# THE INFLUENCE OF BEE VENOM ON THE OSMOTIC FRAGILITY OF YOUNG RABBIT RBC

I. HORT

*Department of Anatomy and Physiology, The Hebrew University, Rehovot, Israel*

THE resistance of erythrocytes to hemolysis is clinically measured by various classical tests,[1] and recently by a new method using the fragiligraph.[2–5] By this method the influences of bee venom on the osmotic fragility of human RBC were studied.[5] The venom was found to increase the osmotic fragility and induced the division of the RBC into two populations of different osmotic fragility patterns. The fragiligrams were very typical, suggesting an auxiliary test to prove venom activity in blood.

In this study, the influence of bee venom on the osmotic fragility of young rabbit red-blood cells, which are normally dividing into two populations, was established.

Twelve rabbits, 1-3 months of age, were studied. Blood samples were taken from the central vein of the ear by vein puncture and collected in heparinized capillary tubes of a type used for microhematocrit.

Normal fragiligrams were obtained by a method based on gradual hemolysis in hypotonic NaCl solutions.[2-5]

The influence of the venom was studied by mixing 1 ml. of buffered isotonic NaCl solution containing 20 gama per ml. of bee venom with different volumes, ranging from 1 to 999 ml. of 1 : 10 RBC suspension in buffered isotonic NaCl solution (Concentrations 1 : 1–1 : 1000 in the tables). After 20-30 seconds, 0·075 ml. of the suspension was introduced into a container cell for recording, by the same method used for normal fragiligrams (Test 1 and time interval 1 in the tables). A few minutes later, a second and sometimes a third record from the same sample was made (Tests 2 and 3 time intervals 2 and 3 in the tables).

The relative values of the RBC populations were obtained by a direct planimetration of the area under the derivative curves.

In the fragiligram (Cumulative curves in Fig. 1), the degree of hemolysis (Ordinate-%) recorded as a function of time during which the venom was present in the RBC suspensions, and the hemolysis took place (Abscissa—minutes). The time values were transferred to concentration values of NaCl solutions.

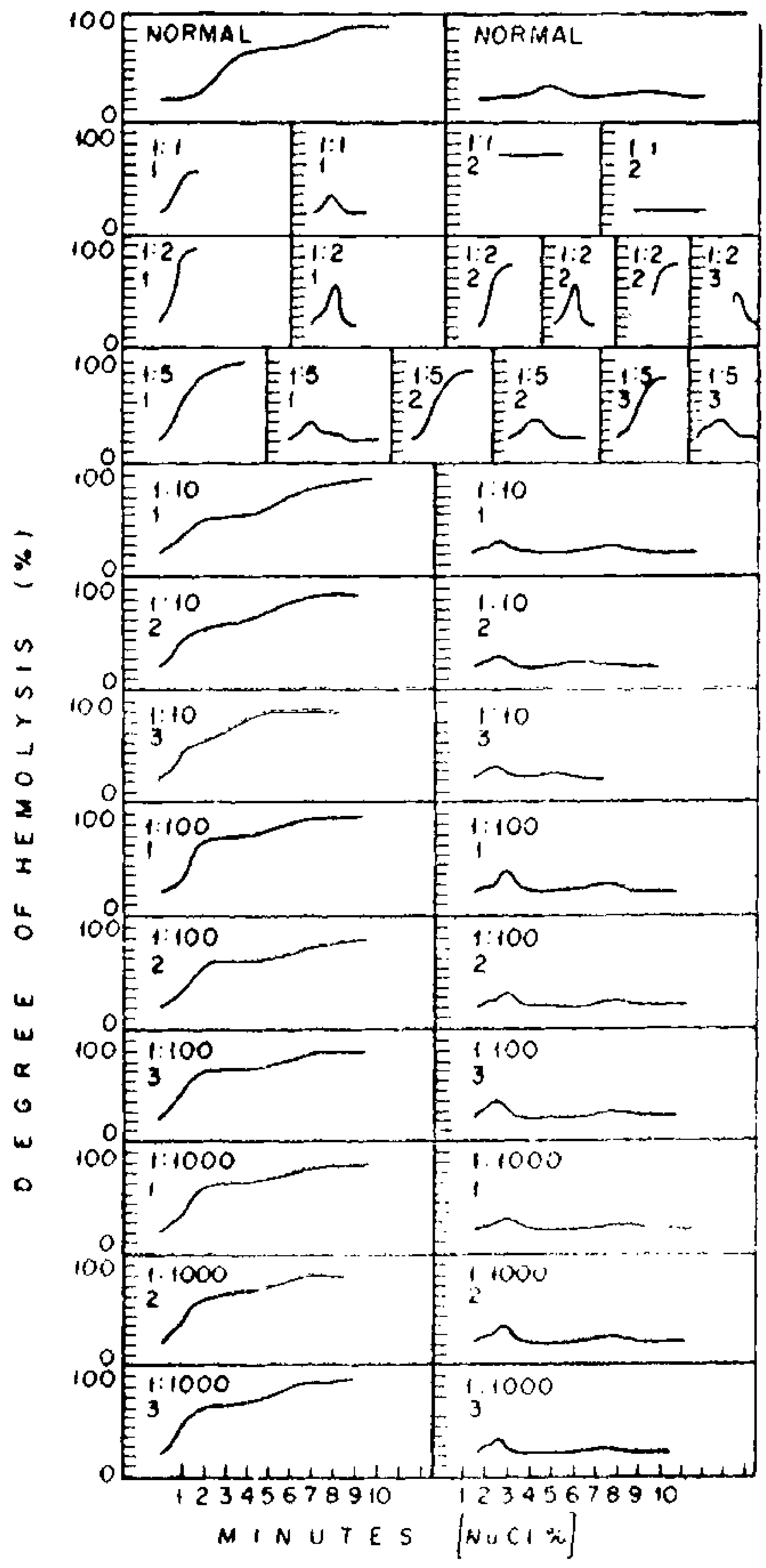



FIG. 1. The fragiligrams and their derivatives obtained from the controls and the treatment studies from rabbits one to three months of age.

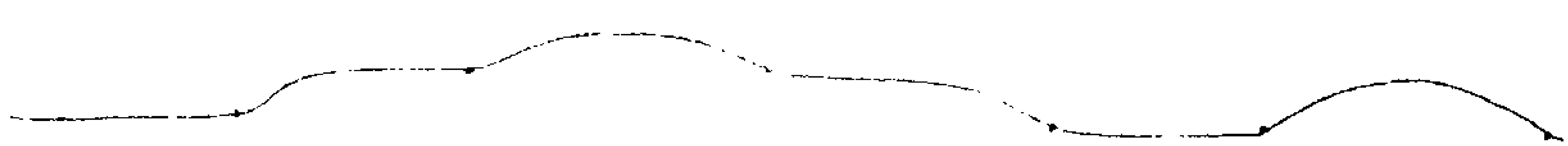
The fragiligrams, the derivative curves and the fragility values for rabbit in normal conditions and in the presence of the bee venom with indications to the important point on the curves, are presented in Fig. 1 and Table I. The relative values of the RBC population are presented in Table II,

TABLE I

*Osmotic fragility of erythrocytes from the controls and the treatment studies from rabbits 1-3 months of age*

| Concentration | Test No. | Time interval minutes | Minimum resistance I: Time (minutes) | Minimum resistance I: NaCl (%) | Maximum resistance Ia: Time (minutes) | Maximum resistance Ia: NaCl (%) | Maximum resistance Ib: Time (minutes) | Maximum resistance Ib: NaCl (%) | Maximum resistance Ic: Time (minutes) | Maximum resistance Ic: NaCl (%) | Minimum resistance II: Time (minutes) | Minimum resistance II: NaCl (%) | Maximum resistance I: Time (minutes) | Maximum resistance I: NaCl (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls | 1 | 0·50 ±0·03 | 2·1 ±0·05 | 0·52 | .. | .. | .. | .. | 4·6 ±0·23 | 0·32 | 6·7 ±0·47 | 0·24 | 11·0 ±2·01 | 0·18 |
| 1 : 1 | 1 | 0·20 ±0·01 | 0·3 ±0·01 | 0·80 | .. | .. | .. | .. | 1·4 ±0·10 | 0·59 | .. | .. | .. | .. |
| | 2 | 10·25 ±0·60 | 0·0 ±0·00 | 0·90 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1 : 2 | 1 | 0·50 ±0·02 | 0·0 ±0·00 | 0·90 | 0·5 ±0·03 | 0·78 | .. | .. | 1·3 ±0·09 | 0·61 | .. | .. | .. | .. |
| | 2 | 6·30 ±0·10 | 0·0 ±0·00 | 0·90 | 0·5 ±0·05 | 0·78 | .. | .. | 1·3 ±0·08 | 0·61 | .. | .. | .. | .. |
| | 3 | 12·20 ±1·01 | 0·0 ±0·00 | 0·90 | .. | .. | .. | .. | 0·9 ±0·13 | 0·68 | .. | .. | .. | .. |
| 1 : 5 | 1 | 0·50 ±0·03 | 0·2 ±0·03 | 0·86 | 0·7 ±0·02 | 0·74 | 1·5 ±0·30 | 0·58 | 2·5 ±0·23 | 0·46 | .. | .. | .. | .. |
| | 2 | 9·50 ±0·95 | 0·2 ±0·04 | 0·86 | 0·5 ±0·03 | 0·78 | 1·9 ±0·28 | 0·52 | 2·3 ±0·20 | 0·48 | .. | .. | .. | .. |
| | 3 | 17·70 ±2·30 | 0·2 ±0·01 | 0·86 | 0·7 ±0·01 | 0·74 | .. | .. | 2·3 ±0·21 | 0·48 | .. | .. | .. | .. |
| 1 : 10 | 1 | 0·50 ±0·11 | 0·4 ±0·05 | 0·70 | 0·9 ±0·02 | 0·68 | .. | .. | 2·4 ±0·18 | 0·47 | 4·4 ±0·31 | 0·33 | 8·7 ±0·41 | 0·20 |
| | 2 | 16·50 ±0·93 | 0·2 ±0·03 | 0·86 | 0·7 ±0·06 | 0·74 | .. | .. | 1·9 ±0·10 | 0·52 | 3·2 ±0·27 | 0·40 | 7·1 ±0·25 | 0·23 |
| | 3 | 37·00 ±3·45 | 0·2 ±0·02 | 0·86 | 0·7 ±0·10 | 0·74 | .. | .. | 2·0 ±0·31 | 0·53 | 2·8 ±0·29 | 0·42 | 5·1 ±0·13 | 0·29 |
| 1 : 100 | 1 | 30 ±0·05 | 0·3 ±0·02 | 0·82 | 0·9 ±0·20 | 0·68 | .. | .. | 2·5 ±0·20 | 0·46 | 5·2 ±0·40 | 0·29 | 8·6 ±0·29 | 0·21 |
| | 2 | 10·70 ±1·09 | 0·3 ±0·02 | 0·82 | 1·0 ±0·32 | 0·67 | .. | .. | 2·5 ±0·15 | 0·46 | 5·8 ±0·21 | 0·27 | 8·6 ±0·12 | 0·21 |
| | 3 | 23·90 ±2·92 | 0·2 ±0·01 | 0·86 | 0·5 ±0·10 | 0·21 | .. | .. | 2·0 ±0·13 | 0·53 | 5·5 ±0·38 | 0·28 | 8·0 ±0·20 | 0·22 |
| 1 : 1000 | 1 | 0·30 ±0·02 | 0·2 ±0·01 | 0·86 | 0·8 ±0·21 | 0·69 | .. | .. | 2·5 ±0·15 | 0·46 | 5·2 ±0·33 | 0·29 | 8·0 ±0·25 | 0·22 |
| | 2 | 12·50 ±2·35 | 0·2 ±0·01 | 0·86 | 0·8 ±0·17 | 0·69 | .. | .. | 2·5 ±0·20 | 0·46 | 4·8 ±0·18 | 0·30 | 7·8 ±0·43 | 0·23 |
| | 3 | 23·00 ±5·31 | 0·2 ±0·04 | 0·86 | 0·6 ±0·10 | 0·76 | .. | .. | 2·4 ±0·30 | 0·47 | 4·4 ±0·25 | 0·32 | 7·4 ±0·31 | 0·23 |

Referent point on the derivative curve



The normal fragiligrams and the normal derivatives are bimodal as typical for normal young rabbit RBC populations.[6] The fragility values are within the normal range.

An increased fragililty of the RBC populations, fusion of the two populations into one, the appearance of a third population and changes in the relative values of the populations are found according to the influence of the different venom concentrations and tests. The fragility of the RBC is increased and the fusion of the populations is more prominent, moving from the lowest to the highest venom concentration and from the first to the third test.

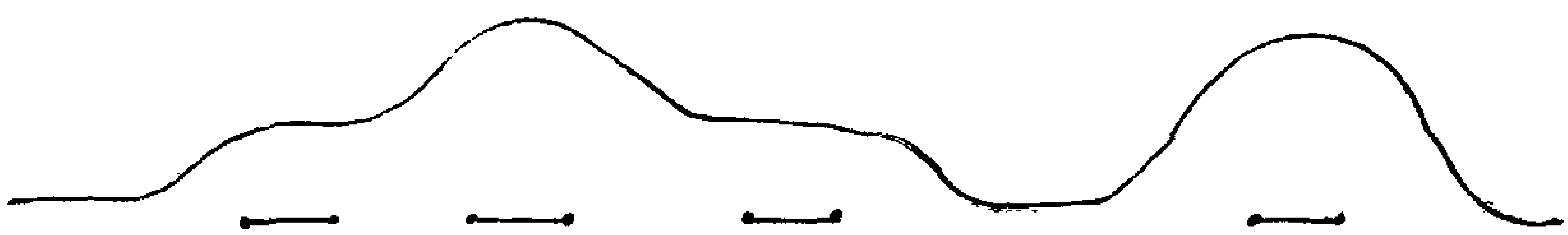
The osmotic fragility of rabbit RBC has recently been studied.[6,7] According to the authors, the fragiligrams of young rabbit

TABLE II

*The relative values of the RBC populations obtained from rabbit 1-3 months of age*

| Concentration | Test No. | Time interval minutes | Population I (%) | | | Population II (%) |
|---|---|---|---|---|---|---|
| | | | Ia | Ib | Ic | |
| Controls | 1 | 0·50±0·03 | .. | 52·6±8·3 | | 47·3±5·1 |
| 1 : 2 | 1 | 0·50±0·02 | 13·9±2·8 | 86·1±6·6 | | .. |
| | 2 | 6·30±0·10 | 21·9±5·1 | 78·0±5·7 | | .. |
| 1 : 5 | 1 | 0·50±0·03 | 12·9±1·9 | 61·2±4·7 | 25·8±2·5 | .. |
| | 2 | 9·50±0·95 | 9·4±1·7 | 84·3±5·9 | 6·2±1·0 | .. |
| | 3 | 17·70±2·30 | 17·8±3·1 | 82·2±7·1 | .. | .. |
| 1 : 10 | 1 | 0·50±0·11 | 13·5±2·5 | 43·2±9·3 | | 43·2±3·3 |
| | 2 | 9·50±0·93 | 18·5±4·0 | 40·7±9·5 | | 40·7±4·7 |
| | 3 | 37·00±3·79 | 12·5±2·0 | 50·0±9·9 | | 37·5±4·1 |
| 1 : 100 | 1 | 0·30±0·05 | 9·3±1·6 | 65·1±6·3 | | 25·5±6·2 |
| | 2 | 0·70±1·09 | 11·1±2·9 | 57·8±6·9 | | 31·1±3·4 |
| | 3 | 23·90±2·92 | 15·6±3·5 | 51·3±1·3 | | 25·1±2·2 |
| 1 : 1000 | 1 | 0·30±0·02 | 16·7±2·4 | 56·2±7·7 | | 23·3±2·5 |
| | 2 | 12·50±2·35 | 12·5±1·3 | 62·5±5·4 | | 25·0±4·3 |
| | 3 | 23·00±5·31 | 12·5±2·3 | 53·1±2·7 | | 34·3±3·1 |

Referent point on the derivative curve



are bimodal, indicating two erythrocyte populations; the "Adult" and "Fœtal" types. Similar results were obtained in the controls of this study.

The relative values of "Young" (64·1% ± 7·3) and "Old" (36·5% ± 7·3) rabbit RBC were previously obtained by the electronmicroscope.[8] The same values were measured in the fragiligrams of normal young rabbits (Controls of Table II). It may be, that the electronmicroscipe differentiates between "Adult" and "Fœtal" RBC and not between "Young" and "Old" RBC. The differentiation between "Young" and "Old" erythrocytes which are both components of "Adult" RBC type, might be achieved by the fragility test in the presence of bee venom or its active factors, since they induce the division of the "more fragile population" (Population 1 in Table II) which was proved to be identical with the "Adult" RBC,[6] into two populations.

The division of human RBC, by the osmotic fragility into two populations, has been studied in various conditions,[9-11] including the influence of bee venom.[5] The fragiligrams were bimodal, indicating two RBC populations, both of increased fragility or one of them of an increased fragility and the other of a decreased fragility. The results of the treatment in this experiment were compatible with those found by the influence of the bee venom on human RBC. The osmotic fragility was increased and the normal "more fragile population" (The "Adult" RBC) was divided into two populations (Concentrations 1 : 10-1 : 1,000).

Similar to the findings for human RBC, the fragiligrams of rabbit RBC in the presence of bee venom were typical and reproducible. In addition, the fragiligrams obtained were found to be a function of both the venom concentration and the time during which the venom was present in the RBC suspensions (Tests 1, 2 and 3).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# ORIENTED ADSORPTION OF ALIPHATIC NORMAL ALCOHOLS IN THE MONOLAYER ON FIBROUS SILICA GEL

K. SUBBA RAO AND BHAGWAN DAS

*Department of Chemistry, Birla Institute of Technology and Science, Pilani (Rajasthan) India*

THE subject of adsorption is one of the oldest and yet it has remained new in view of the voluminous amount of researches which are being published. The adsorbent and adsorbate materials used by different workers are numerous and varied in composition and the adsorption isotherms obtained have large variety of shapes. The BET classification[1] of the different isotherms into 5 typical categories has been a distinct advance and simplification. On the theoretical interpretation of adsorption, at the time when Langmuir looked upon adsorption as purely monomolecular and Zsigmondy, McGavack and Patrick as purely capillary condensation, neither of them being completely successful in explaining all the diverse facts of adsorption, the multimolecular adsorption theory proposed by Brunauar, Emmett and Teller has been a furthed advance in the subject. The sorption-desorption hysteresis has still remained a vexed and unsolved problem.

Monsanto Company, U.S.A., has produced a new form of silica ærogel of trade name Santocel C. This is a fine loose dusty powder, white in colour. Its air volume is 94%.[2] The particles are composed of submicroscopic fibres of 25 to 35 Å diameter and approximately 330 Å apart with a specific surface of 600 sq. m. per gm. This product is essentially meant for use as a flatting agent in protective and decorative coatings.[3] It has been used by Puddington[4] in making stopcock lubricant in glycerine for organic vapours. This new form of silica-fibrous silica gel (Santocel C) has been used as the adsorbent and methyl, ethyl, *n*-propyl, *n*-butyl and *n*-amyl alcohols as adsorbates in the present study of the nature of adsorption in the monolayer and sorption-desorption hysteresis.

Quartz fibre spring technique[5] has been employed in the present investigations. Fibrous silica gel was heated to 250° C. for 2 hrs. in order to remove any organic vapour and the activated gel was used in studying a series of sorptions and desorptions of the aliphatic alcohols at 35° C. The study was continued upto 3rd or 4th cycle. In each system there has been permanent and reproducible hysteresis loop and the loops have been presented in Fig. 1 in which the volume of alcohol adsorbed per 100 gm. of gel is plotted against the relative vapour pressure of the alcohol.

The isotherms of all the five alcohols have clearly defined "Knees". According to BET theory, the "Knee" signifies the transition from monomolecular to multimolecular adsorption. By the application of BET equation to the isotherms, the monolayer capacity for each alcohol is determined. From the monolayer capacity and knowing the cross-section area of the alcohol molecule, the specific surface of the fibrous silica gel has been calculated. There are three possible values for the cross-section depending upon the shape of the molecule and mode of adsorption. The molecule may be assumed to be spherical and the diameter D spherical[6] and the cross-section are obtained from molecular weight and density. The alcohol molecule being linear, there are two modes of oriented adsorption perpendicular and parallel to surface. The cross-section of the linear molecule is $(4{\cdot}55)^2$ Å$^2$. Knowing