (abscissa – min) during which the venom was present in the RBC suspension. The time values were transferred to concentration values by the established curve[4].

The fragiligrams of the control tests and the fragility values are summarized in Figure 1 and Table I. All time (concentration) – hemolysis curves had a sigmoidal pattern and the derivative curves were unimodal as typical of a continuous distribution of a heterogeneous population. The fragility values were within the range of normal blood. Hemolysis began at 0.41 $\pm$ 0.04% NaCl and was completed at 0.28 $\pm$ 0.04% NaCl.

[1] J. V. DACIE and S. M. LEWIS, *Practical Haematology* (J. and A. Churchill, London 1963).

[2] D. DANON, A. NEVO and Y. MARIKOVSKY, Res. Counc. Israel Bull. *6E*, 36 (1956).

[3] D. DANON, E. K. FREI, Y. F. FREI and Y. LIPKIN ,Inst. of Electrical Electronic Engineering, Transactions on Bio-Medical Electronics, 10, 24 (1963).

[4] D .DANON, J. clin. Path. *16* ,377 (1963).