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.

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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.

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Table I. Osmotic fragility of erythrocytes from normal blood

Minimum resistance						Maximum resistance					
Min	S.D.	Range	NaCl%	S.D.	Range	Min	S.D.	Range	NaCl%	S.D.	Range
3.08	0.23	2.75–3.25	0.41	0.04	0.40–0.44	5.46	0.31	5.00–6.00	0.28	0.04	0.20–0.30

S.D., standard deviation.

Table II. Osmotic fragility of erythrocytes in the presence of bee venom

Minimum resistance						Maximum resistance I					
Min	S.D.	Range	NaCl%	S.D.	Range	Min	S.D.	Range	NaCl%	S.D.	Range
0.46	0.41	0.13–1.50	0.79	0.10	0.58–0.88	1.39	0.58	1.00–2.50	0.59	0.10	0.46–0.68
						Maximum resistance II					
						Min	S.D.	Range	NaCl%	S.D.	Range
						2.93	0.43	2.75–3.75	0.43	0.03	0.36–0.45

S.D., standard deviation.

Table III. The relative values (%) of the RBC population obtained by the osmotic fragility test in the presence of bee venom

More fragile population			Less fragile population		
%	S.D.	Range	%	S.D.	Range
28.0	9.5	20.0–37.5	73.6	23.4	62.5–82.0

S.D., standard deviation.

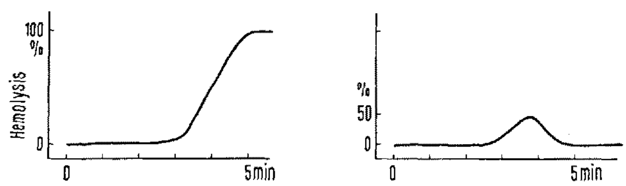

Fig. 1. The fragiligrams (left) and the derivatives (right) from normal blood.

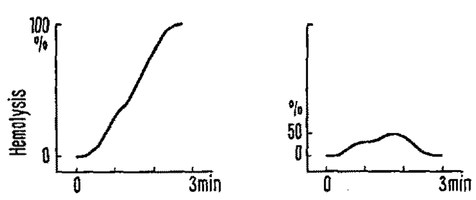

Fig. 2. The fragiligrams (left) and the derivatives (right) of erythrocytes in the presence of bee venom.

The fragiligrams of the treatment tests and the fragility values are summarized in Figure 2 and Table II. The cumulative curves and their derivatives were all bimodal, indicating 2 erythrocyte populations. Minimum resistance was at $0.79 \pm 0.1\%$ NaCl. The maximum resistance of the more fragile population was at $0.59 \pm 0.1\%$ NaCl and that of the less fragile population, at $0.43 \pm 0.03\%$ NaCl. These values indicated an increased fragility of the RBC in the presence of bee venom as compared with normal blood. The relative values of the 2 RBC populations obtained by direct planimetration are summarized in Table III. The more fragile population represents $28.01 \pm 9.56\%$ and the more resistant are $73.63 \pm 2.34\%$ of the RBC population.

The fragiligrams obtained in this study are of a new pattern showing 2 separate populations of the RBC of increased fragility. The relative values obtained for the 2 populations by direct planimetration of the derivative curves are similar to the values for 'old' and 'young' erythrocytes obtained in electron microscope studies[5]. Hence it may be that the division of the RBC into 2 populations is due to the 2 known factors acting in the hemolytic mechanism of bee venom[6,7] or the age distribution of the RBC population. However, the fragiligrams were so very typical and reproducible as to suggest an auxiliary test to prove venom activity in blood.

Résumé. Le venin des abeilles augmente la réfraction osmotique des globules rouges du sang humain. Il divise la population cellulaire en 2 groupes de réfraction osmotique. Ces 2 groupes semblent représenter les globules rouges «jeunes» et «vieux». La courbe obtenue peut servir de test spécifique révélant l'action du venin des abeilles sur le sang.

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