estimate by the equation (predictability) indicates that the equation is quite satisfactory as a statistical model. The correlation coefficient of 0.998 indicates a good fit; a value of r of 1.000 would be a perfect fit of the data to the line described by the equation. Thus, the molar diamagnetic susceptibility for an aromatic molecule can be expressed to a good level of approximation by an order of magnitude increase in the molecular electrophilic superdelocalizability. A major advantage of this method over use of Pascal constants, etc. in estimating diamagnetic susceptibilities is that a constitutive correction appropriate to a given molecule is made 'automatically' as a consequence of the linear combination of atomic orbital – molecular orbital calculation.

Self polarizabilities[14], localization energies[15] or Z-numbers[16] might also replace superdelocalizabilities[17] in the correlation as these are known[18] to be highly intercorrelated. We find however that S^E values are easier to obtain than the others. A word of caution is now necessary. The correlation we have obtained may not necessarily be of fundamental physical significance. Superdelocalizability was originally derived[19] from perturbation theory as a reactive index for favorable charge-transfer transition state for a reaction whereby a weak π bond was formed between attacking reagent and specific atom of substrate. Diamagnetic susceptibility reflects interaction of organic molecule's electrons with a magnetic field. Statistically, ΣS^E may vary in the same direction as a more physically meaningful quantity or it may be related in a more complex manner to physically significant parameters (cf. reference[20]). Nevertheless while the 'true' physical meaning of the correlation may be suspect at this time, the correlation should prove useful in estimating diamagnetic susceptibilities for aromatic compounds. A striking feature of the correlation is that a single expression is all that is required for complex molecules that include alternate hydrocarbons, heterocyclics, and substituted benzenes. $-X_M$ values have been used in nuclear magnetic resonance[21], to calculate London dispersion forces[22], and to describe molecular interactions in biological systems[23, 24].

Conclusion. The diamagnetic susceptibility of an aromatic molecule may be predicted from its molecular electrophilic superdelocalizability calculated from Hückel molecular orbital theory. Correlation between the two variables was given by the least squares expression $-X_M = 10.00\,\Sigma S^E + 0.28$ for 44 aromatic compounds consisting of substituted benzenes and nonalternate hydrocarbons and heteroatomic substances. Diamagnetic susceptibilities have been used in calculation of London forces and of molecular interactions in biological systems.

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PRO EXPERIMENTIS

A Method for Automatic Recording of Serum Lysozyme Activity with the Fragiligraph

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Summary. A quick and simple method for the estimation of lysozyme activity using the Fragiligraph, was described. Diminution of turbidity in a suspension of *Micrococcus lysodeikticus* produced by the addition of standard lysozyme (hen egg white) or serum sample, was continuously recorded for 5 min by the Fragiligraph. The normal mean serum lysozyme activity value obtained by this method is 6,80 µg/ml ± 1.85.

Serum lysozyme level is considered to be a reflection of the turnover of neutrophilic granulocytes[2, 3]. High values were reported in conditions with increased turnover or destruction of neutrophilic granulocytes, such as neutropenia due to hypersplenism[4, 5], polycythemia vera[6] and megaloblastic anemia[7]. Low values were reported in neutropenia due to hypoplastic bone marrow[4, 5]. Serum and urine lysozyme activity has recently been introduced as a useful test in the differential diagnosis of acute leukemia[8, 9]. Since lysozyme is filtered by the glomerulus and almost completely reabsorbed by proxymal tubular cells[10], the serum and urinary lysozyme activity can be used for monitoring renal function.

Immunological or bacteriolytic methods are currently used for human lysozyme activity determination in

[1] We gratefully acknowledge the excellent technical assistence of Miss M. Touma.

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biological fluids. Since lysozymes from different species are immunologically different, the use of immunological methods has been limited by the short supply of human crystalline lysozyme. Thus, bacteriolytic methods (turbidometric or lyso-plate) have been widely used.

When a certain amount of lysozyme is added to a suspension of *Micrococcus lysodeikticus*, the turbidity of the suspension is gradually diminished as a result of bacterial lysis by the lysozyme. This process, which is generally measured by spectrophotometric methods, can be recorded automatically by the Fragiligraph, which is an instrument primarily designed for the study of the osmotic fragility of red blood cells[11] and which was subsequently adapted for a variety of other clinical tests and studies[12,13].

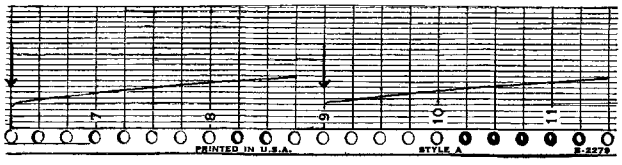

Fig. 1. Automatically recorded curve obtained in 5 min by a Fragiligraph. 0.2 ml of a solution containing 7.5 µg of egg white lysozyme were added (arrow) to a 1.8 ml of bacterial suspension containing 15 mg/100 ml phosphate buffer. The increased light transmission represents increased bacterial lysis.

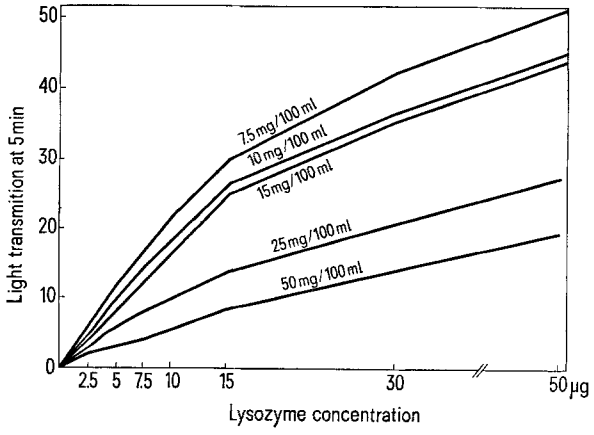

Fig. 2. Bacterial lysis by standard lysozyme solution as expressed by light transmission at 5 min with different concentrations of bacterial suspensions. The degree of light transmission is given in arbitrary units, the light transmission reached at the plateau with 7.5 mg/100 ml of bacterial suspension being taken as 50%. Note the linearity of the curve obtained when 15 mg of bacteria in 100 ml of buffer were used.

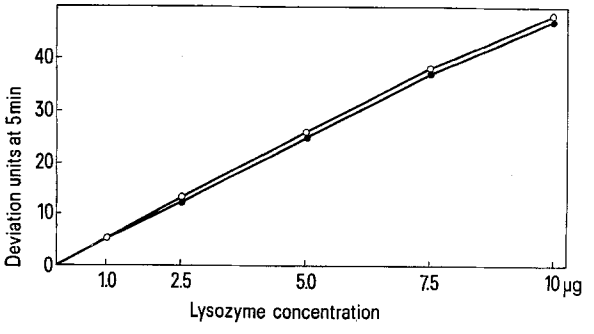

Fig. 3. The range obtained by 6 successive standard curves performed with the same bacterial suspension of 15 mg/100 ml buffer solution and a standard enzyme solution. The narrow range indicates satisfactory reproducibility.

In the present communication, we report a modification of the turbidometric method of lysozyme determination[14,15], in which the rate of lysis is automatically recorded by the Fragiligraph.

A suspension of ultraviolet-killed, dried *Micrococcus lysodeikticus* (Difco) in sodium phosphate buffer (pH 6.25) was used as a substrate for the enzymatic assay. The suspension was prepared at least 6 h before use and kept at 4 °C for not more than 4 days. Crystallized egg white lysozyme (Difco) in sodium phosphate buffer was used to establish the standard for enzymatic activity. The dilutions were prepared before each assay from a stock solution stored at 4 °C. In preliminary experiments, amounts of 1; 2.5; 5; 7.5; 10; 15; 30; and 50 µg in 0.2 ml of normal saline were used in each assay. Later it was found that only the first 5 solutions were necessary for practical purposes. Fragiligraph Model D_2 (Elmedix, Tel-Aviv, Israel), equipped with a 'linearizer' and holder for a plastic cuvette, was used; 1.8 ml of the bacterial suspension was introduced into the cuvette containing a magnetic bar. The baseline was adjusted to the first line of the recording paper, and the recorder switched to the 'on' position. The magentic bar was then automatically activated. After a few sec of stirring and recording at baseline level, 0.2 ml of the standard solution or of the serum sample was added. The increasing light transmission as a function of time was recorded for 5 min. Each assay was performed twice, and the deviations obtained were practically identical (Figure 1).

Figure 2 shows the rate of bacterial lysis as expressed by increased light transmission, with different concentrations of bacterial suspensions. The light transmission at 5 min was plotted against different concentrations of standard lysozyme, this for each bacterial concentration. The lower the bacterial concentrations used, the higher was the light transmission (Figure 2). It can be seen that a decrease in the bacterial lysis at 5 min took place with lysozyme concentrations higher than 15 µg in every one of the bacterial concentrations tested. It can be seen in the left part of Figure 2 that there is a deviation from linearity with very high (50 and 25 mg) or very low (7.5 mg) bacterial concentrations. The optimal concentration of bacteria was, therefore, chosen as 15 mg per 100 ml of

Table I. Serum lysozyme activity in different types of leukemia

Type of leukemia	Serum lysozyme (µg/ml)	Urinary lysozyme (µg/ml)
Acute undifferentiated leukemia	0	0
Acute myeloid leukemia	5.0	0
Acute erythroleukemia	5.0	N.D.
Chronic myeloid leukemia (blastic crisis)	32.8	N.D.
Acute promyelocytic leukemia	50.0	1.6
Acute myelomonocytic leukemia	57.0	1.1
Acute monocytic leukemia	105.0	110.0
Acute monocytic leukemia	187.5	N.D.
Chronic monocytic leukemia	425.0	N.D.

Both morphological and cytochemical criteria were employed for the diagnosis of the different types of leukemia. N.D. = not done.

Table II. Serum and urinary lysozyme activity in chronic renal failure

Patient No.	Urea (mg/100 ml)	Cr. clearance ml/min	Serum lysozyme (μg/ml)	Urinary lysozyme (μg/ml)
1	42	60	25.0	0.8
2	63	25.0	50.0	1.5
3	108	10.0	65.0	20.0
4	208	6.0	45.0	5.6
5	171	4.9	44.0	25.0
6	250	3.2	37.5	5.45
7	232	3.0	62.5	30.0
8	261	2.6	45.0	40.0
9	320	2.5	83.0	21.5
10	258	1.4	72.0	100.0

The number of neutrophilic granulocytes were in the normal range in every case. Note the high levels of serum lysozyme in patients with low levels of creatinin clearance. Patients with high urinary lysozyme had other signs of functional tubular impairment.

buffer, resulting in a final concentration of 13.5 mg/100 ml after the addition of 0.2 ml of the enzyme solution. Figure 3 represents the range of the variability of typical standard curves obtained when a bacterial suspension of 15 mg/100 ml was used. However, it is recommended that a standard curve be established for every day on which tests or experiments are to be performed. We found that the addition of NaCl did not improve either the linearity or the reproductibility of the reaction. Therefore, we did not include the addition of salt to the assay system, as Parry and others did[14].

Procedure. A standard curve is established as described above, using 5 different concentrations of enzyme and a bacterial suspension of 15 mg/100 ml. Each assay was recorded for 5 min. (This period of time was chosen because after 5 min the curve changes and tends to approach a plateau.) The tests are now performed in a similar manner, with 0.2 ml of serum being added to the bacterial suspension instead of 0.2 ml of the standard enzyme. The level from the baseline at 5 min is measured and compared to the standard curve. Each test is performed twice and the deviation obtained at 5 min is usually identical.

We tested the serum lysozyme activity of 85 healthy hospital personnel aged between 20 and 60. The mean lysozyme value found was 6.80 μg/ml ± 1.85. These results are in agreement with values reported in the literature for normal serum lysozyme activity, using egg white lysozyme as standard and turbidometric method[3].

We have currently been testing the serum and urinary lysozyme activity of patients suffering from different types of leukemia and renal diseases. The results obtained with the method presently described are within the range of those reported in the literature for the above-mentioned clinical conditions (Tables I and II).

When the serum lysozyme activity was higher than 15 μg/ml, the recorded curve was not linear in the first 5 min; the samples were therefore diluted in these cases with phosphate buffer until a straight line was recorded within the first 5 min, and the dilution was taken into account. In this manner, a simple, rapid and reproducible test is performed using small amounts of serum, which has also the advantage of recorded results that can be added to the protocol or to the case report.

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An Improved Method for Flow Dialysis Studies with Highly Increased Diffusion Rates[1]

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Summary. An improved flow dialysis procedure with highly increased diffusion rates has been developed allowing the study of small changes in the rate of diffusion. The application of the method described with only a few individual experiments, and with the use of small amounts of biological material, gives much information about binding systems.

It is known that for several reasons[2] flow dialysis could be an excellent approach for ligand polymer binding. Some investigations made with this technique have confirmed this thesis, although the sensitivity of the methods used[3–13] was disappointingly low. The same experience was confirmed during the development of our flow dialysis cell. It appears that the drawbacks of the method are caused by an uncontrolled turbulence inside the compartment containing the diffusant. By introducing a laminar flow in both compartments of the dialysis cell, the rate of diffusion increased 200 times and more compared with earlier published results[8].

The high reproducibility, flexibility and sensitivity of this method allows one to study small changes in the rate of diffusion due to parameters such as temperature, pH, ionic strength, viscosity, binding proteins and their various conformations. This allows a rapid screening of

[1] This work was supported by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung (grant No. 3.1650.73).
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